



Biochemical Characterization using Multivariate Analysis in Fruits of Date Palm (*Phoenix dactylifera* L.) Cultivars in North-Western Agroclimatic Zone of Tamil Nadu

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ABSTRACT

Background: The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated fruit crops. The dates are a great source of energy and easily absorbed carbohydrates because they are high in sugars, such as fructose and glucose. An experiment was conducted to assess the biochemical attributes of fifteen Date palm cultivars (*Phoenix dactylifera* L.) from the North-western region of Tamil Nadu. Six qualitative traits were analyzed, including TSS (°Brix), titratable acidity (%), ascorbic acid (mg/g), total sugar (%), reducing sugar (%) and non-reducing sugar (%).

Methods: The research experiment was carried out on 15 different date palm cultivars at Ariyakulam, Dharmapuri district, Tamil Nadu in the year 2024-2025. The experiment was laid out in a randomized block design (RBD). The 15 genotypes were considered as treatments, such as Barhee, Khalas, Anand, Mijenas, Khadraway, Sayas, Maktoom, Elite, Kanijee, RB-1, Noor, Medjool, 0.91, 101 and Ajwa. Five fruits/genotype and five genotypes per treatment were selected randomly and the following observations, like TSS (°Brix), titratable acidity (%), ascorbic acid (mg/g), total sugar (%), reducing sugar (%) and non-reducing sugar (%) were recorded. The Dendrogram and PCA-biplot, were generated using the CRAN (Comprehensive R Archive Network) programme version 4.5.0 and the data was analyzed using OPSTAT, considered statistically significant at $P = 0.05$.

Result: The results showed that the high TSS (31°Brix) was found in cultivar Ajwa, whereas high ascorbic acid (0.34 mg/g), total sugar (0.67%) and reducing sugar (0.74%) respectively, were recorded in cultivar Medjool. A multivariate technique has been found very helpful in identifying various germplasms with desirable traits. Based on the findings from the study, it is concluded that a diverse array of variability is observed in different cultivars. Ajwa cultivar recorded TSS (31°Brix) and Medjool (Ascorbic acid (0.34 mg/g), total sugar (0.67%) and reducing sugar (0.74%). The performance of the studied Date palm cultivars is well-suited in a water-scarce area and the coastal belt of the southern part of India. Therefore, it can be recommended for commercial cultivation in regions facing water scarcity and soil salinity.

Key words: Date palm, Dendrogram, Principal component analysis, Qualitative traits.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated fruit crops. It serves as a vital part of the diet in many countries across the Middle East and North Africa (Chao and Krueger, 2007). It is a monocotyledonous, dioecious plant with 18 pairs of diploid chromosomes ($2n = 2x = 36$) with an estimated 670 Mb genome size (Al-Dous *et al.*, 2011). They have been growing along the Tigris and Euphrates rivers since 4000 BC and are believed to have originated in Mesopotamia (modern-day Iraq). Around 3500 BC, date palm cultivation spread into Egypt and by 2000 BC, it had also reached India, Western North Africa and Southern Europe (Zohary and Hopf, 2000).

Date palm fruit is a single-seeded, rectangular berry with fleshy mesocarp and membrane endocarp. The dates are a great source of energy and easily absorbed carbohydrates because they are high in sugars, such as fructose and glucose. Dates are a highly nutritious and energy-rich fruit, offering around 282 kcal per 100 g and including other essential nutrients such as sugar (60-65%), vitamin A (10 IU), vitamin B complexes, trace level of fat (0.2-0.5%) and protein (2.3-5.6%). Additionally, dates provide significant dietary fibre (6.4-11.5%) and

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important minerals such as iron (8%), calcium (4%), potassium (9%), magnesium (12%) and zinc (3%). Their natural sweetness and nutrient density make them a healthy and delicious choice (USDA Nutrient Database, 2021).

Five stages of date fruit development are distinguished: hababouk, kimri, khalal, rutab and tamar. According to Hussain *et al.* (2020), fruits at the edible khalal stage acquire the proper size, shape and color, whereas those in the hababouk and kimri stages are green and unfit for human consumption. The rutab stage (partially ripened) and the tamar stage (completely ripened, either manually sun-dried or on the tree for dry cultivars) come after the khalal stage. Markhand *et al.* (2010), reported that the semi-dry and dry types of dates have low tannin content and have a sweet taste are typically consumed at the tamar stage, while the soft types are consumed at the khalal stage (Baliga *et al.*, 2011) described that during the Khalal stage, the date fruit becomes yellow or red and it becomes delicious and edible. However, during the Rutab stage, most date varieties produce soft, sweet and delectable fruits. Whereas, the Tamar, the final stage of maturation, occurs when the date's water content falls below 24% and turns dark brown Alissa (2016).

