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# Effect of Cultivar Type and Ripening on the Polyphenol Content of Date Palm Fruit

Noura M. S. Eid,[†] Balqees Al-Awadi,[†] David Vauzour,[§] Maria J. Oruna-Concha,[†] and Jeremy P. E. Spencer*[,†]

[†]Molecular Nutrition Group, Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, University of Reading, Reading RG6 6AP, United Kingdom

[§]Department of Nutrition, Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom

**ABSTRACT:** Date palm (*Pheonix dactylifera*) fruit contains an array of polyphenols, although how these levels alter with cultivar type and fruit ripening is unclear. Utilizing HPLC and LC-ESI-MS/MS, this study define and quantify an array of hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids in three common cultivars of dates (Ajwa, Barni, and Khalas) at the main ripening stages (*kimri*, *khalal*, *rutab*, and *tamr*). Polyphenols were at highest concentration at earlier stages of ripening, with concentrations reducing with ripening. The khalal stage of the Ajwa cultivar contained significantly higher ($P < 0.001$) levels of polyphenols than measured in the Barni and Khalas dates at the same degree of ripening. Furthermore, the Ajwa cultivar was the only one to contain significant quantities of anthocyanidins, in particular at the khalal stage. These data suggest dates are a significant source of polyphenols, especially if the earlier edible ripening stages are consumed or utilized as food ingredients.

**KEYWORDS:** *date palm fruit, antioxidant activity, phenolic compounds, HPLC, LC/MS (ESI−)*

## INTRODUCTION

Dates, the fruit of the date palm *Pheonix dactylifera*,[1] are one of the most commonly consumed fruits in the Middle East and North Africa,[2] although they are also cultivated and consumed in other regions of the world, including North America and Europe.[3] In the former they are considered a staple food, providing calories and nutrients,[4] and numerous biological activities have been described with respect to potential health effects, including antioxidant activity,[5−8] antimutagenic effects,[5] anti-inflammatory activity,[9] gastroprotective actions,[10] and anticancer activity.[11] In addition to their carbohydrate content of around 73% of the dry weight (predominately monosaccharides such as glucose, fructose, and sucrose), dates contain protein (3%) and a significant amount of fiber (5.2%), including relatively high amounts of pectin hemicellulose, lignin, resistant starch, and soluble fiber (6.4−11.5%). Indeed, around 100 g of dates (seven to nine fruits) provides the RDA for dietary fiber intake.[1] Furthermore, they also contain an array of micronutrients, including minerals, such as calcium, magnesium, copper, phosphorus, sodium, zinc, fluorine, selenium, potassium, iron, and sulfates, and vitamins, including vitamins A, B, and C.[12,13] For example, consumption of 17 g of dates fulfills the RDA for selenium.[6]

In addition to these macro- and micronutrients, dates have been reported to contain an array of (poly)phenols, including phenolic acids (gallic acid, protocatechuic acid, hydroxybenzoic acid, and vanillic acid), hydroxycinnamates (caffeic acid, ferulic acid, and *p*-coumaric acid), flavonoid glycosides (luteolin, apigenin, quercetin, and isorhamnetin), and proanthocyanidins oligomers (type A and type B),[3,4,6−8,14−27] which may be, in part, responsible for their beneficial effects. However, their polyphenol content (and their macro- and micronutrient levels) will be dependent on a number of factors,[28] with cultivar and degree of ripeness likely to be major determinants. There are a large variety of date palm cultivars grown around the world, although three of the most commonly consumed varieties in Saudi Arabia and the rest of the Middle East are Barni, Khalas, and Ajwa, which differ in size, color, and taste.[29] The main ripening stages are known worldwide by their Arabic names: *kimri* (unripe), *khalal* (full-size, slightly crunchy; edible), *rutab* (ripe, soft; edible), and *tamr* (ripe, reduced moisture; edible).[30] Previous research has shown that the sensory, chemical, and functional composition of dates is significantly altered during date ripening,[18,30,31] with levels of reducing sugars increasing and vitamin, mineral, and fiber levels decreasing steadily.[28,32−34] However, little is known about how the polyphenol content of dates alters during ripening. The levels of polyphenols in grapes is known to increase in the early stages of ripening and fall just prior to harvest,[35] whereas the levels of anthocyanins in berries increase significantly with ripening and hydroxycinnamate levels decrease.[36] In the present study, we have investigated the impact of both cultivar and ripening on the polyphenol content and profile of dates.

## MATERIALS AND METHODS

**Chemicals.** Chemicals included hydrochloric acid, methanol, sodium fluoride, diethyl ether, Amberlite XAD-16 (20−60 mesh), Fehling's reagents, Folin−Ciocalteu reagent, sodium carbonate, sodium acetate trihydrate, acetic acid, 2,4,6-tripyridyl-*s*-triazine (TPTZ), ferric chloride hexahydrate, ascorbic acid, iron sulfate heptahydrate. Phenolic compounds standards included gallic, proto-

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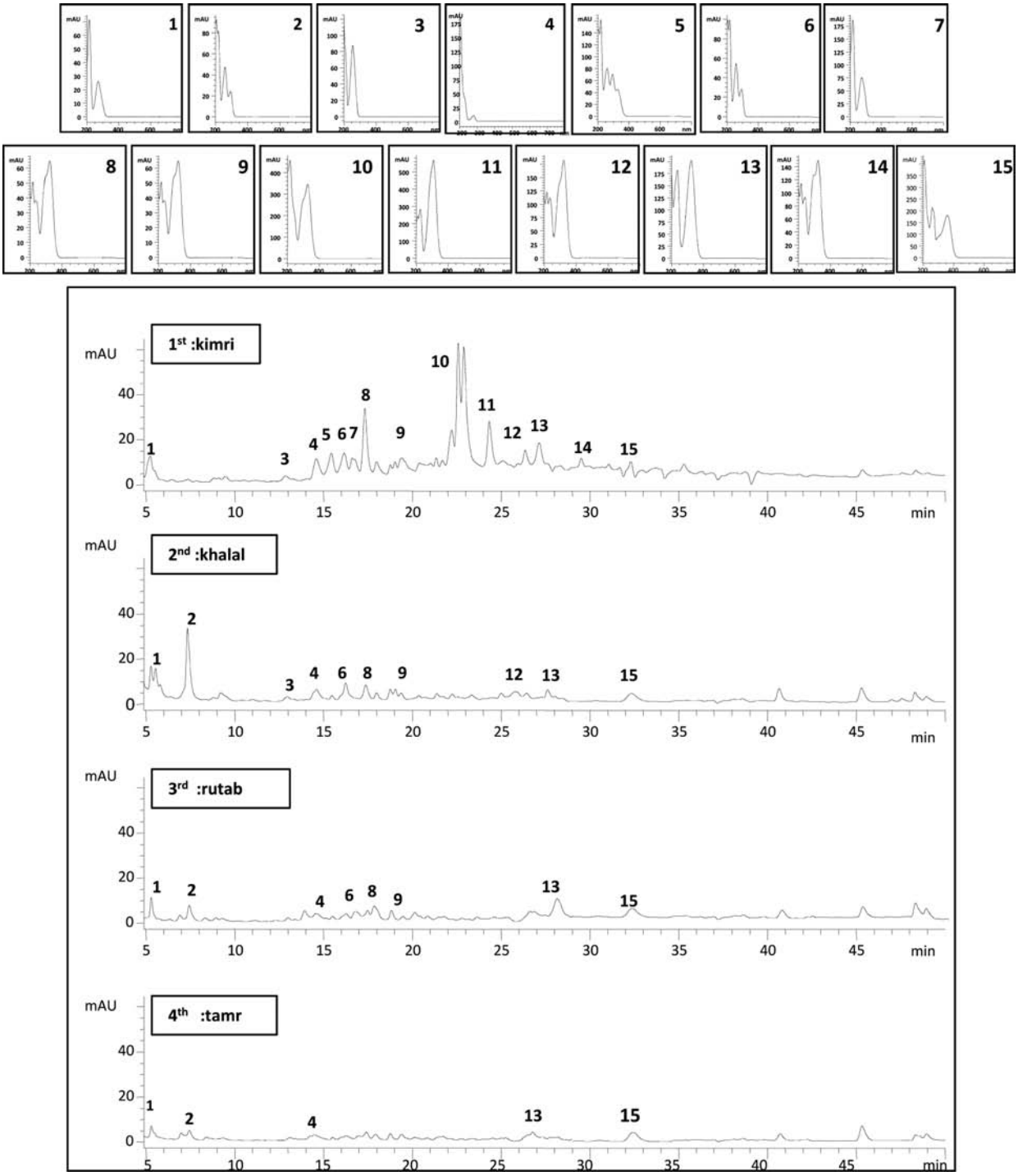


**Figure 1.** Typical HPLC chromatogram of the analysis of hydroxybenzoic acids, hydroxycinnamic acids, and flavonoid glycosides present in dates at their main ripening stages (kimri, khalal, rutab, and tamr). Data were collected at 254 nm. (1) Gallic acid; (2) protocatechuic acid; (3) hydroxybenzoic acid; (4) catechin; (5) vanillic acid; (6) isovanillic acid; (7) syringic acid; (8) chlorogenic acid; (9) caffeic acid; (10) caffeic acid derivatives; (11) caffeic acid isomers; (12) *p*-coumaric acid; (13) ferulic acid; (14) sinapic acid; (15) isoferulic acid; (16) rutin.

catechuic, *p*-hydroxybenzoic, vanillic, isovanillic, caffeic, syringic, *p*-coumaric, ferulic, isoferulic, and sinapic acids, (+)-catechin, (−)-epicatechin, rutin, myricetin, quercetin, luteolin, naringenin, kaempferol, isorhamnetin, apigenin, and petunidin. All materials were purchased from (Sigma Aldrich, UK).

**Dates.** Three different varieties of fresh date (Ajwa, Barni, and Khalas) were harvested at the four main ripening stages (kimri, khalal, rutab, and tamr) (Al-Gudaibi, Al-Qassim, Saudi Arabia). The first stage of maturation was harvested in May, with the remaining maturation stages collected between June and September 2010. The stage of ripening was determined according to the time of harvest, where fruit color, size, and texture all vary (kimri, small, green and hard; khalal, large, yellow or red, and crunchy; rutab, brown and soft; tamr, dark brown and dry). All fruits were rapidly cooled after harvest and transported to the United Kingdom at 4 °C. Samples were stored at −20 °C prior to analysis.

**Extraction of Polyphenols.** Dates (100 g) were pitted, weighed, and homogenized in 300 mL of methanol/water (4:1) containing 10% (v/v) of NaF (1 M) to inhibit polyphenol oxidase.[3] Extracts were stirred for 2 h at 20 °C and then filtered through a sintered funnel

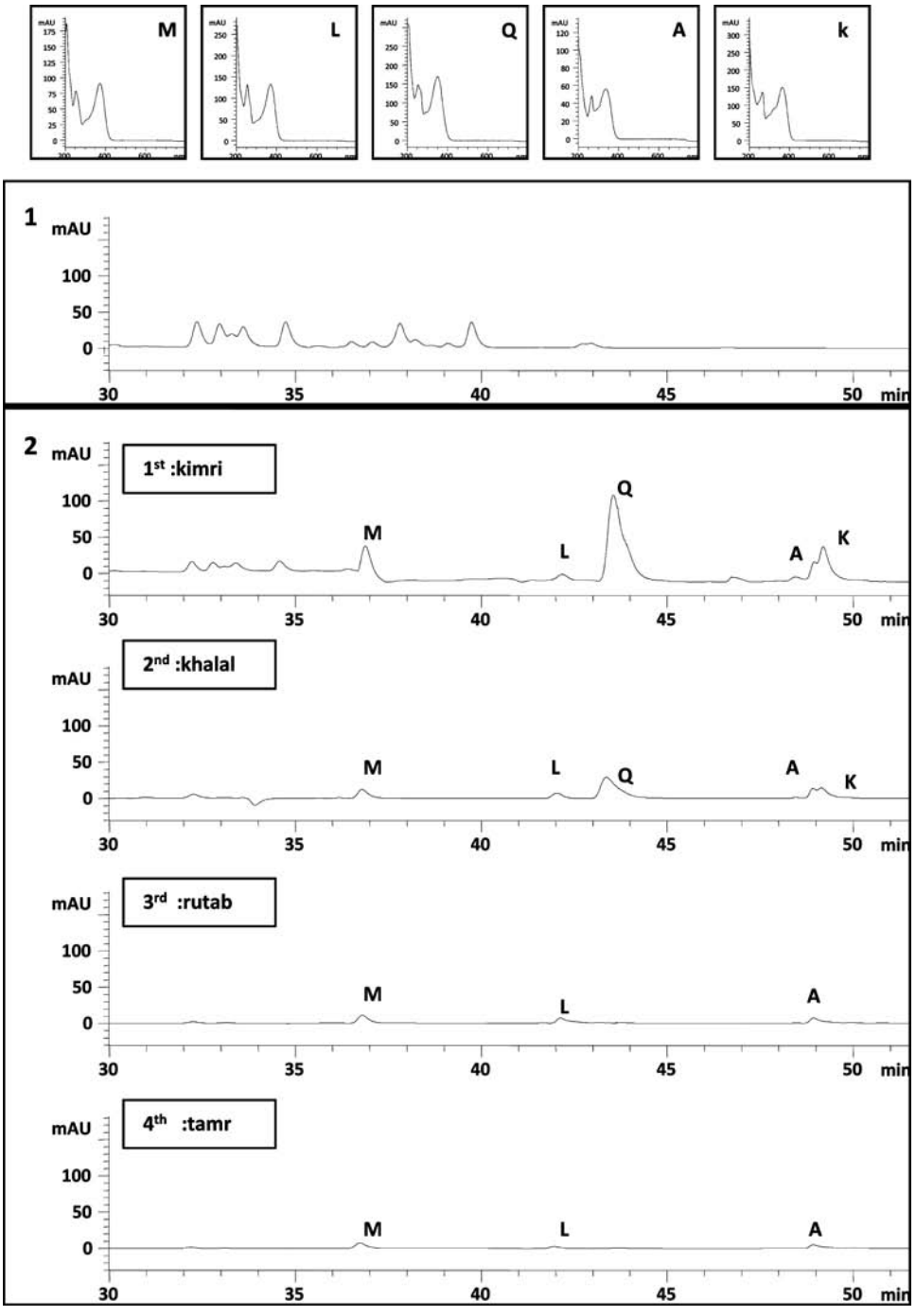


**Figure 2.** Typical HPLC chromatogram of the analysis of flavonoid aglycones in dates (kimri, khalal, rutab, and tamr) following acid hydrolysis of date methanolic extracts (5 M HCl for 1 h). All chromatograms were collected at 356 nm. (1) Example HPLC chromatogram of a date extract prior to hydrolysis; (2) example HPLC chromatograms of date extracts (kimri, khalal, rutab, and tamr) after acid hydrolysis. M, myricetin; L, luteolin; Q, quercetin; A, apigenin; K, kaempferol.

(porosity = 1) (Sigma Aldrich, UK) to remove solids. Aqueous methanol extracts were concentrated under vacuum using a rotator vacuum evaporator (ORME Scientific Ltd.), and the remaining residue was diluted in acidified water (pH 2; HCl) to a concentration of 300 mg/mL. Sugars were removed by adding 3 g of the extract to an XAD-16 resin-packed column (50 cm L × 2.2 cm diameter).[37] Elution of sugars was achieved by addition of 100 mL of acidified water (pH 2; HCl), followed by 300 mL of distilled water at a constant rate (0.5 mL/min) using a diaphragm-metering pump (STEPDOS, Scientific Laboratory Suppliers). The Fehling test was carried out to ascertain the presence of sugars in the water extracts.[38] Following removal of all sugars, the elution of phenolics was achieved by addition of 400 mL of methanol at a flow rate of 0.5 mL/min. The eluent collected was concentrated using a rotator vacuum evaporator at 40 °C and the concentrated extract stored at −80 °C until analysis. From 3 g of date fruit approximately 300 mg of dried material remained following extraction, which contained polyphenols and other small molecular weight components.

**Quantification of Phenolics, Hydroxycinnamates, and Flavonoids.** For quantitative determination of polyphenol levels, acid hydrolysis was performed to cleave flavonoid glycosides prior to analysis. One hundred microliters of methanol extract was added to 900 μL of 50% (v/v) acidified aqueous methanol (5 M HCl) and incubated for 1 h at 70 °C. Extracts were filtered through a 0.45 μm


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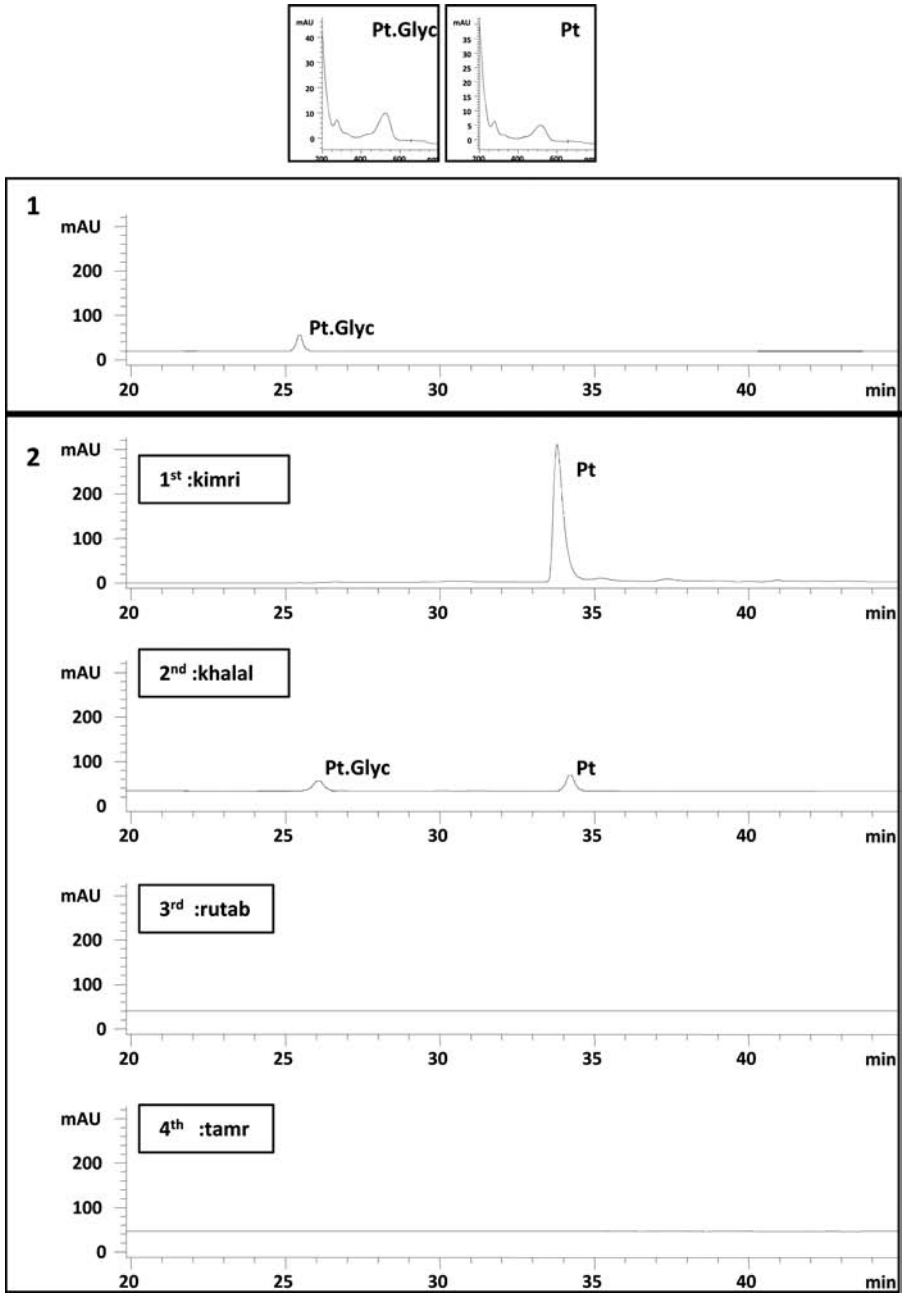


**Figure 3.** Typical HPLC chromatogram of the analysis of anthocyanidins following acid hydrolysis (5 M HCl; 1 h). All chromatograms were collected at 520 nm. (1) Example HPLC chromatogram of a date extract prior to hydrolysis; (2) example HPLC chromatograms of date extracts (kimri, khalal, rutab, and tamr) after acid hydrolysis. Pt Glyc, petunidin glycoside; Pt, petunidin.

acrodisc filter prior to injection (50 $\mu$L) onto the HPLC system. HPLC analyses were performed using an Agilent 1100 series linked to a diode array detector. Date extracts (100 mg mL$^{-1}$) were separated using a C18 Nova Pak column (250 mm × 4.6 mm i.d., 5 $\mu$m particle size), fitted with a guard column C18 NovaPak (Waters Ltd., Elstree, UK). The mobile phase consisted of (A) 5 M hydrochloric acid (0.1%) in 5% aqueous methanol and (B) 5 M hydrochloric acid (0.1%) in aqueous acetonitrile (1:1) and was pumped through the column at 0.7 mL min$^{-1}$. Samples (50 $\mu$L) were injected and separated using the following gradient system (min/% B): 0/5, 5/5, 40/50, 55/100, 59.9/100, and 60/5 for detection of all compounds. The eluent was monitored by photodiode array detection at 254, 280, 320, 370, and 520 nm and spectra of products obtained over the 220−600 nm range. Phenolic compounds were characterized by their retention time and by comparison with known phenolic standards (0−100 $\mu$M; $R > 0.995$). All data were analyzed using ChemStation software.

**LC-ESI-MS/MS Analysis.** Methanol extracts were also analyzed by LC-MS/MS utilizing electrospray ionization (ESI). This consisted of an Agilent 1200 HPLC system equipped with a binary pump, degasser, autosampler, thermostat, column heater, photodiode array detector, and an Agilent 1100 series LC/MSD mass trap spectrometer. Separation of samples was achieved using a Zorbax SB C18 column (2.1 × 100 mm; 1.8 $\mu$m; Agilent, Santa Clara, CA, USA), and HPLC conditions were as follows: injection volume, 5 $\mu$L; column temperature, 25 °C; binary mobile system, (A) 0.1% aqueous formic acid and (B) 0.1% formic acid in acetonitrile; flow rate, 0.2 mL/min. A series of linear gradients were used for separation (min/%B): 0/10, 3/10, 15/40, 40/70, 50/70, and 65/10. Mass spectrometry was performed in the negative ion mode (scan range, $m/z$ 100−800 Da; source temperature, 350 °C). All solvents used were of LC-MS grade.

**Total Phenolics and Antioxidant Capacity.** The total content of phenolic compounds in the column methanol eluent was analyzed

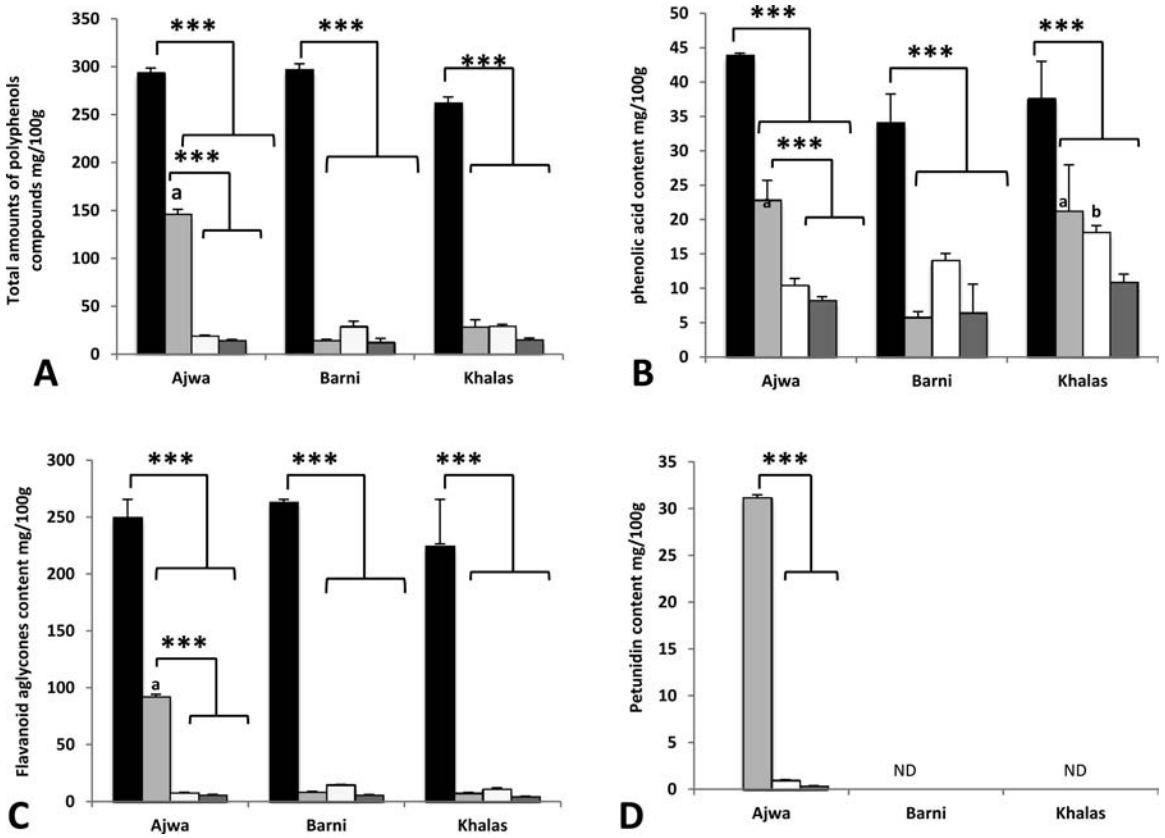


**Figure 4.** