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Comparison of phenolic composition in Australian-grown date fruit (Phoenix dactylifera L.) seeds from different varieties and ripening stages

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ABSTRACT

In this research, different seeds of Australian-grown date palm (*Phoenix dactylifera* L.) were studied to evaluate the antioxidant potential and analyze their phenolic constituents. Phenolic compounds were extracted from seeds of various Australian-grown date varieties at different ripening stages. Eight varieties of date seeds (Zahidi, Medjool, Deglet nour, Thoory, Halawi, Barhee, Khadrawy, and Bau Strami) at three ripening stages (*Kimri*, *Khalal,* and *Tamar*) were investigated in this study. Date seeds at *Khalal* (9.87–16.93 mg GAE/g) and *Tamar* (9.20–27.87 mg GAE/g) stages showed higher total phenolic content than those at *Kimri* stage (1.81–5.99 mg GAE/g). For antioxidant assays like DPPH, FRAP, ABTS, RAP, FICA, and TAC, date seeds at *Khalal* and *Tamar* stages also showed higher antioxidant potential than *Kimri* stage. However, date seeds at *Kimri* stage (55.24–63.26 mg TE/g) expressed higher radical scavenging activity than *Khalal* (13.58–51.88 mg TE/g) and *Tamar* (11.06–50.92 mg TE/g) stages. Phenolic compounds were characterized using LC-ESI-QTOF-MS/MS, revealing the presence of 37 different phenolic compounds, including 8 phenolic acids, 18 flavonoids, and 11 other phenolic compounds. Further, phenolic compounds were quantified using LC-DAD, revealing that Zahidi variety of date seeds exhibited the highest content during the *Kimri* stage. In contrast, during the *Khalal* and *Tamar* stages, Deglet nour and Medjool date seeds displayed higher concentrations of phenolic compounds. The results indicated an increase in phenolic content in date seeds after the *Kimri* stage, with significant variations observed among different date varieties.

1. Introduction

In the arid landscapes of the Middle East and North Africa, where relentless sun and scorching winds prevail, a resilient and fruitful companion stands tall – the date palm (*Phoenix dactylifera* L.) (Chao & Krueger, 2007). The production trend of dates has witnessed stable growth in recent decades. With advancements in agricultural practices, including irrigation systems and improved cultivation techniques, the yield of date palms has increased significantly. In 2021, the global date production reached an impressive 9.66 million metric tons (Statista, 2023). This increase in production has not only contributed to food security in the regions where date palms thrive but has also made dates a sought-after export commodity on the international market. Also, the land area dedicated to date palms is increasing in Australia, and date palm products are becoming important players in the market (Sirisena, Ng, & Ajlouni, 2015).

Date seeds represent an average reduction of 10 % in the weight of the whole fruit during date palm product production (Salomón-Torres et al., 2019). Every year, the global date industry generates a staggering amount of date seeds, considering that the world production of dates reached 9.66 million tons in 2021, this would potentially result in over 900,000 tons of date seeds being generated (Statista, 2023). However, only a small fraction of these seeds has a purpose beyond their role as remnants of consumed dates. Traditionally, date seeds have been relegated to the status of agricultural waste, often discarded after the fruit is consumed or used as animal feeds after mass production (Oladzad, Fallah, Mahboubi, Afsham, & Taherzadeh, 2021). This neglect stems from several factors, including a lack of awareness about the potential applications of date seeds, the challenge of extracting and processing them efficiently, and limited research into their beneficial properties. As

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Table 1
Date seed weight at different ripening stages and varieties (before and after freeze drying) and the percentage of removed moisture.

	Ripening stage								
	Kimri			*Khalal*			*Tamar*		
	Initial weight/g	Final weight/g	Removed moisture, %	Initial weight/g	Final weight/g	Removed moisture, %	Initial weight/g	Final weight/g	Removed moisture, %
Zahidi	14.0	4.8	65.7	snc	snc	–	7.8	7.2	7.7
Medjool	10.6	6.8	35.8	8.3	5.8	30.1	7.5	6.7	10.7
Degelet nour	13.4	5.4	59.7	10.8	5.0	53.7	8.4	7.8	7.1
Thoory	16.3	7.7	52.8	9.3	6.7	28.0	6.9	6.5	5.8
Halawi	10.7	4.6	57.0	9.9	7.2	27.3	7.2	6.3	12.5
Barhee	6.7	3.0	55.2	7.3	5.0	31.5	6.4	5.7	10.9
Khadrawy	12.9	5.9	54.3	6.9	5.3	23.2	7.3	6.9	5.5
Bau Strami	16.7	8.1	51.5	9.8	6.8	30.6	6.9	6.3	8.7

snc, sample not collected.

a result, much of the potentially valuable by-products of date palm production has been wasted.

However, within the seemingly unremarkable seeds lie components that possess potential applications spanning various industries. Researchers and innovators had recognized the potential value contained within date seeds. One of the most significant aspects of date seeds is their phenolic content. Phenolic compounds showcase their antioxidant abilities by removing harmful free radicals and reactive oxygen species (ROS), hindering the oxidation process. This action can potentially prevent diseases related to oxidative stress, such as enhancing control over blood sugar levels, reducing hypertension, and enhancing lipid profiles (Attia et al., 2021). Radfar et al. (2019) reported that the phenolic contents for the Iranian date seed extracts ranged from 1483 to 3377 mg GAE/100 g dry weight. The substantial phenolic content found in date seeds offers numerous pharmacological benefits, such as anti-inflammatory, chemopreventive, and antimutagenic effects (Alkhoori et al., 2022; Selim et al., 2021). Nonetheless, Shahdadi, Mirzaei, and Daraei Garmakhany (2015) noted that the content of phenolic compounds in dates differed both quantitatively and qualitatively. Further, the total phenolic content varies with different ripening stages. For instance, for the Mozafati variety of date in the *Khalal*, *rutab*, and *Tamr* stages, phenolic content showed a trend of *Tamr* stage < *Rutab* stage < *Khalal* stage. Similarly, the variety of date palm also had a significant influence. In their research, regardless of the ripening stage, the phenolic content of Mozafati remains consistently higher than that of Karudeh.

The phenomenon of phenolic content and quality in seeds affected by ripening stages and varieties was also observed in other fruits. In previous studies, a similar concept has been conveyed regarding the investigation of phenolic compounds within mango seed kernels. Alañón, Pimentel-Moral, Arráez-Román, and Segura-Carretero (2021) explored the variations in phenolic compounds across five ripening stages for three mango cultivars, namely Keitt, Kent, and Osteen. Their findings revealed that the Keitt samples exhibited elevated levels of iriflophenone glucoside, maclurin C-glucoside, maclurin digalloyl glucoside, mangiferin, 5-galloyl quinic acid and trigalloyl glucose during the initial three ripening stages. Conversely, seed kernels from the Osteen variety displayed higher concentrations of hexa- and hepta-gallotannins, with their levels gradually decreasing throughout the maturation process. Therefore, both cultivar and ripening stage factors significantly impact the phenolic composition within mango seed kernels. Same for grape seeds, Obreque-Slier, López-Solís, Castro-Ulloa, Romero-Díaz, and Peña-Neira (2012) conducted an assessment of the phenolic composition of seeds from Carménère, Merlot, Cabernet Franc, and Cabernet Sauvignon grape varieties at four distinct ripening stages. The study revealed that the initial total phenol content ranged from 23.8 mg EAG/g seed to 28.5 mg EAG/g seed. After ripening, the final total phenol content decreased to a range between 10.2 and 15.1 mg EAG/g seed. Carménère grape seeds consistently exhibited the highest total phenol content at every stage of ripening, while Cabernet Sauvignon grape seeds showed the opposite. All of these provided evidence for substantial differences in phenolic composition within different varieties of grape seeds throughout the ripening process.

Previously, AlFaris et al. (2021) had also reported a compendium of 22 studies conducted since 2019 on the phenolic compounds in date palm fruits. The encompassed articles included different date palms produced in country (10 countries, including Algeria and Bahrain), varieties (ranging from 1 to 15 varieties), various storage conditions (storage durations range from 1 day to 12 months, with storage temperatures including 4 °C, −20 to −18 °C, and −40 °C) and different extraction solutions including methanol, ethanol, acetone, ethyl acetate and distilled water. The resultant amalgamation of these studies reported an average total phenolic content ranging from 4.36 to 753.3 mg GAE/100 g DW in the investigated date palm samples. In addition, Khatib et al. (2022) conducted a study on five widely consumed Arabian date palm varieties (Sukkari, Ajwa, Segae, Barrny, and Khalas), focusing on the analysis of phenolic compounds and polysaccharides and discovered that the total phenols content in five varieties varied between 20 and 50 mg/100 g DW. Zihad et al. (2021) also investigated the antioxidant potential of three date palm varieties in Saudi Arabia (Ajwah, Safawy, and Sukkari) and conducted an analysis of their phenolic constituents. Their study revealed that all three date extracts demonstrated strong scavenging activity against DPPH and hydroxyl radicals, with IC_{50} values ranging from 103 to 177 μg/mL and 1.1 to 1.55 mg/mL, respectively. They also showed significant total antioxidant capacity (IC_{50}: 87–192 μg/mL). UPLC-QTOF-MS identified 22 compounds in the date varieties, including common phenolics, flavonoids, sterols, and phytoestrogens.

Although numerous research reports investigated the evolving antioxidant capacity of different date pulp varieties as they mature, limited attention has been given to understanding the changes in the antioxidant capacity of date seeds during various stages of ripening and there is no systematic research of dates grown locally in Australia. Therefore, this research was aimed at the evaluation of the potential antioxidant activity, estimation, and identification of phenolic compounds in the Australian-grown date seeds at three ripening stages. Specifically, date seeds of eight Australian grown varieties (Zahidi, Medjool, Deglet nour, Thoory, Halawi, Barhee, Khadrawy, and Bau Strami) at three ripening stages (*Kimri*, *Khalal,* and *Tamar*) were involved in this research. Nonspecific colorimetric methods were used to preliminarily evaluate total phenol, flavonoid, condensed tannin content, and antioxidant potential, prior to a more specific phenolic characterization by LC-ESI-QTOF-MS/MS and LC-DAD analysis.

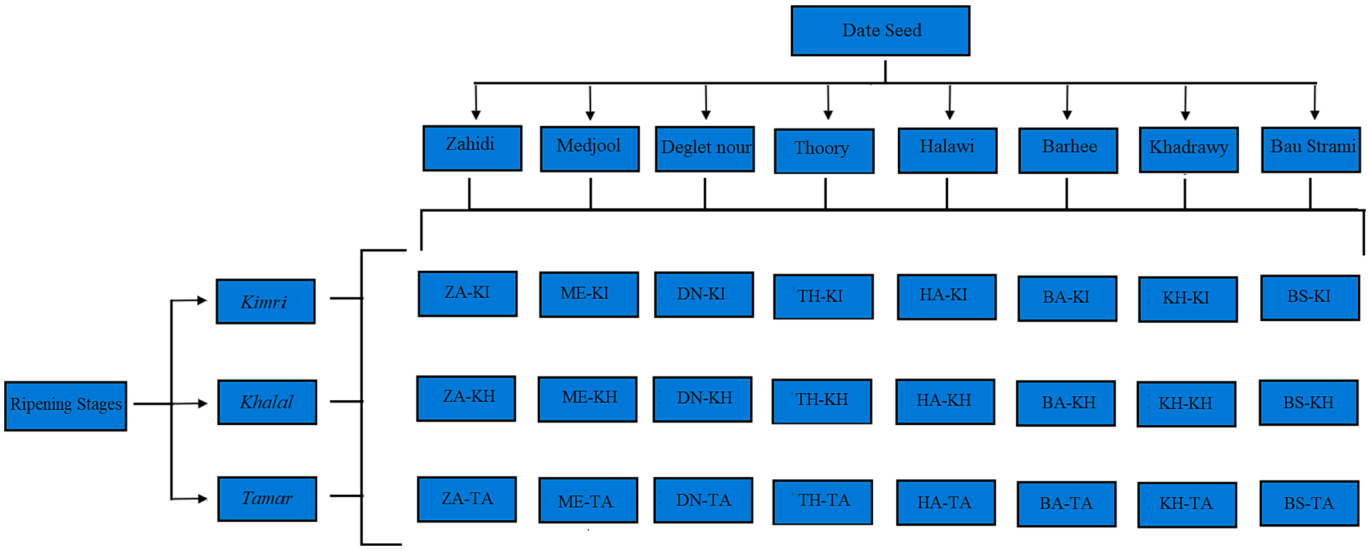

Fig. 1. The sample abbreviations of each ripening stage.

2. Material and methods

2.1. Chemical and reagents

The chemicals used for this study were mostly of analytical grade and purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Folin and Ciocalteu's phenol reagent, gallic acid, L-ascorbic acid, vanillin, hexahydrate aluminium chloride, quercetin, catechin, DPPH, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ABTS, and alizarin were bought from the Sigma-Aldrich (Castle Hill, NSW, Australia). Sodium carbonate anhydrous was purchased from Chem-Supply Pty Ltd. (Adelaide, SA, Australia) and 98 % sulfuric acid from RCI Labscan (Rongmuang, Thailand). Methanol, acetonitrile, ferric chloride (Fe [III]Cl_3•$6H_20$), hydrated sodium acetate, hydrochloric acid, and glacial acetic acid were purchased from Thermo Fisher Scientific Inc (Scoresby, Victoria, Australia).

2.2. Sample preparation

Eight varieties of dates (Zahidi, Medjool, Deglet nour, Thoory, Halawi, Barhee, Khadrawy, and Bau Strami) with three ripening stages (*Kimri*, *Khalal,* and *Tamar* based on colour and collection time) collected in year 2023 were used in this study (the *Khalal* stage of variety Zahidi was not available). Date samples in this study were supplied by "The Dessert Fruit Company, Australia". Each whole date sample weighed over 100 g and arrived at the laboratory within three days after collection and stored in a −20 °C freezer. First, all samples were thawed together, and the flesh and seeds were separated manually. Then, the seeds were crushed and freeze dried for 72 h. After freeze-drying, the date seeds were powdered with a grinder (Laobenhang, model 400Y, Yongkang, Zhejiang, China). Initial and final weight of samples and the percentage of removed moisture are shown in Table 1. The seed samples were then stored at −20 °C.

2.3. Extraction of phenolics

The extracts of seeds were prepared by mixing 3 g of sample powder with 30 mL solvent (70 % ethanol). The solvent was chosen based on a previous study by our group, which showed excellent phenolic compound extraction performance (Subbiah et al., 2023). Formic acid (0.1 %) was added to break the cell wall and increase the permeability, followed by 5-min ultrasonication in an ice water bath using a cell disruptor (Branson, model Digital Sonifier 450) at an amplitude of 40 %. After extraction, the date extracts were centrifugated (Hettich, ROTI-NA380R, Tuttlingen, Baden-Württemberg, Germany) at 8000 rpm for 15 min (4 °C). The supernatant was collected after centrifugation and stored at −20 °C. The sample abbreviations of each variety and ripening stage are shown in Fig. 1.

2.4. Phenolic compound estimation

2.4.1. Determination of total phenolic content (TPC)

The TPC value of date seeds extracts was determined using the Folin–Ciocalteu method mentioned by Slinkard and Singleton (1977) with some modifications. In this assay, 25 μL of extract was added to a 96-well microplate and then mixed with 200 μL of Milli-Q® water and 25 μL 25 % (*v/v*) Folin–Ciocalteu reagent. The mixture was incubated at 25 °C for 5 min. Subsequently, 25 μL of 10 % (*w/w*) sodium carbonate solution was added with continued incubation in a dark environment for 1 h at 25 °C. Absorbance at 756 nm was then measured in triplicate. The calibration curve built in this test was based on ethanolic gallic acid (0–200 μg/mL). The results of the samples are expressed as milligram gallic acid equivalents (GAE) per fresh weight (mg GAE/$g_{f.w.}$) ± standard deviation (SD).

2.4.2. Determination of total flavonoid content (TFC)

The TFC value of date seeds extracts was determined using a modified $AlCl_3$ colorimetric-based assay described by Christ and Müller (1960). Extract (80 μL) was added to a 96-well microplate and then 80 μL of 2 % aluminium chloride solution and 120 μL of 50 g/L sodium acetate solution were added for 2.5 h incubation at room temperature. After incubation, the absorbance at 440 nm was determined in triplicate. The calibration curve built in this test is based on ethanolic quercetin (0–50 μg/mL). The results of the samples are expressed as milligram quercetin equivalents (QE) per fresh weight (mg QE/$g_{f.w.}$) ± SD.

2.4.3. Determination of total condensed tannin (TCT)

The TCT value of date seeds extracts was determined using a modified assay mentioned by Price, Van Scoyoc, and Butler (1978). Extract (25 μL) was added to a 96-well microplate, and then 150 μL 4 % vanillin solution and 25 μL 32 % ethanol diluted sulphuric acid were added. The solutions were incubated for 15 min at 25 °C, and then the absorbance at 500 nm was read in triplicate. The calibration curve built was based on methanolic catechin (0–1000 μg/mL) and extraction yields were expressed as milligram catechin equivalents (CE) per fresh weight (mg CE/$g_{f.w.}$) ± SD.

2.5. Antioxidant activities

2.5.1. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay (DPPH)

The free radical scavenging activity of date seeds extracts was measured via DPPH assay (Blois, 1958). 25 μL of extract and 275 μL 0.1 mM DPPH radical methanol solution were added to a 96-well microplate and allowed to stand at 25 °C for 30 min. Then, the absorbance was measured at 517 nm in triplicate. The calibration curve was built based on aqueous Trolox (0–200 μg/mL) and extraction yields were expressed as milligram Trolox equivalents (TE) per fresh weight (mg TE/$g_{f.w.}$) ± SD.

2.5.2. Ferric reducing/antioxidant power assay (FRAP)

The FRAP value of date seeds extracts was measured by an adopted assay described by Benzie and Strain (1996). FRAP reagent was prepared fresh daily by mixing 300 mM sodium acetate buffer, 10 mM TPTZ, and 20 mM ferric chloride in a ratio of 10:1:1 (*v/v/v*). 20 μL of extract and 280 μL of FRAP reagent were added to a 96-well microplate, allowed to stand at 37 °C for 10 min, min and then measured the absorbance at 593 nm in triplicate. The calibration curve was built based on aqueous Trolox (0–200 μg/mL) and extraction yields were expressed as mg TE/$g_{f.w.}$ ± SD.

2.5.3. Chelating ability of ferrous ion assay (FICA)

The ferrous ion chelating ability of date seeds extracts was determined using an adopted assay mentioned by Dinis, Madeira, and Almeida (1994). The following reagents were added to a 96-well microplate: 15 μL of extract, 50 μL of 2 mM aqueous solution of Fe[II] (diluted 1:15 v/v), and 50 μL of 5 mM aqueous solution of ferrozine (diluted 1:6 v/v). The microplate allowed to stand at 25 °C for 10 min. Then, the absorbance at 562 nm was measured in triplicate. The calibration curve was built based on aqueous ethylenediaminetetraacetic acid (EDTA) (0–50 μg/mL), and extraction yields were expressed as milligram EDTA equivalents (EE) per fresh weight (mg EE/$g_{f.w.}$) ± SD.

2.5.4. 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (ABTS)

The ABTS radical scavenging ability of date seeds extracts was determined using an adopted assay described by Re et al. (1999). $ABTS^+$ was prepared at room temperature via the reaction between 1.25 mL 7 mM ABTS and 22 μL 140 mM potassium persulfate solution and allowed to stand in a dark environment for 16 h. Afterwards, 0.5 mL $ABTS^+$ solution was diluted in 45 mL ethanol to obtain absorbance between 0.75 and 0.78 at 734 nm. 10 μL of extract and 290 μL of $ABTS^+$ solution were added to a 96-well microplate and allowed to stand at 25 °C for 6 min and then the absorbance at 734 nm was measured in triplicate. The calibration curve was built based on aqueous TROLOX (0–500 μg/mL). Extraction yields were expressed as mg TE/$g_{f.w.}$ ± SD.

2.5.5. Hydroxyl radical scavenging activity assay (•OH-RSA)

The •OH-RSA value of date seeds extracts was determined using an adapted assay based on the principle described by Salgado, Melin, Contreras, Moreno, and Mansilla (2013). 50 μL of extract, 50 μL of $FeSO_4 \cdot 7H_2O$ and 50 μL 6 mM H_2O_2 were added to a 96-well microplate, and allowed to stand at 25 °C for 10 min. Subsequently, 50 μL 6 mM 3-Hydroxybenzoic acid solution was added, allowed to stand at the same temperature in a dark environment for 10 min. The absorbance was measured at 510 nm in triplicate. The calibration curve was built based on aqueous Trolox (0–300 μg/mL). Extraction yields were expressed as mg TE/$g_{f.w.}$ ± SD.

2.5.6. Reducing power assay (RPA)

The RPA value of date seeds extracts was determined using an adopted assay described by Oyaizu (1986). Buffer was prepared by mixing 0.2 M $Na_2HPO_4 \cdot 7H_20$ and 0.2 M $Na_2HPO_4 \cdot H_20$ (3.74 mL/6.24 mL). 10 μL of extract, 10 μL buffer, and 25 μL of 1 % (*w/v*) $K_3[Fe(CN)_6]$ aqueous solution were added to a 96-well microplate, and allowed to stand at 25 °C for 20 min. Subsequently, 25 μL of trichloroacetic acid (10.0 %, *w/v*) was added to terminate the reaction. Then, 85 μL of Milli-Q® water and 8.5 μL ferric chloride (0.1 % *w/v*) were added and allowed to stand at the same temperature in dark environment for 15 min. The absorbance at 750 nm was measured in triplicate. The calibration curve built in this test was based on aqueous Trolox (0–500 μg/mL). Extraction yields were expressed as mg TE/$g_{f.w.}$ ± SD.

2.5.7. Determination of total antioxidant capacity (TAC)

The TAC value of date seed extracts was determined using an adopted assay (Prieto, Pineda, & Aguilar, 1999). 40 μL of extract and 260 μL of phosphomolybdate reagent (0.6 M H_2SO_4, 0.028 M sodium phosphate and 0.004 M ammonium molybdate) were added to a 96-well microplate and allowed to stand at 95 °C for 90 min. After being cooled at 25 °C for 10 min, the absorbance was measured at 695 nm in triplicate. The calibration curve built in this test was based on ethanolic ascorbic acid (0–300 μg/mL). Extracts were expressed as milligram ascorbic acid equivalents (AAE) per fresh weight (mg AAE/$g_{f.w.}$) ± SD.

2.6. Characterization of phenolic compounds using LC-ESI-QTOF-MS/MS analysis

LC-ESI-QTOF-MS/MS carried out the extensive characterization of phenolic compounds of twenty three date seed samples using the method described by Allwood, Evans, Austin, and McDougall (2020) and Zhu et al. (2022). Characterization of phenolic compounds was carried out using an Agilent 1200 series of HPLC (Agilent Technologies, Santa Clara, CA, USA) connected via electrospray ionization source (ESI) to the Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS (Agilent Technologies, Santa Clara, CA, USA). HPLC buffers were sonicated using a 5 L Digital Ultrasonic water bath (Power sonic 505, Gyeonggi-do, Korea) for 10 min at 25 °C. The separation was carried out using a Synergi Hydro-Reverse Phase 80°A, LC column 250 × 4.6 mm, 4 μm (Phenomenex, Torrance, CA, 202 USA) with temperature of 25 °C and sample temperature at 10 °C. The sample injected was 20 μL. Since the system was binary solvent: mobile phase A, 100 % Milli-Q® water added with 0.1 % formic acid, and mobile phase B, acetonitrile/Milli-Q® water/formic Acid (95:5:0.1), at a flow rate of 0.3 mL/min. The gradient was as follows: 0–4 min hold 2 % B, 4–10 min 2–5 % B, 10–50 min 5–45 % B; 50–52 min 45–100 % B, 52–58 min hold 100 % B, 58–60 min 100–2 % B, 60–70 min hold 2 % B for HPLC equilibration. Both positive and negative modes were applied for peak identification. Nitrogen gas was used as a nebulizer and drying gas at 45 psi, with a flow rate of 5 L/min at 300 °C. Capillary and nozzle voltage were placed at 3.5 kV and 500 V, respectively. The mass spectra were obtained at the range of 50–1300 amu. Further, MS/MS analyses were carried out in automatic mode with collision energy (10, 15, and 30 eV) for fragmentation. Data acquisition and analyses were performed using Agilent LC-ESI-QTOF-MS/MS Mass Hunter workstation software (Qualitative Analysis, version B.03.01, Agilent).

2.7. Quantification of phenolic compounds through LC-DAD analysis

The quantification of phenolic compounds was carried out by HPLC (Waters Alliance 2690, Chromatograph Separation Module) along with a diode array detector (DAD) (Model 2998, Waters), which was set at λ 280, 313 and 350 nm with 1.25 scan/s (peak width = 0.2 min) (Schieber, Keller, Streker, Klaiber, & Carle, 2002). The sample separation was performed using a Synergi Hydro-Reverse Phase 80°A LC column with dimensions of 250 × 4.6 mm and 4 μm particle size (Phenomenex, Torrance, CA). The column temperature was maintained at 25 °C while the sample temperature was kept at 30 °C. A 15-μL sample was injected into the system. The system used a binary solvent consisting of mobile phase A, which was made up of 95 % Milli-Q® water and 5 %

Table 2
The antioxidant assays of different ripening stage and variety of date seeds.

Ripening Stages	Antioxidant Assays	Sample Name							
		Zahidi	Medjool	Deglet nour	Thoory	Halawi	Barhee	Khadrawy	Bau Strami
***Kimri* stage**		**ZA-KI**	**ME-KI**	**DN-KI**	**TH-KI**	**HA-KI**	**BA-KI**	**KH-KI**	**BS-KI**
	TPC (mg GAE/g)	4.34 ± 0.28^{b}	1.81 ± 0.05^{d}	5.99 ± 0.47^{a}	2.08 ± 0.17^{d}	3.33 ± 0.16^{c}	5.79 ± 0.22^{a}	3.89 ± 0.32^{bc}	4.11 ± 0.28^{b}
	TCT (mg CE/g)	37.08 ± 1.12^{c}	16.81 ± 0.86^{e}	46.72 ± 2.20^{b}	21.43 ± 1.32^{e}	27.90 ± 2.59^{d}	58.16 ± 0.59^{a}	50.32 ± 3.72^{b}	44.28 ± 3.02^{b}
	TFC (mg QE/g)	1.15 ± 0.08^{a}	0.40 ± 0.04^{de}	0.60 ± 0.01^{bc}	0.50 ± 0.03^{cd}	0.28 ± 0.02^{f}	0.63 ± 0.05^{b}	0.29 ± 0.02^{ef}	0.54 ± 0.05^{bc}
	DPPH (mg TE/g)	10.11 ± 0.33^{b}	6.43 ± 0.37^{c}	0.87 ± 0.05^{f}	6.92 ± 0.10^{c}	2.35 ± 0.18^{de}	12.06 ± 0.38^{a}	1.98 ± 0.14^{e}	2.82 ± 0.17^{d}
	FRAP (mg TE/g)	17.81 ± 1.68^{c}	7.21 ± 0.49^{e}	21.66 ± 1.32^{ab}	8.55 ± 0.66^{e}	12.74 ± 0.98^{d}	24.23 ± 1.60^{a}	19.30 ± 0.40^{bc}	18.14 ± 0.91^{c}
	ABTS (mg AAE/g)	9.56 ± 0.26^{ab}	4.62 ± 0.28^{f}	8.42 ± 0.32^{bc}	6.64 ± 0.01^{de}	6.02 ± 0.35^{e}	10.57 ± 0.72^{a}	7.91 ± 0.53 ^{cd}	7.78 ± 0.76^{cd}
	$^{\bullet}OH^{-}$RSA (mg TE/g)	61.31 ± 1.19^{abc*}	63.26 ± 1.77^{ab*}	57.06 ± 1.51^{cd*}	55.24 ± 3.95^{d*}	66.01 ± 1.96^{a*}	62.85 ± 1.23^{ab*}	60.74 ± 0.87^{abc*}	59.42 ± 0.26^{bcd*}
	RAP (mg TE/g)	8.25 ± 0.69^{b}	2.53 ± 0.11^{d}	9.81 ± 0.81^{a}	2.03 ± 0.15^{d}	5.49 ± 0.48^{c}	11.20 ± 0.84^{a}	7.23 ± 0.57^{b}	8.04 ± 0.55^{b}
	FICA (mg EE/g)	1.19 ± 0.10^{a}	1.00 ± 0.01^{bc}	0.97 ± 0.01^{c}	1.07 ± 0.06^{abc}	1.15 ± 0.09^{abc}	1.19 ± 0.06^{a}	1.17 ± 0.05^{ab}	1.16 ± 0.07^{ab}
	TAC (mg AAE/g)	0.74 ± 0.02^{d}	0.76 ± 0.04^{d}	1.63 ± 0.03^{b}	0.88 ± 0.06^{cd}	0.45 ± 0.01^{e}	2.47 ± 0.22^{a}	1.00 ± 0.02^{c}	0.86 ± 0.02^{cd}
***Khalal* stage**		**ZA-KH**	**ME-KH**	**DN-KH**	**TH-KH**	**HA-KH**	**BA-KH**	**KH-KH**	**BS-KH**
	TPC (mg GAE/g)	snc	13.26 ± 0.47^{b*}	16.93 ± 1.09^{a}	9.87 ± 0.99^{c}	10.98 ± 0.35^{c}	14.91 ± 0.29^{b*}	16.93 ± 0.37^{a*}	11.17 ± 0.60^{c*}
	TCT (mg CE/g)	snc	96.05 ± 3.50^{cd*}	137.22 ± 7.23^{ab*}	82.73 ± 1.01^{d}	83.90 ± 6.44^{d}	119.00 ± 10.58^{bc}	158.55 ± 14.40^{a*}	96.05 ± 7.83^{cd*}
	TFC (mg QE/g)	snc	2.11 ± 0.05^{d*}	2.97 ± 0.23^{b}	1.70 ± 0.04^{ef}	1.67 ± 0.15^{f}	2.49 ± 0.08^{c}	3.34 ± 0.07^{a*}	2.05 ± 0.16^{de}
	DPPH (mg TE/g)	snc	15.65 ± 0.49^{a*}	12.62 ± 0.56^{b}	9.34 ± 0.24^{c*}	5.44 ± 0.44^{e}	15.90 ± 0.42^{a*}	7.69 ± 0.70^{d}	5.08 ± 0.49^{e}
	FRAP (mg TE/g)	snc	68.77 ± 5.80^{bc*}	70.96 ± 3.54^{b}	55.16 ± 0.83^{de}	56.33 ± 5.04^{cde}	67.41 ± 6.05^{bcd*}	129.15 ± 4.82^{a*}	53.39 ± 4.45^{e*}
	ABTS (mg AAE/g)	snc	19.69 ± 0.50^{c*}	24.25 ± 0.05^{b}	17.11 ± 0.91^{c*}	19.45 ± 0.59^{c}	18.42 ± 0.74^{c}	27.94 ± 1.94^{a*}	18.56 ± 0.99^{c*}
	$^{\bullet}OH^{-}$RSA (mg TE/g)	snc	43.06 ± 2.31^{b}	34.32 ± 1.26^{c}	51.88 ± 1.96^{a}	48.49 ± 2.88^{ab}	35.28 ± 3.15^{c}	13.58 ± 0.23^{d}	45.62 ± 0.74^{b}
	RAP (mg TE/g)	snc	25.18 ± 0.58^{de*}	32.08 ± 0.12^{b}	20.84 ± 0.76^{f}	23.37 ± 0.47^{ef}	28.30 ± 1.07^{c}	37.46 ± 0.77^{a}	26.91 ± 1.76^{cd*}
	FICA (mg EE/g)	snc	1.45 ± 0.06^{a*}	1.39 ± 0.02^{a}	1.39 ± 0.07^{a}	1.41 ± 0.03^{a}	1.20 ± 0.07^{b}	1.19 ± 0.07^{b}	1.16 ± 0.09^{b}
	TAC (mg AAE/g)	snc	4.99 ± 0.26^{c*}	7.21 ± 0.50^{b}	3.63 ± 0.36^{d}	4.42 ± 0.42^{cd}	6.51 ± 0.39^{b*}	8.55 ± 0.38^{a*}	5.00 ± 0.34^{c}
***Tamar* stage**		**ZA-TA**	**ME-TA**	**DN-TA**	**TH-TA**	**HA-TA**	**BA-TA**	**KH-TA**	**BS-TA**
	TPC (mg GAE/g)	15.05 ± 0.56^{bc*}	11.70 ± 1.04^{de}	27.87 ± 2.05^{a*}	11.67 ± 0.88^{de*}	17.79 ± 0.79^{b*}	10.45 ± 1.33^{e}	14.16 ± 0.75^{cd}	9.20 ± 0.10^{e}
	TCT (mg CE/g)	130.23 ± 7.33^{a*}	82.38 ± 2.58^{de}	130.79 ± 10.61^{a}	92.58 ± 7.01 ^{cd*}	91.10 ± 7.84 ^{cd*}	109.68 ± 7.17^{bc*}	124.93 ± 4.36^{ab}	71.19 ± 5.70^{e}
	TFC (mg QE/g)	3.17 ± 0.01^{c*}	2.04 ± 0.07^{e}	5.03 ± 0.18^{a*}	2.13 ± 0.03^{e*}	3.68 ± 0.22^{b*}	2.71 ± 0.14^{d*}	3.12 ± 0.18^{c}	2.07 ± 0.07^{e*}
	DPPH (mg TE/g)	16.40 ± 0.18^{a*}	13.87 ± 0.28^{b}	15.99 ± 0.87^{a*}	2.61 ± 0.06^{f}	6.96 ± 0.58^{e*}	15.67 ± 0.26^{a}	11.93 ± 0.53^{c*}	10.13 ± 0.93^{d*}
	FRAP (mg TE/g)	82.28 ± 5.50^{ab*}	59.33 ± 5.62^{de}	94.69 ± 8.84^{a*}	60.40 ± 1.20^{de*}	93.15 ± 2.28^{a*}	65.79 ± 5.33^{cd}	77.19 ± 0.21^{bc}	52.18 ± 1.86^{e}
	ABTS (mg AAE/g)	25.75 ± 0.72^{b*}	18.71 ± 0.16^{c}	28.37 ± 0.23^{a*}	16.53 ± 0.02^{d}	28.29 ± 0.55^{a*}	20.81 ± 1.81^{c*}	24.20 ± 0.56^{b}	16.47 ± 0.41^{d}
	$^{\bullet}OH^{-}$RSA (mg TE/g)	25.98 ± 0.44^{c}	42.79 ± 2.91^{b}	15.66 ± 1.24^{d}	50.92 ± 2.00^{a}	11.06 ± 0.14^{e}	27.69 ± 0.31^{c}	29.34 ± 1.06^{c}	43.14 ± 1.14^{b}
	RAP (mg TE/g)	35.71 ± 0.85^{c*}	20.53 ± 1.11^{e}	43.74 ± 3.08^{ab*}	24.44 ± 0.18^{de*}	39.36 ± 1.36^{bc*}	28.65 ± 0.37^{d*}	44.25 ± 1.35^{a*}	23.38 ± 2.01^{e}
	FICA (mg EE/g)	1.36 ± 0.02^{ab*}	1.33 ± 0.08^{b}	1.42 ± 0.10^{ab*}	1.41 ± 0.09^{ab*}	1.56 ± 0.09^{a*}	1.21 ± 0.09^{b*}	1.25 ± 0.09^{b*}	1.35 ± 0.07^{ab*}
	TAC (mg AAE/g)	6.42 ± 0.32^{b*}	3.94 ± 0.18^{c}	8.61 ± 0.71^{a*}	4.18 ± 0.24^{c*}	7.60 ± 0.71^{ab*}	4.66 ± 0.34^{c}	6.89 ± 0.42^{b}	4.69 ± 0.23^{c*}

The data are shown as mean ± standard deviation (n = 3); $^{a-e}$ indicates the means in a row with significant difference ($p < 0.05$) using a one-way analysis of variance (ANOVA) and Tukey's test; * indicates the highest value among the three ripening stages. The standards and samples were mentioned in abbreviations. GAE, gallic acid equivalents; QE, quercetin equivalents; CE, catechin equivalents; AAE, ascorbic acid equivalents; TE, Trolox equivalents; EE, EDTA equivalents; snc, sample not collected.

acetonitrile, and mobile phase B, which was made up of 50 % Milli-Q® water and 50 % acetonitrile. The flow rate was set at 0.6 mL/min. The gradient conditions used were as follows: 0–10.8 min hold 5 % B, 10.8–18.8 min 5–10 % B, 18.8–22.8 min 10–20 % B; 22.8–30.0 min 20–25 % B, 30.0–38.0 min 25–30 % B, 38.0–42.2 min 30–40 % B, 42.2–45.0 min 40–45 % B, 45.0–50.0 min 45–100 % B, 50.0–60.0 min hold 100 % B, 60.0–62.0 min 100–5 % B for HPLC equilibration. Individual phenolic compounds were determined using calibration curves

generated from standards that were produced from commonly found 12 phenolic compounds present in dates (Gallic acid, *p*-hydroxybenzoic acid, Catechin, Caffeic acid, Syringic acid, Epicatechin, Coumaric acid, *trans*-ferulic acid, sinapic acid, Procyanidin A2, Quercetin, and Kaempferol). All aspects of instrument control, data acquisition, and chromatography processing were conducted with Empower software (2010). Venn diagrams based on the phenolic compounds quantified through LC-DAD were built, accompanied with a Heat map that could assist visualize the correlation between the content of phenolic compounds and the extraction methods.

2.8. Statistical analysis

One- and two-way analysis of variance (ANOVA) and Tukey's honestly significant differences (HSD) multiple rank test at $p \leq 0.05$ were used to analyse the mean differences of triplicated tested data among samples. One-way ANOVA was carried out by Minitab for Windows version 19.0 (Minitab, LLC, State College, PA, USA). Two-way ANOVA was carried out by Statistix for Windows version 8.1 (Statistix, Tallahassee, Florida, USA). Pearson's correlation coefficient at $p \leq 0.05$ and a principal component analysis (PCA) graph were applied to analyse the correlations between the content of phenolic compounds and antioxidant activities.

3. Results and discussion

3.1. Phenolic content estimation (TPC, TFC and TCT)

The phenolic content of each date seed sample determined by the TPC, TFC, and TCT are presented in Table 2. Overall, the TPC, TFC, and TCT values increased with the ripening stage. Among varieties, Deglet nour showed the highest total phenolic content among all three ripening stages. Varieties Barhee, Khadrawy, and Deglet nour exhibit the highest condensed tannin content at *Kimri*, *Khalal*, and *Tamar* stages, respectively. For the total flavonoid content, Zahidi, Khadrawy, and Deglet nour were the highest at *Kimri*, *Khalal*, and *Tamar* stages, respectively.

In TPC assay, Deglet nour had the highest value in all three ripening stages (5.99 mg GAE/g, 16.93 mg GAE/g, and 27.87 mg GAE/g) indicating Deglet nour seeds had the highest phenolic compound among all eight varieties tested in this assay. In contrast, Medjool, Halawi, and Bau Strami date seeds had the lowest TPC among all three ripening stages (1.81 mg GAE/g, 9.87 mg GAE/g, and 9.20 mg GAE/g, respectively). At the same time, we found that the TPC value of four varieties were higher than the other two ripening stages at *Khalal* stage (Medjool: 13.26 mg GAE/g; Barhee: 14.91 mg GAE/g; Khadrawy: 16.93 mg GAE/g; Bau Strami: 11.17 mg GAE/g) while there were also four varieties reaching the highest at *Tamar* stage (Zahidi: 15.05 mg GAE/g; Deglet nour: 27.87 mg GAE/g; Thoory 11.67 mg GAE/g; Halawi: 17.79 mg GAE/g). This phenomenon indicated that the phenolic compound in date seeds reached a peak between the ripening and half-ripening stage and the phenolic compounds may start to degrade after this peak, as also reported by Al-Mssallem, Alqurashi, and Al-Khayri (2020).

Concentration of TCT in Khadrawy at *Khalal* stage was the highest (158.55 mg CE/g) while at *Tamar* stage, Zahidi date seed had the highest TCT value (130.23 mg CE/g). The distribution of the highest TCT values across all varieties and ripening stages followed the similar trend as TPC. We found four varieties of the highest TCT value in *Khalal* stage (Medjool, Deglet nour, Khadrawy, and Bau Strami) and four in *Tamar* stage (Zahidi, Thoory, Halawi, and Barhee). Before reaching full ripeness, date fruits have a bitter taste due to soluble tannins. As the fruit ripens, tannins concentration decreases, resulting in a less astringent flavor (Ghnimi, Umer, Karim, & Kamal-Eldin, 2017). It is reasonable to assume that the content of condensed tannins in date seeds follows the same pattern as other fruits. In the case of grapes, the soluble condensed tannins present in the seeds show a slight decrease in variation as the grape berries mature, while the insoluble tannins exhibit a steady increase before véraison (corresponds to the *Khalal* stage in date ripening) and stayed high afterward. Comparatively, a modest rise in sugar content in developing grape berries encouraged the transformation of seed tannins into more complex polymer structures (Wang et al., 2023).

At the *Kimri* stage, Zahidi date seeds had a significantly higher TFC of 1.15 mg QE/g compared to other varieties. At the *Khalal* stage, Khadrawy had the highest TFC (3.34 mg QE/g), while at the *Tamar* stage, Deglet Nour had the highest TFC content (5.03 mg QE/g). Among the three ripening stages, six varieties (Zahidi, Deglet nour, Thoory, Halawi, Barhee, and Bau Strami) at *Tamar* stage had the highest TFC than other ripening stages. This observation is inconsistent with the findings of Mohamed Lemine et al. (2014) reporting a decline in flavonoid content in date fruit over the course of maturation. Methanolic extracts from fruits of six date palm varieties commonly cultivated in Mauritania were analysed for their flavonoid content at two stages of ripeness suitable for consumption. Flavonoid levels were higher in the earlier *Khalal* stage, compared to the fully ripe *Tamar* stage, regardless of the date palm variety. Because of the current lack of relevant studies, we hypothesize that this occurrence can be explained by two factors: (i) in contrast to the fruit pulp, which aims to attract birds or other organisms to spread the seeds upon maturation, seeds themselves require a constant accumulation of antioxidant substances to protect against insects and pathogens (Lei et al., 2021). (ii) Not all varieties of date seeds exhibit a reduction in flavonoid content as the fruit matures.

3.2. Antioxidant estimation

The antioxidant capacity of date seed samples was tested by using DPPH, FRAP, ABTS, RPA, $^{\bullet}$OH-RSA, FICA, and TAC assays. The results (shown in Table 1) demonstrated the capacity of these date seed samples to scavenge free radicals. Reactive oxygen species can damage biomolecules, but date seed's antioxidants can counteract ROS during metabolic processes. In other words, these antioxidants act as agents that scavenge free radicals, bind to metals, and supply hydrogen atoms and this process reduces oxidative stress and prevents harm to human body (Attia et al., 2021). In this study, the phenolic compounds were tested for their ability to scavenge free radicals using different methods like DPPH, $^{\bullet}$OH-RSA, and ABTS assays. Metal chelation properties were analysed using FICA, and the FRAP test focused on the capacity to donate electrons, reducing the Fe^{3+}-TPTZ complex to the Fe^{2+}-TPTZ complex.

At *Kimri* stage, Barhee date seed samples showed higher antioxidant capacity than other varieties. BA-KI variety exhibited significantly higher antioxidant activity compared to other varieties. Specifically, it showed a DPPH value of 12.06 mg TE/g, FRAP value of 24.23 mg TE/g, ABTS value of 10.57 mg AAE/g, $\bullet OH^{-}$RSA value of 62.85 mg TE/g, RAP value of 11.20 mg TE/g, FICA value of 1.19 mg EE/g, and TAC value of 2.47 mg AAE/g. Simultaneously, we observed a significant trend while comparing $^{\bullet}OH^{-}$RSA values at various stages of maturity. It is evident that as the fruit matures, the radical scavenging ability decreases. For instance, in the case of the Deglet nour variety, the $^{\bullet}OH^{-}$RSA values from low to high maturity stages were 57.06 mg TE/g, 34.32 mg TE/g, and 15.66 mg TE/g, respectively.

At *Khalal* stage, Khadrawy date seeds had the most antioxidant assays with the highest values which include FRAP (129.15 mg TE/g), ABTS (27.94 mg AAE/g), RAP (37.46 mg TE/g), and TAC (8.55 mg AAE/g). At *Tamar* stage, Deglet nour date seeds had the highest value of DPPH (15.99 mg TE/g), FRAP (94.69 mg TE/g), ABTS (28.37 mg AAE/g), RAP (43.74 mg TE/g), FICA (1.42 mg EE/g), and TAC (8.61 mg AAE/g). These values were significantly higher than other varieties and were the highest among all the three ripening stages of Barhee dates. Previous studies have suggested that theoretically, the phenolic compounds in dates should decrease with the fruit's ripening, leading to a decline in the antioxidant capacity (Al-Mssallem et al., 2020). However, among the eight varieties of date seeds tested in our study, only the most common

Table 3
Results of the two-way ANOVA on antioxidant assays of the 8 varieties (factors: 'Ripening stage' and 'Sample variety').

Test	Sources	*df*	MS	*F*	*p*
TPC	'Ripening stage'(A)	2	749.26	1380.28	<0.001
	'Sample variety'(B)	7	93.62	172.46	<0.001
	A × B	14	54.87	101.09	<0.001
	Error	48	0.54		
TCT	'Ripening stage'(A)	2	31642.50	832.23	<0.001
	'Sample variety'(B)	7	3986.60	104.85	<0.001
	A × B	14	2483.20	65.31	<0.001
	Error	48	38.00		
TFC	'Ripening stage'(A)	2	36.39	3206.43	<0.001
	'Sample variety'(B)	7	2.23	196.19	<0.001
	A × B	14	2.09	184.33	<0.001
	Error	48	0.01		
DPPH	'Ripening stage'(A)	2	234.71	1225.24	<0.001
	'Sample variety'(B)	7	97.41	508.50	<0.001
	A × B	14	58.75	306.71	<0.001
	Error	48	0.19		
FRAP	'Ripening stage'(A)	2	22024.40	1553.81	<0.001
	'Sample variety'(B)	7	1622.20	114.44	<0.001
	A × B	14	1488.30	105.00	<0.001
	Error	48	14.20		
ABTS	'Ripening stage'(A)	2	1375.21	2511.06	<0.001
	'Sample variety'(B)	7	89.07	162.64	<0.001
	A × B	14	97.78	178.54	<0.001
	Error	48	0.55		
•OH–RSA	'Ripening stage'(A)	2	6472.63	2078.08	<0.001
	'Sample variety'(B)	7	629.93	202.24	<0.001
	A × B	14	491.10	157.67	<0.001
	Error	48	3.11		
RAP	'Ripening stage'(A)	2	4127.43	3628.69	<0.001
	'Sample variety'(B)	7	297.72	261.75	<0.001
	A × B	14	186.15	163.66	<0.001
	Error	48	1.14		
FICA	'Ripening stage'(A)	2	0.44	89.93	<0.001
	'Sample variety'(B)	7	0.21	44.24	<0.001
	A × B	14	0.26	54.72	<0.001
	Error	48	0.01		
TAC	'Ripening stage'(A)	2	156.07	1413.82	<0.001
	'Sample variety'(B)	7	13.43	121.63	<0.001
	A × B	14	8.52	77.17	<0.001
	Error	48	0.11		

variety, Medjool, followed this pattern. Further validation in future research is necessary to confirm our assumption regarding possible species differences.

The two-way ANOVA results (Table 3) also indicated that the ripening stage and variety had a significant effect on the antioxidant capacity of date seeds ($p < 0.05$). The statistical analysis results of the phenolic content and antioxidant capacity data confirm the extent to which they were affected by the ripening stage, allowing us to compare the differences between date seeds of different varieties.

3.3. Correlation between phenolic compounds and antioxidant assays

The correlation between phenolic content and antioxidant activities was performed with Pearson's correlation test (Table 4). A principal components analysis (PCA, Fig. 2) was also performed to find out the similarities and differences among all the fractions and the relationship among the antioxidant potential assays. A total of 86.48 % variability of the initial data can be explained by the first two factors (F1 and F2). From Fig. 2, we found that antioxidant assays including ABTS, TAC, RPA, and FRAP were highly related to the estimated phenolic compounds (TPC, TCT, and TFC). This high correlation indicates that phenolic compounds present in date seed extracts had a strong antioxidant capacity and these assays are strongly related to each other. However, FICA and DPPH compared to other antioxidant assays had a lower correlation with the estimated phenolic compounds (DPPH-TPC: $r = 0.557$; DPPH-TCT: $r = 0.567$; DPPH-TFC: $r = 0.602$; FICA-TPC: $r = 0.673$; FICA-TCT: $r = 0.536$; FICA-TFC: $r = 0.654$). Also, the quantitative analysis of phenolic acids and flavonoids via HPLC was poorly correlated with most of the antioxidant assays and estimated phenolic compounds. This might indicate that we have not quantified all the phenolic acids and flavonoids from the seed extracts, which requires improvement.

Based on the information presented in Fig. 2 and Table 4, there was a significant correlation between phenolic compounds and the antioxidant capacity of date seed samples, which aligns with previous reports (Suleria, Barrow, & Dunshea, 2020). However, •OH-RSA showed a negative correlation with all three estimated phenolic compounds (•OH-RSA-TPC: $r = -0.878$; •OH-RSA-TCT: $r = -0.850$; •OH-RSA-TFC: $r = -0.940$). This phenomenon is inconsistent with previous experimental findings. According to Ge et al. (2021), •OH-RSA may exhibit a positive correlation with other antioxidant tests as well as the content of phenolic compounds. However, in our experiment, •OH-RSA showed a highly negative correlation. Considering that the •OH-RSA is based on a colorimetric reduction reaction, we speculate that 3-Hydroxybenzonic acid might preferentially react with other substances present in the date seed extract, leading to its oxidation and the eventual formation of coloured complexes or precipitates with $FeSO_4{\cdot}7H_2O$. Further experimentation is needed to properly evaluate the antioxidant capacity of date seeds.

3.4. LC-ESI-QTOF-MS/MS based characterization of phenolic compounds

The qualitative analysis of the phenolic compounds from date seed extracts was conducted using LC-ESIQTOF-MS/MS in negative and positive ionization modes (Table 5). 37 different phenolic compounds were identified in all samples including 8 phenolic acids, 18 flavonoids, and 11 other phenolic compounds.

3.4.1. Phenolic acids

A total of 8 phenolic acids were identified, including 1

Table 4
Pearson's correlation between antioxidant capacity by different antioxidant assays.

	TPC	TCT	TFC	DPPH	FRAP	ABTS	•OH-RSA	RAP	FICA	TAC	Phenolic acids
TCT	0.873**										
TFC	0.963**	0.875**									
DPPH	0.557	0.567*	0.602*								
FRAP	0.894**	0.934**	0.914**	0.490*							
ABTS	0.927**	0.926**	0.955**	0.565*	0.964**						
•OH-RSA	−0.878**	−0.850**	−0.940**	−0.530*	−0.930**	−0.937**					
RAP	0.923**	0.925**	0.956**	0.538*	0.935**	0.973**	−0.918**				
FICA	0.673**	0.536	0.654**	0.398*	0.627**	0.711**	−0.545	0.650**			
TAC	0.942**	0.940**	0.948**	0.556*	0.957**	0.969**	−0.931**	0.968**	0.624**		
Phenolic acids	0.727**	0.686**	0.705**	0.471*	0.682**	0.716**	−0.573*	0.635*	0.668**	0.688**	
Flavonoids	0.667**	0.649**	0.781**	0.360*	0.800**	0.794**	−0.903**	0.792**	0.471*	0.757**	0.318

** Significant correlation with $p \leq 0.01$;
* Significant correlation with $p \leq 0.05$.

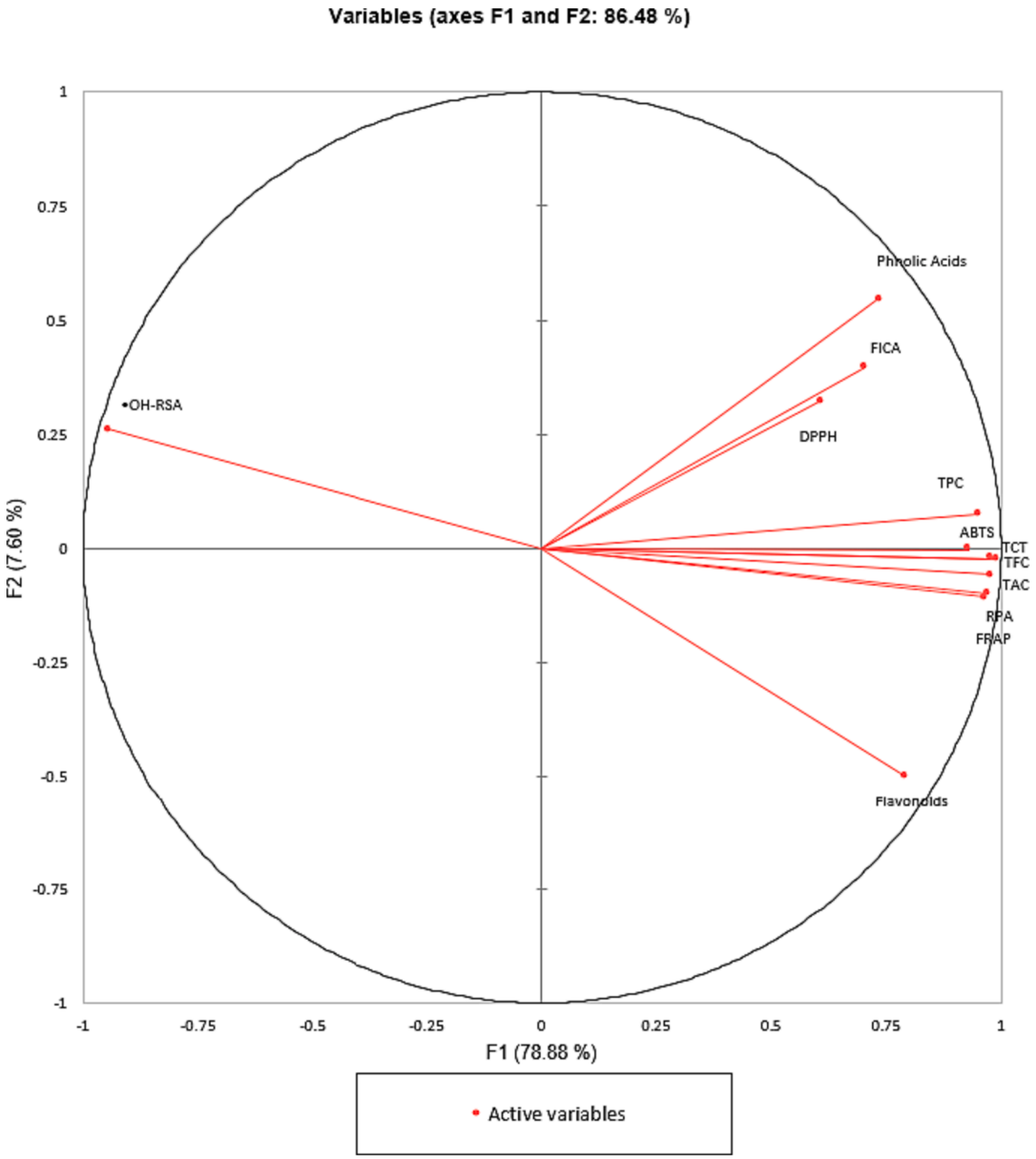

Fig. 2. Principal component analysis (PCA) of the phenolic content (TPC, TFC, TCT, Phenolic Acids, Flavonoids) and antioxidant activities (DPPH, ABTS, FRAP, RPA, OH-RSA, TAC) of eight date seeds from three ripening stages.

hydroxybenzoic acids, 4 hydroxycinnamic acids, 1 hydroxyphenylacetic acids and 2 hydroxyphenylpentanoic acid.

3.4.1.1. Hydroxybenzoic acids. One hydroxybenzoic acid was identified in this test which was tentatively identified as 3,4-*O*-Dimethylgallic acid (Compound **1**). This compound was detected at both positive and negative mode with an observed $[M + H]^+$ *m/z* at 199.0581 existed in sample ME-KI, ME-TA, HE-KH, DN-KI, DN-KH, and BS-KH. The compound 3,4-O-dimethylgallic acid is a major metabolite of gallic acid, previously found in fermented papaya extracts and present in various parts of the fruit (Yücetepe, Altin, & Özçelik, 2021).

3.4.1.2. Hydroxycinnamic acids. Hydroxycinnamic acid is the largest group among all the subclass of phenolic acids detected in this study. We observed four hydroxycinnamic acids, mainly in *Khalal* and *Tamar* stages. During the *Kimri* stage, only compounds **3** and **5** were present. Compound **2** was tentatively identified as 1-Sinapoyl-2-feruloylgentiobiose (RT = 9.322 min with *m/z* 723.2147), which could be found from date seeds of Deglet nour and Bau Strami verities at *Khalal* stage. The compound 1-sinapoyl-2-feruloylgentiobiose has been found in avocados and in large quantities in *Brassica* oleracea, including varieties like broccoli and cabbage (Fan et al., 2022). 5–5′-Dehydrodiferulic acid (Compound **3**) and 5-Feruloylquinic acid (Compound **5**) were both only detected in positive mode with an observed $[M + H]^+$ *m/z* at 387.1067 and 369.1180. These two compounds were widely present in six varieties of date seeds, with most samples being from *Khalal* and *Tamar* stages.

3.4.1.3. Hydroxyphenylacetic acids. In this study, only one compound was found in this subclass of phenolic acid, which is compound **6** detected in BS-KI, ME-TA, and DN-KI samples, characterized in both modes. Compound **6** was found in three varieties of date seeds from

Table 5
Characterization of phenolic compounds in date seeds by LC-ESI-QTOF-MS/MS.

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI+/ESI-)	Molecular Weight	Theoretical (*m/z*)	Observed (*m/z*)	Error (ppm)	MS/MS Product ion	Sample
	Phenolic acids									
	Hydroxybenzoic acids									
1	3,4-*O*-Dimethylgallic acid	$C_9H_{10}O_5$	56.342	**$[M+H]^+$	198.051	199.0583	199.0581	−1.0	153, 139, 125, 111	*ME-KI, ME-TA, HE-KH, DN-KI, DN-KH, BS-KH
	Hydroxycinnamic acids									
2	1-Sinapoyl-2-feruloylgentiobiose	$C_{33}H_{40}O_{18}$	9.322	**$[M-H]^-$	724.2216	723.2143	723.2147	0.6	529, 499	*DN-KH, BS-KH
3	5–5′-Dehydrodiferulic acid	$C_{20}H_{18}O_8$	62.340	$[M+H]^+$	386.0993	387.1066	387.1067	0.3	369	*BS-TA, DN-TA, TH-TA, HA-KH, HA-KI, BA-KH, KH-KH, KH-TA, BS-KH
4	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	62.679	**$[M-H]^-$	516.1261	515.1188	515.1163	−4.9	187, 192	*DN-TA, KH-KH, HA-KH, BA-KH, BA-TA, BS-KH
5	5-Feruloylquinic acid	$C_{17}H_{20}O_9$	60.976	$[M+H]^+$	368.1109	369.1182	369.1180	−0.5	192, 193	*HA-TA, ZA-KI, ME-KI, ME-KH, ME-TA, TH-KI, TH-TA, HA-KH, BA-KH, BA-TA, KH-KH
	Hydroxyphenylacetic acids									
6	3,4-Dihydroxyphenylacetic acid	$C_8H_8O_4$	59.260	**$[M-H]^-$	168.0419	167.0346	167.0354	4.8	149, 123	*BS-KI, ME-TA, DN-KI
	Hydroxyphenylpentanoic acids									
7	Dihydroferulic acid 4-*O*-glucuronide	$C_{16}H_{20}O_{10}$	47.649	$[M-H]^-$	372.1027	371.0954	371.0955	0.3	195	*HA-TA, ZA-KI, DN-TA, BA-KI, KH-TA, BS-TA
8	5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone	$C_{11}H_{12}O_5$	69.166	$[M-H]^-$	224.0705	223.0632	223.0632	0.0	205, 163	*DN-TA, ZA-KI, ZA-TA, ME-KI, ME-KH, ME-TA, DN-KI, DN-KH, TH-KI, TH-KH, TH-TA, HA-KH, HA-TA, BA-KI, KH-KI, KH-KH, KH-TA, BS-KI, BS-KH, BS-TA
	Flavonoids									
	Anthocyanins									
9	Cyanidin 3-*O*-(6′′-malonyl-3′′-glucosyl-glucoside)	$C_{30}H_{33}O_{19}$	9.642	$[M+H]^+$	697.1635	698.1708	698.1706	−0.3	449, 180, 88	*BA-KI, TH-TA, HA-KI, HA-KH, HA-TA, BA-KH, KA-KH
	Flavanols									
10	Theaflavin 3,3′-*O*-digallate	$C_{43}H_{32}O_{20}$	42.664	**$[M-H]^-$	868.1475	867.1402	867.1395	−0.8	715, 563, 545	*KH-KH, ZA-KI, ME-KH, DN-KI, DN-TA, TH-KI, TH-KH, TH-TA, HA-KI, HA-KH, BA-KI, BA-KH
11	4′′-*O*-Methylepigallocatechin 3-*O*-gallate	$C_{23}H_{20}O_{11}$	54.227	**$[M-H]^-$	472.1041	471.0968	471.0949	−4.0	169, 319	*TH-KH, KH-TA
12	4′-*O*-Methylepigallocatechin	$C_{16}H_{16}O_7$	56.573	**$[M+H]^+$	320.0879	321.0952	321.0950	−0.6	92, 121	*BA-TA, ZA-KI, TH-TA, KH-KH
	Flavanones									
13	Narirutin	$C_{27}H_{32}O_{14}$	10.954	$[M-H]^-$	580.1800	579.1727	579.1730	0.5	271	*DN-TA, TH-KH
	Flavones									
14	Luteolin 7-*O*-(2-apiosyl-glucoside)	$C_{26}H_{28}O_{15}$	57.032	$[M+H]^+$	580.1417	581.1490	581.1489	−0.2	419, 401, 383	*ZA-TA, ZA-KI, ME-KI, ME-KH, ME-TA, TH-KH, HA-KI, HA-KH, KH-TA, BA-KI, BS-KI
15	Neodiosmin	$C_{28}H_{32}O_{15}$	60.309	$[M+H]^+$	608.1783	609.1856	609.1853	−0.5	301, 286	*DN-KI, ZA-KI, ME-KI, ME-TA, BA-KH, BS-KH
16	Gardenin B	$C_{19}H_{18}O_7$	65.139	$[M+H]^+$	358.1037	359.1110	359.1121	3.1	344, 329, 311	DN-TA
17	Apigenin 7-*O*-glucuronide	$C_{21}H_{18}O_{11}$	65.736	$[M+H]^+$	446.0824	447.0897	447.0901	0.9	271, 253	DN-KH
	Flavonols									
18	Quercetin 3′-sulfate	$C_{15}H_{10}O_{10}S$	8.506	**$[M-H]^-$	381.9992	380.9919	380.9922	0.8	79	*ZA-KI, BS-TA
19	Quercetin 3-*O*-(6′′′-malonyl-glucoside) 7-*O*-glucoside	$C_{30}H_{32}O_{20}$	10.698	$[M+H]^+$	712.1455	713.1528	713.1534	0.8	187, 359	BA-TA
20	Quercetin 3-*O*-xylosyl-rutinoside	$C_{32}H_{38}O_{20}$	55.299	**$[M-H]^-$	742.1934	741.1861	741.1864	0.4	479, 317	*KH-TA, ZA-TA, ME-TA, DN-TA

(*continued on next page*)

Table 5 (*continued*)

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI+/ESI-)	Molecular Weight	Theoretical (*m/z*)	Observed (*m/z*)	Error (ppm)	MS/MS Product ion	Sample
	Isoflavonoids									
21	Tectorigenin 7-sulfate	$C_{16}H_{12}O_9S$	7.847	**$[M-H]^-$	380.0206	379.0133	379.0131	−0.5	299	*BA-KI, DN-KH, DN-TA
22	3′-*O*-Methylviolanone	$C_{18}H_{18}O_6$	9.020	$[M-H]^-$	330.1113	329.1040	329.1025	−4.6	314, 299, 284, 256	HA-TA
23	6′′-*O*-Malonylglycitin	$C_{25}H_{24}O_{13}$	11.306	$[M+H]^+$	532.1182	533.1255	533.1258	0.6	285, 270, 253	*DN-KH, ZA-KI, BA-KH, KH-TA
24	6′′-*O*-Acetylglycitin	$C_{24}H_{24}O_{11}$	12.466	$[M+H]^+$	488.1301	489.1374	489.1379	1.0	285, 270	*BA-KI, ME-KI, ME-KH, TH-KI, HA-KH, HA-TA, BA-KH, BS-TA
25	2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone	$C_{17}H_{14}O_6$	58.021	$[M+H]^+$	314.0820	315.0893	315.0890	−1.0	300, 282	ME-TA
26	3′-Hydroxydaidzein	$C_{15}H_{10}O_5$	59.142	$[M+H]^+$	270.0549	271.0622	271.0622	0.0	253, 241, 225	*ME-KI, ZA-KI, ZA-TA, ME-KH, ME-TA, DN-KI
	Other phenolic compounds									
	Alkylmethoxyphenols									
27	4-Vinylsyringol	$C_{15}H_{14}O_3$	55.798	$[M+H]^+$	242.0924	243.0997	243.0998	0.4	225, 211, 197	*KH-KI, KH-KH, BS-KI, BS-KH
	Curcuminoids									
28	Curcumin	$C_{21}H_{20}O_6$	33.932	$[M-H]^-$	368.1237	367.1164	367.1164	0.0	217	*TH-TA, ZA-TA, HA-TA, BS-TA
	Furanocoumarins									
29	Isopimpinellin	$C_{13}H_{10}O_5$	56.983	$[M+H]^+$	246.0535	247.0608	247.0607	−0.4	232, 217, 205, 203	*ME-TA, ME-KI, ME-KH, DN-KI
	Hydroxybenzaldehydes									
30	*p*-Anisaldehyde	$C_8H_8O_2$	27.508	$[M+H]^+$	136.0513	137.0586	137.0587	0.7	122, 109	*BS-TA, ZA-TA, ME-KH, ME-TA, DN-KI, HA-KI, BA-KI
	Hydroxyphenylpropenes									
31	Eugenol	$C_{10}H_{12}O_2$	62.932	$[M+H]^+$	164.0829	165.0902	165.0901	−0.6	31	*HA-KH, TH-KH, TH-TA, BA-KH
	Tyrosols									
32	3,4-DHPEA-AC	$C_{10}H_{12}O_4$	69.474	$[M-H]^-$	196.0724	195.0651	195.0651	0.0	135	*BS-TA, BS-KI
	Stilbenes									
33	Resveratrol 5-*O*-glucoside	$C_{20}H_{22}O_8$	10.550	$[M-H]^-$	390.1337	389.1264	389.1261	−0.8	227	*KH-TA, ME-TA, DN-KI, DN-TA, TH-KH, BA-KH, BA-TA, BS-KI
34	4-Hydroxy-3,5,4′-trimethoxystilbene	$C_{17}H_{18}O_4$	64.667	$[M+H]^+$	286.1224	287.1297	287.1295	−0.7	271, 241, 225	*BA-KI, ZA-KI, HA-KI
	Lignans									
35	Schisandrin	$C_{24}H_{32}O_7$	10.822	**$[M+H]^+$	432.2142	431.2069	431.2063	−1.4	415, 384, 361	*HA-KI, ZA-TA, ME-KK, TH-KI, HA-TA, BA-TA, BS-KI
36	Schisandrin B	$C_{23}H_{28}O_6$	61.843	$[M+H]^+$	400.1910	401.1983	401.1999	4.0	386	DN-KH
37	Schisandrol B	$C_{23}H_{28}O_7$	65.687	$[M+H]^+$	416.1866	417.1939	417.1938	−0.2	224, 193, 165	*BA-KH, ME-KI, DN-TA, TH-KH, TH-TA, KH-KI, KH-KH, BS-TA

*Compound was detected in more than one sample; data presented in this table are from an asterisk sample. **Compounds were detected in both negative $[M-H]^-$ and positive $[M+H]^+$ modes of ionization while only single mode data was presented. Date seed samples were mentioned in abbreviations.

Kimri and *Tamar* stages, tentatively identified as 3,4-Dihydroxyphenylacetic acid with the $[M-H]^-$ *m/z* at 167.0354. Das, Acharya, and De (2017) reported the existence of this compound in ethyl acetate extract of date palm fruits after alkaline processing.

3.4.1.4. Hydroxyphenylpentanoic acids. Two hydroxyphenylpentanoic acids were identified and both were characterized in negative mode. Compound **7** was tentatively identified as Dihydroferulic acid 4-*O*-glucuronide with the precursor ion is observed at *m/z* 371.0955 and was confirmed by the product ion at *m/z* 195 in MS/MS analysis. According to Hong, Wang, Barrow, Dunshea, and Suleria (2021), this compound could be identified from genus *Prunus* fruits plums and apricots. Compound **8** was tentatively identified as 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (RT = 69.166 min with *m/z* 223.0632) and in the MS/MS spectra, the product ion at *m/z* 205 and *m/z* 163 proven the existence of this compound. Compound **8** existed in a wide range of samples and for Medjool, Deglet nour, Thoory, Khadrawy, and Bau Strami date seeds in all three ripening stages.

3.4.2. Flavonoids

Flavonoid conjugates were the main phenolic compounds detected in date extracts and a total of 18 flavonoids were identified, including 6 subtypes: 1 anthocyanin, 3 flavanols, 1 flavanone, 4 flavones, 3 flavonols, and 6 isoflavonoids. Flavones and isoflavonoids were the main subtypes in this study.

3.4.3. Anthocyanins

Compound **9** was the only compound found in this subclass of flavonoids and was tentatively identified as Cyanidin 3-*O*-(6′′-malonyl-3′′-glucosyl-glucoside). This compound was detected only in positive mode with $[M+H]^+$ *m/z* at 698.1706 confirmed by the product ion at *m/z* 449, 180, and 88. This compound was identified in TH-TA, HA-KI, HA-KH, HA-TA, BA-KI, BA-KH, and KA-KH samples mainly in *Khalal* and *Tamar* stages. This compound also existed in all three ripening stages of Halawi date seeds.

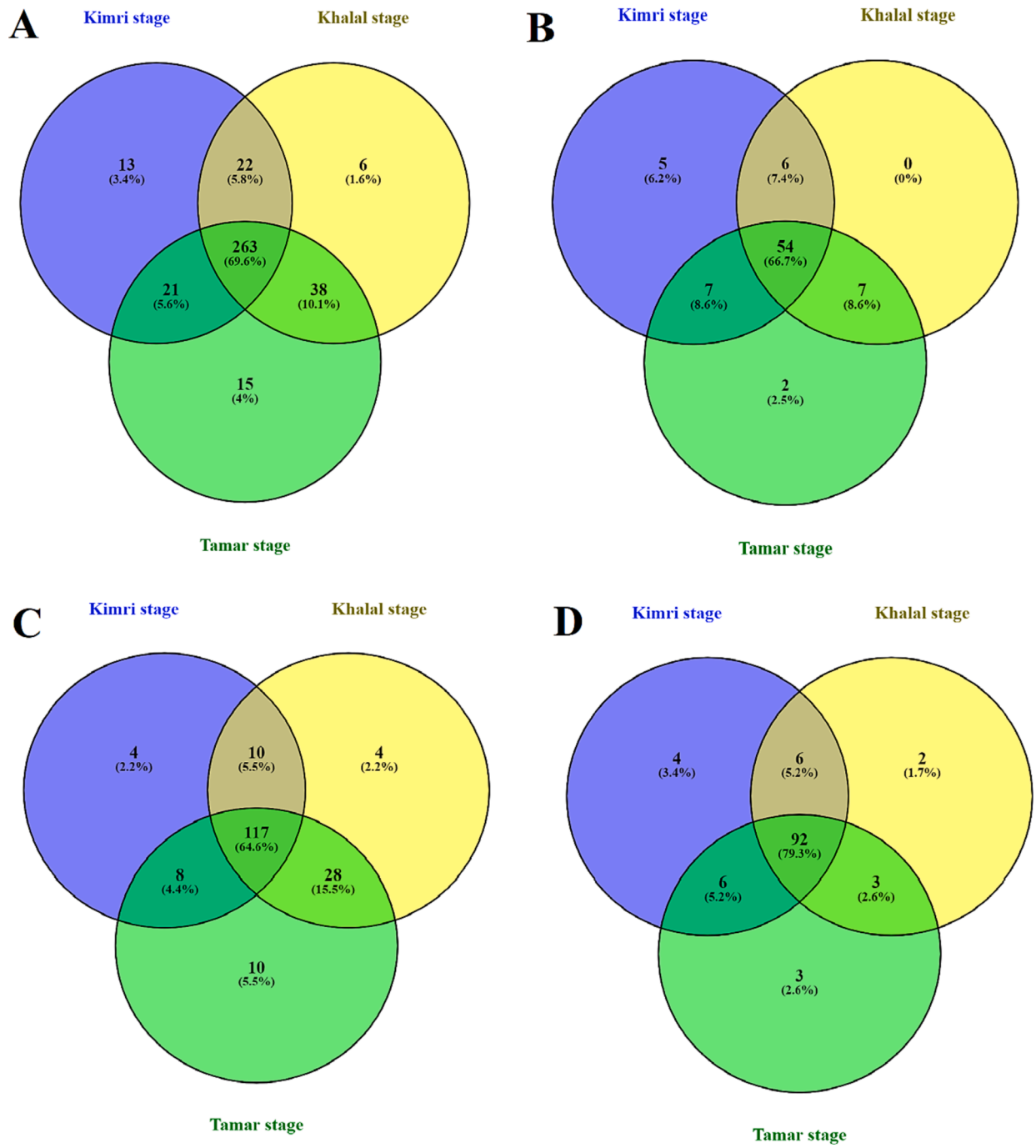

Fig. 3. Venn diagram of phenolic compounds presented in different ripening stage of date seed samples. **(A)** shows comparison of total phenolic compounds present in different ripening stage. **(B)** shows comparison of total phenolic acids present in different ripening stage. **(C)** shows comparison of flavonoids present in different extraction ripening stage. **(D)** shows comparison of other phenolic compounds present in different ripening stage.

3.4.4. *Flavanols*

A total of 3 flavanols was detected in this study. Compound **10** existed in all varieties of date seeds except Bau Strami and mainly existed in the two early stages of ripening (*Kimri* and *Khalal* stages). This compound was tentatively identified as Theaflavin 3,3′-*O*-digallate due to the precursor ion observed at *m/z* 867.1395 and was confirmed by the product ion at *m/z* 715, 563, and 545 in MS/MS analysis. Compound **11** was tentatively identified as 4′′-*O*-Methylepigallocatechin 3-*O*-gallate at *m/z* 471.0949 from TH-KH and KH-TA samples. This compound was confirmed by the product ions of *m/z* 319 and *m/z* 169, indicating galloyl group and methyl-(epi)gallocatechin (Henriques et al., 2016). Compound **12** existed in both modes among BA-TA, ZA-KI, TH-TA, and KH-KH samples, and was tentatively identified as 4′-*O*-methyl-epigallocatechin with the precursor ion $[M + H]^+$ at *m/z* 321.0950. A previous study reported a southern African plant called *Elaeodendron transvaalense* also contained this compound (Khumalo, Sadgrove, Van Vuuren, & Van Wyk, 2019).

3.4.5. *Flavanones*

Compound **13** was the only flavanones found in this test which was identified as Narirutin (RT = 10.954 min with *m/z* 579.1730) in DN-TA and TH-KH samples. This compound was further confirmed by the product ion at *m/z* 271, representing the loss of rhamnosyl group (146 Da) (Hai-Qiang, Yun-Xiang, Yi-Ning, Ruo-Liu, & Shu-Fang, 2019).

3.4.6. Flavones

Flavones is the second largest group of flavonoids in this test with a total of 4 compounds identified from date seed samples. Compound **14** was tentatively identified as Luteolin 7-*O*-(2-apiosyl-glucoside) which was detected only at positive mode with an observed $[M + H]^+$ *m/z* at 581.1489 and confirmed by produced product ion at *m/z* 419, 401, and 383. This compound existed in all varieties of date seeds except Deglet nour and existed in all three ripening stages of Medjool date seeds. Neodiosmin (compound **15**) was another compound widely detected in the varieties of date seeds. We identified this flavonoid from extracts of date seeds belonging to five different varieties. However, there were no commonalities in terms of types and maturity stages among the date seeds from which these extracts were derived. Neodiosmin (RT = 60.309 min with *m/z* 609.1853) was further confirmed by produced product ion at *m/z* 301 and 286.

3.4.7. Flavonols

Compound **18** was tentatively identified as Quercetin 3′-sulfate at both modes, based on the observed $[M + H]^+$ *m/z* values of 380.9922. This compound was further confirmed by the produced product ion at *m/z* 79, representing the loss of SO_3 group (80 Da) (Kleinenkuhnen, Büchel, Gerlich, Kopriva, & Metzger, 2019). Quercetin 3′-sulfate, a metabolite of quercetin found in ZA-KI and BS-TA, has been shown to possess antioxidant properties at levels found naturally in the body (Thilakarathna, Rupasinghe, & Needs, 2013). Compound **20** was detected with a precursor ion at *m/z* 741.1864 only in date seed samples harvested during the *Tamar* stage. This compound was identified as Quercetin 3-*O*-xylosyl-rutinoside. It produced product ions at *m/z* 479 and *m/z* 317, resulting from the removal of two pentoses and an extra loss of a hexose (Nebieridze, Skhirtladze, Kemertelidze, & Ganzera, 2017).

3.4.8. Isoflavonoids

Isoflavonoid is the largest group among all the subclass of flavonoids detected in this study with a total of 6 identified compounds. Compound **21** was tentatively identified as Tectorigenin 7-sulfate (RT = 7.847 min with *m/z* 379.0131), further confirmed by produced product ion at *m/z* 399 in MS/MS analysis. Compound **22** was only detected in sample HA-TA and tentatively identified as 3′-*O*-methylviolanone with an observed $[M - H]^-$ *m/z* at 329.1025. Confirmation of the compound was further supported by its negative MS/MS spectrum, which revealed product ions at *m/z* 314, *m/z* 299, *m/z* 284, and *m/z* 256, corresponding to CH_3, $2CH_3$, $3CH_3$, and $3CH_3$-CO, respectively (Li, Zhang, Liao, Fan, & Cheng, 2021). Compounds **23** and **24** were tentatively identified as 3′-*O*-Methylviolanone and 6′′-*O*-Acetylglycitin under the condition of *m/z* of 533.1258 and 489.1379, respectively. Compound **25** was tentatively characterized as 2′,7-dihydroxy-4′,5′-dimethoxyisoflavone under a positive ion mode with an *m/z* of 315.0890 and a retention time of 58.021 min. This compound was found only in ME-TA sample and has been reported to be present in *Lepidium sativum* (Kadam, Palamthodi, & Lele, 2018).

3.4.9. Other phenolic compounds

A total of 11 other phenolic compounds were identified in this test. Among them, there is 1 compound under each of 6 subclasses, 2 compounds under stilbenes, and 3 compounds under lignans. Compounds **27** and **32** were only detected in date seed samples from *Tamar* stage while compound **27** was allocated for 4-Vinylsyringol based on the $[M + H]^+$ *m/z* at 243.0998 and compound **32** was tentatively identified as 3,4-DHPEA-AC based on the $[M - H]^-$ *m/z* at 195.0651. Compound **30** (RT = 27.508 min with *m/z* 137.0587) was tentatively identified as *p*-Anisaldehyde due to the precursor ion observed at *m/z* 137.0587 and was confirmed by the product ion at *m/z* 122 and 109 in MS/MS analysis. Compound **30** was the phenolic compound existed in the most varieties in this test including Deglet nour, Halawi, Barhee, Khadrawy, and Bau Strami and most of the samples at *Kimri* and *Khalal* stages.

3.4.10. Stilbenes

Two compounds were detected in this subclass. Compound **33** was tentatively identified as Resveratrol 5-*O*-glucoside at negative mode with an observed $[M - H]^-$ *m/z* at 389.1261. This compound was widely existed in sample HA-TA, BA-KI, KH-KI, ZA-KH, DN-KH, ZA-TA, ME-TA, and BA-TA and was previously identified from a Tunisian desert plant called *Calligonum azel* Maire (Bannour et al., 2017). Compound **34** was identified in sample BS-KH, ZA-KI, and HA-KH with positive ionization mode at *m/z* 287.1295. The produced product ions at *m/z* 271, 241, and 225 further identified the compound as 4-Hydroxy-3,5,4′-trimethoxystilbene.

3.4.11. Lignans

Lignan was the largest subclass of other phenolic compounds, with three identified compounds. Compound **35** was detected in the $[M + H]^+$ mode at *m/z* 431.2063 and was preliminarily characterized as Schisandrin. This compound could be detected in shiitake mushroom and needle mushrooms as anti-inflammatory compound (Chu et al., 2023). Compound **36** (RT = 65.687 min with *m/z* 417.1938) and **37** (RT = 61.843 min with *m/z* 401.1999) were tentatively characterized as Schisandrol B and Schisandrin B.

3.5. Distribution of phenolic compounds from LC-MS – Venn diagram

To explore the distribution of phenolic compounds in date seeds, Venn diagrams were made among the type of phenolic compounds and the ripening stages of date seed (Fig. 3). Phenolic compounds were divided into four groups (total phenolic compounds, total phenolic acids, total flavonoids, and other phenolic compounds), ripening stages were divided into three groups (*Kimri*, *Khalal*, or *Tamar* stage).

A total of 378 phenolic compounds were found in all date seed extracts through LC-ESI-QTOF-MS as shown in Fig. 3**A**. We found that 263 (69.6 %) phenolic compounds existed in all three ripening stages of date seeds while *Tamar* stage had the largest number of unique phenolic compounds (15) followed by *Kimri* stage (13). Fig. 3**B** illustrates the distribution of phenolic acids among all three stages, showing a total 54 (66.7 %) phenolic acids existed in all three ripening stages of date seeds. At *Kimri* stage, date seed samples had the highest percentage of unique phenolic acids (6.2 %), which decreased at *Khalal* (0 %) and *Tamar* (2.5 %) stages. Phenolics tended to decrease with the ripening stage of the fruit, which is consistent with a previous report (Al-Mssallem et al., 2020). We analyzed Fig. 3**C** to observe the changing numbers of flavonoids during fruit ripening, noting a continuous increase in flavonoid quantities within date seeds during ripening. A total of 117 flavonoids (64.6 % of the total) were identified across the three maturity stages. Specifically, during the *Kimri* stage, 22 flavonoids were found that were not shared across the three stages. The number of not shared flavonoid types increased to 42 during the *Khalal* stage and further to 46 during the *Tamar* stage. Compared with phenolic acids and flavonoids, other phenolic compounds identified in date seeds among all three ripening stages seemed to be consistent (Fig. 3**D**). A total of 92 phenolic compounds were identified from all three ripening stages of date seeds, with 16, 11, and 12 being unusual in *Kimri*, *Khalal*, and *Tamar* stages, respectively.

After comparing the phenolic content among groups, we identified that flavonoid is the largest group of phenolic compounds in date seeds, followed by other phenolic compounds, and finally phenolic acids. Therefore, we can conclude that flavonoids are the primary phenolic compounds in date seeds, which are a varied group of plant compounds known for their antioxidant properties also renowned for their anti-inflammatory effects which are frequently linked to a decreased likelihood of chronic diseases (Alkhoori et al., 2022). Phenolic acids, on the other hand, are the least common phenolic compounds found in date seeds.

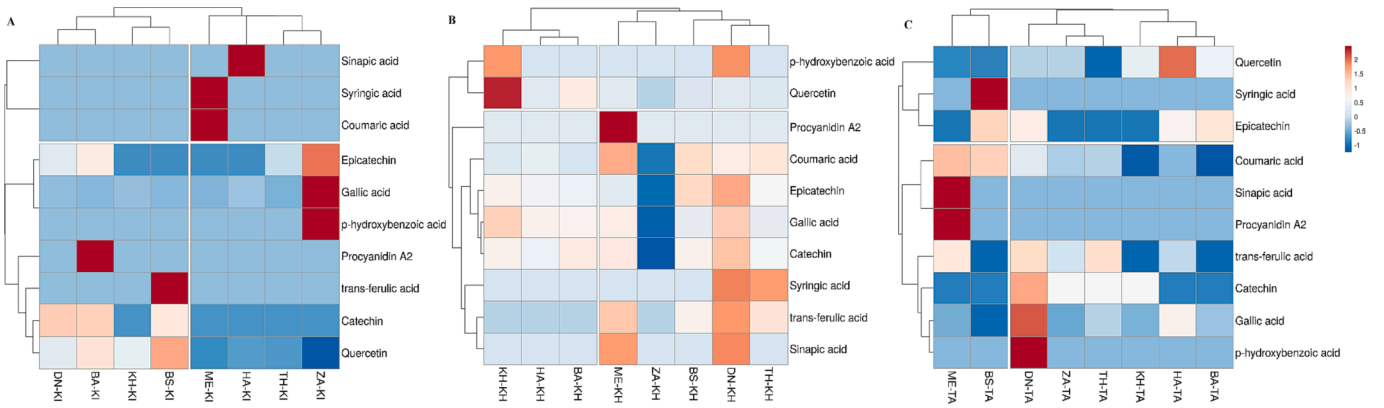

Fig. 4. Heatmap showing phenolic compounds distribution and concentration among eight varieties of *Kimri* (A), *Khalal* (B), and *Tamar* (C) date samples. Red boxes' mean concentrations are higher than the mean value among samples. Blue boxes mean lower concent. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.6. Heat map and hierarchical clustering phenolic compound analysis

The heat map was constructed to analyze the hierarchical clustering of the LC-DAD analyzed phenolic compounds in the twenty-three extracts of date seeds (Fig. 4). The correlation between samples and compounds was represented by the measurable distance and the average concentration of each sample, the rows and columns clustered. The tightest clusters will be clustered in tree order first.

According to the results of phenolic compound concentration of *Kimri* stage date seeds (Fig. 4**A)**, the Zahidi variety had a very exceptional concentration of epicatechin, gallic acid, and *p*-hydroxybenzoic acid which were all over 1.5 times of the average concentration of these tested phenolic compounds. Medjool date seeds came second, which had the highest concentration of syringic acid and coumaric acid. Barhee, Bau Strami, and Halawi had the high concentration (more than 2 times of the average concentration) of procyanidin A2, *trans*-ferulic acid, and sinapic acid, respectively. Compared with *Kimri* and *Tamar* stages, the difference in phenolic content among various date seed samples was significantly diminished **(**Fig. 4**B)**. This is symbolized by the significant reduction in the blue segment, representing phenolic compounds below the average concentration. However, among these samples, date seeds of the Deglet nour variety still exhibit evident distinctiveness. Only three specific phenolic compounds (Quercetin, Procyanidian A2, and Coumaric acid) in this variety exhibited markedly low levels in comparison to the others, which maintained levels above twice the average. At this ripening stage, date seeds of the Khadrawy and Medjool varieties demonstrated exceptional phenolic content in the Quercetin and Procyanidin A2, respectively, surpassing that of all other date seed samples. As shown in Fig. 4**C**, the difference in phenolic content widened once again at the *Tamar* stage, evident from the distinct color variations. Date seeds of the Deglet nour variety contained a higher number of phenolic compounds compared to the average level. Following closely, the Medjool variety exhibited the highest content in sinapic acid and Procyanidian A2. The date seeds of the Bau Strami variety had the highest content in Syringic acid, while date seeds of the Halawi variety had the highest quercetin content.

4. Conclusion

The analysis of Australian grown date seed samples from different varieties and ripening stages using advanced techniques such as LC-ESI-QTOF-MS/MS and LC-DAD revealed a diverse range of phenolic compounds. Our findings demonstrated that the antioxidant capacity of date seeds increases as they ripen. LC-ESI-QTOF-MS/MS analysis identified 37 different phenolic compounds in various extracts, many of which are known for their positive impact on human health due to their antioxidant properties. Particularly, Deglet nour and Medjool varieties of date seeds were rich sources of phenolic compounds at the *Khalal* and *Tamar* ripening stages. Our findings highlight the significant potential of date seed extracts derived from specific varieties and ripening stages, indicating their suitability as food processing agents and nutritional supplements. However, there is a gap in the existing literature regarding the relationship between phenolic content in date seeds and their ripening stage. Future research should focus on exploring this aspect to advance the utilization of date seeds in waste valorization efforts.

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CRediT authorship contribution statement

Linghong Shi: Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft. **Ziyao Liu:** Methodology, Data curation, Writing – review & editing, Visualization. **Claudia Gonzalez Viejo:** Supervision, Writing – review & editing. **Farhad Ahmadi:** Data curation, Supervision, Validation, Visualization, Writing – review & editing. **Frank R. Dunshea:** Writing – review & editing. **Hafiz A.R. Suleria:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114096.

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