TSS (total soluble solids) increases significantly due to the accumulation of sugars as water content decreases, e.g., >60 °Brix indicates full ripeness in some varieties. The total sugar rises continuously as a starch is hydrolyzed and sucrose is broken down into simple sugars. At the tamar stage, sugars may exceed 70% of fresh weight. Reducing sugar intake by increasing invertase enzyme activity, which breaks down sucrose into glucose and fructose. High reducing sugar content gives dates their intense sweetness and hygroscopic nature. Non-reducing sugar, mainly sucrose, increases the early khalal stage and then declines sharply at the rutab and tamar stages (Benzohra *et al.*, 2022). This is due to enzymatic hydrolysis into reducing sugars.

Due to its high sugar content (70-85% fresh weight), which is mostly made up of reducing sugars like fructose and glucose and just a little quantity of non-reducing sugars like sucrose, date flesh is an easily accessible source of energy (Al-Farsi and Lee, 2007; Rastegar *et al.*, 2012). According to Ismail *et al.* (2006), dates are often eaten at the rutab and tamar stages. Al-Jasass *et al.* (2015) claim that the color of the dates is an important factor that helps consumers determine the quality of the dates. Additionally, the physical properties of dates, such as colour, shape, size and texture, are crucial in determining their quality and biochemical investigations can be employed to assess their nutrient content.

The date palm chemical composition varies depending on some characteristics, including variety, area, climate, fertilization level and cultural practices (Al-Rawahi *et al.*, 2005). Hence, the objective of this study is to examine the biochemical properties of 15 date palm cultivars from North-western Tamil Nadu.

MATERIALS AND METHODS

The research experiment was carried out on 15 different date palm cultivars at Ariyakulam, Dharmapuri district, Tamil Nadu in the year 2024-2025. The experiment was laid out in the randomized block design (RBD) with three replications. The 15 genotypes were considered as treatments, such as Barhee, Khalas, Anand, Mijenas, Khadraway, Sayas, Maktoom, Elite, Kanjee, RB-1, Noor, Medjool, 0.91, 101 and Ajwa (Jenito *et al.*, 2025). Five fruits/ genotype and five genotypes per treatment were selected randomly and the following observations, namely, TSS (°Brix), titratable acidity (%), ascorbic acid (mg/g), total sugar (%), reducing sugar (%) and non-reducing sugar (%) were recorded. The Dendrogram and PCA-biplot were generated using the CRAN (Comprehensive R Archive Network) programme version 4.5.0 and the data was analyzed using OPSTAT, considered statistically significant at $P = 0.05$.

Biochemical characteristics

Total soluble solids (TSS °brix)

The total soluble solids content in the pulp was determined by using a digital refractometer.

Titratable acidity %

Titratable acidity was estimated by the A.O.A.C. (2012) method, pipette out a 5/ mL aliquot of the produced filtrate into a conical flask and add a few drops of phenolphthalein indicator. The solution was titrated with 0.1/N NaOH until a slight pink color developed and lasted at least 30 seconds. The amount of NaOH used (titration value) was noted.

Ascorbic acid mg/g

Ascorbic acid was estimated by the volumetric method by Sadasivam and Manickam (1992). Weigh 100 mg of sample and centrifuge and collect the extract. To attain a pink endpoint, pipette 5 mL of the working standard into a 100 mL flask, add 10 mL of 4% oxalic acid and then titrate with dye (V_1 mL). The volume of this color is equivalent to the amount of ascorbic acid. After extracting 0.5-5 g of material in 100 ml of 4% oxalic acid, centrifuge. Titrate with dye (V_2 mL), pipette 5 mL of supernatant, add 10 mL of 4% oxalic acid and note the titration value.

Total sugar %

Total sugar was estimated by the Anthrone method by Hedge and Hofreiter (1962). Weigh 100 mg of sample into a boiling tube. Hydrolyze with 5ml of 2.5N HCl in a boiling water bath for 3 hours. When the effervescence stops, cool and neutralize with solid sodium carbonate. Fill to 100 mL, then centrifuge. For analysis, prepare 0.5 and 1mL aliquots. Assemble standards (0-1 mL) using 1 mL of distilled water. Measure absorbance at 630 nm after heating 4 mL of Anthrone reagent in a boiling water bath for 8 minutes.

Reducing sugars %

Reducing sugar was estimated by the Dinitrosalicylic acid (DNS) method by Miller (1972). Pipette out 0.5 to 3 mL of

alcohol-free extract into test tubes, then fill each tube to 3 mL with water. Add 3ml of Dinitrosalicylic acid (DNS) reagent and mix. Boil for 5 minutes in a boiling water bath. After the color has developed, add 1 mL of 40% Rochelle salt solution (while the contents are still heated) and stir. Cool the tubes with running tap water before measuring the absorbance at 510 nm.

Non-reducing sugar %

Non-reducing sugar was estimated by difference using the following formula:

$$\text{Non-reducing sugar (\%)} = \text{Total sugar (\%)} - \text{reducing sugar (\%)}$$

RESULTS AND DISCUSSION

Total soluble solids (TSS °brix)

The TSS content studied among the 15 cultivars varied significantly, ranging from 9.97 °Brix (Maktoom) to 30.07 °Brix (Ajwa) with a mean of 18.55 °Brix, where the cultivar Ajwa had high TSS (30.07 °Brix) and followed by Medjool (28.10 °Brix) and shows a significant and a coefficient of variation (1.874%) (Table 1). Cultivars like Ajwa and Medjool recorded the highest TSS; similarly, Alqahtani *et al.* (2023) reported cultivar Medjool with high TSS content. The high TSS indicates advanced ripeness and high sugar accumulation, aligning with findings by Rastegar *et al.* (2012) found that TSS gradually increases during the rutab and tamar stages due to enzymatic degradation of polysaccharides into simple sugars.

According to Al-Abdoulhadi *et al.* (2011), the soluble components were primarily sugars, with trace levels of proteins, vitamins, organic acids, free amino acids, essential oils and glycosides. Similarly, Al-Farsi and Lee

(2007) stated that TSS values more than 25 °Brix are common in premium cultivars like Medjool, which is world-renowned for its sweetness and commercial value.

Titrateable acidity %

The amount of titrateable acidity in 15 cultivars where examined, ranging from 0.119% in Maktoom to 0.044% in Ajwa, with a mean of 0.078%. the cultivar Maktoom has with high titrateable acidity content of 0.119%, followed by Sayas with 0.114% and with a significant and coefficient of variation (2.903%) (Table 1). This reveals an inverse association between TSS and acidity, which is similar to the findings of Ismail *et al.* (2006) and Baliga *et al.* (2011). Lower acidity in high TSS cultivars indicates improved accessibility and customer acceptance. This link has been well documented in fruit ripening research in which organic acids are used as substrates for respiration, resulting in a decrease in acidity during the maturity process (Al-Farsi *et al.*, 2007).

Ascorbic acid or vitamin 'C' (mg/g)

Ascorbic acid content in 15 cultivars is investigated, ranging from 0.34 mg/g in Medjool to 0.03 mg/g in Noor, with a mean of 0.18 mg/g. where the cultivar Medjool with high ascorbic acid (0.34 mg/g), followed by Ajwa with the amount of 0.33 mg/g. With a significant and coefficient of variation (6.172%) (Table 1). These findings coincide with Kafkas (2006), Chao and Krueger (2007), Baliga *et al.* (2011), who indicated that date fruits are a modest source of vitamin C, with values varying depending on variety and stage of maturation.

While vitamin C degrades during prolonged ripening due to oxidative processes, some cultivars retain higher levels, potentially due to natural antioxidant capacities (Al-Jasass *et al.*, 2015) and (Popet *et al.*, 2022).

Table 1: Biochemical attributes of the fifteen datepalm (*Phoenix dactylifera* L.) cultivars.

Cultivars	TSS (°Brix)	Ascorbic acid mg/g	Titrateable acidity %	Total sugar %	Reducing sugar %	Non-reducing sugar %
Barhee	14.0	0.28	0.102	0.59	0.68	0.09
Khalas	25.5	0.25	0.052	0.55	0.71	0.16
Anand	19.0	0.24	0.058	0.46	0.58	0.12
Mijenas	18.5	0.14	0.074	0.47	0.63	0.16
Khadraway	18.0	0.17	0.063	0.45	0.66	0.21
Sayas	10.5	0.08	0.114	0.43	0.61	0.18
Makdhoom	10.0	0.12	0.118	0.39	0.64	0.25
Elite	11.5	0.09	0.109	0.42	0.69	0.27
RB-1	16.5	0.07	0.082	0.37	0.58	0.21
Kanijee	12.5	0.04	0.105	0.34	0.62	0.28
Noor	15.0	0.03	0.094	0.32	0.57	0.25
Medjool	28.0	0.34	0.046	0.67	0.74	0.07
0.91	24.5	0.29	0.055	0.52	0.73	0.21
101	24.0	0.27	0.057	0.51	0.72	0.19
Ajwa	31.0	0.33	0.044	0.56	0.65	0.09
C.D.	0.584	0.019	0.004	0.021	0.020	0.031
SE(d)	0.284	0.009	0.002	0.010	0.010	0.015
C.V.	1.874	6.172	2.903	2.607	1.835	9.890

Total sugars, reducing and non-reducing sugars (%)

The total sugar and reducing sugar content were highest in Medjool (0.67%) and Ajwa (0.56%) sugars such as Total sugar, reducing sugar and non-reducing sugar are significant and the coefficient of variance is (2.067%, 1.835, 9.980%) (Table 1). Al-Farsi and Lee (2007) revealed that the sugar proportion in ripe dates could be as high as 80% of the fruit's dry weight. Rastegar *et al.* (2012) found that the accumulation of reducing sugars increased slightly throughout growth but dramatically during maturation and ripening, due to sugar gains and moisture loss.

According to Mortazavi *et al.* (2010), non-reducing carbohydrates such as sucrose were significantly reduced, consistent with the expected enzymatic breakdown during the ripening stages. Sugar buildup, particularly the concentration of high quantities of fructose and glucose (reducing sugar), is a critical physiological mechanism that influences dessert fruit quality.

The hierarchical cluster analysis (HCA) dendrogram generated for the fifteen Date palm (*Phoenix dactylifera* L.) cultivars revealed cluster groupings based on their biochemical profiles (TSS, titratable acidity, ascorbic acid, total sugars, reducing sugars and non-reducing sugars).

Among the three clusters, the I, II and III clusters, cluster I has six cultivars grouped, such as Barhee, Khalas, Anand, Mijenas, Khadrawey and Sayas. Cluster II has four cultivars, namely Maktoom, Elite, RB-1 and Kanjee. Cluster III has five cultivars like Noor, Medjool, 0.91, 101 and Ajwa (Fig 1).

Six cultivars- Barhee, Khalas, Anand, Mijenas, Khadrawey and Sayas were placed together in Cluster I. These cultivars share modest TSS (14-25.5 °Brix) and balanced acidity, suggesting similar maturation patterns and biochemical composition (Fig 1). Ismail *et al.* (2006) and Ahmed *et al.* (2009) found comparable clustering patterns, emphasizing the tendency of date palm kinds with moderate sugar accumulation to cluster together due to similar ripening stages and environmental adaptations.

Maktoom, Elite, RB-1 and Kanjee are all part of Cluster II, which is defined by comparatively low TSS (10-12.5 °Brix) but greater titratable acidity levels (up to 0.118%) (Fig 1). According to research by Baliga *et al.* (2011) and Markhand *et al.* (2010), who studied the effect of genotype-environment interactions on the sugar and acid content of dates, these biochemical characteristics may be associated with delayed maturity or less favourable environmental conditions.

Five cultivars-Noor, Medjool, 0.91, 101 and Ajwa were placed together in Cluster III. These accessions had the greatest levels of ascorbic acid and TSS (24-31 °Brix), indicating advanced ripening and superior fruit quality (Fig 1). Rastegar *et al.* (2012) found that cultivars with high invertase activity cluster together due to enhanced hydrolysis of sucrose into simple sugars during ripening, with Medjool and Ajwa distinguishing out for their higher sugar accumulation. Furthermore, the clustering pattern supports the findings of Al-Farsi and Lee (2007) stated

that premium cultivars, such as medjool, are commercially attractive due to their genetic tendency to produce higher sugar concentrations.

The principal component analysis (PCA) biplot for the biochemical traits of the 15 Date palm (*Phoenix dactylifera* L.) cultivars revealed clear patterns of trait contributions and cultivar grouping.

PC1 revealed 74% total variation and significant positive loadings for reducing sugars, total sugars and TSS (°Brix), demonstrating that these factors influence fruit sweetness. On the positive side of PC1, cultivars such as Medjool and Ajwa had high sugar and TSS contents (Fig 2).

PC2 had an inverse relationship with TSS and sugars and it was mostly related to ascorbic acid and titratable acidity. This shows that cultivars with higher levels of vitamin C may not always contain the maximum sugar content (Fig 2). But cultivar Medjool, which clustered towards higher PC1 and PC2 values, combined high sugars with the highest ascorbic acid (0.34 mg/g) (Gopi *et al.*, 2021).

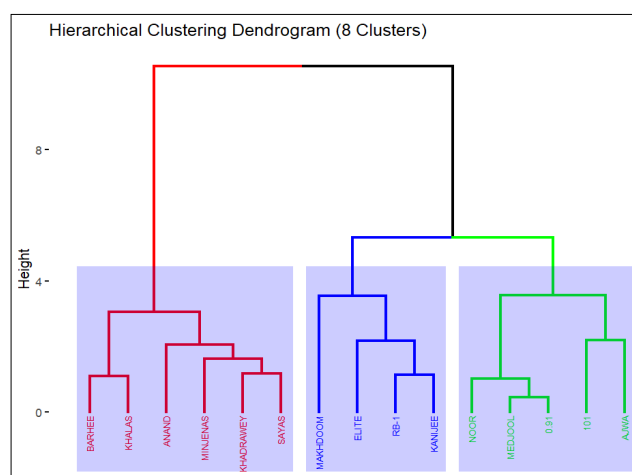


Fig 1: Dendrogram based on the hierarchical clustering using a dissimilarity matrix.

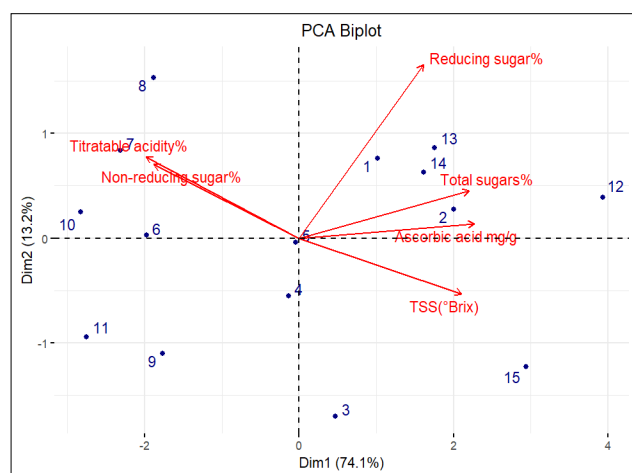


Fig 2: Principal component analysis based on the biplot distribution of fifteen date palm cultivars.

Overall, the biplot analysis showed that the correlation matrix is a useful multivariate tool for assessing the relationships between features in date cultivars and the PCA results validated the correlation coefficient. In a study by Ebrahimi (2022), the Euclidean square distance matrix was used to perform PCA analysis on 68 significant date palm genotypes. The findings showed that 54.57% of the variation was explained by the first ten components. In a study on fifty date fruit cultivars, Ahmad *et al.* (2023) used PCA to analyze certain phenological and biochemical characteristics. They found that among the examined characteristics of date fruit cultivars, the first four components, with eigenvalues larger than 1, showed the most variability and explained 77.04% of the overall variance. Gopi *et al.* (2021) concluded that principal component analysis was used to evaluate the combination of qualitative and quantitative attributes. The total variability described by the five PCs was 84.14 percent, while the variability of the 13 basic components was 99.99%. The genotypes that may be chosen were those with positive PC values in each constituent graph that were more than 1.0.

The biplot analysis revealed that these various cultivars for biochemical traits can be used to improve elite date palm genotypes and selection breeding may be an appropriate technique to achieve further development in these genotypes or their traits.

CONCLUSION

A multivariate technique has been found very helpful in identifying various germplasms with desirable traits. Based on the findings from the study, it is concluded that a diverse array of variability is observed in different cultivars, like Ajwa TSS (31°Brix) and Medjool (Ascorbic acid (0.34 mg/g), total sugar (0.67%) and reducing sugar (0.74%). The performance of the studied Date palm cultivars is well-suited in a water-scarce area and the coastal belt of the southern part of India. Therefore, it can be recommended for commercial cultivation in regions facing water scarcity and soil salinity.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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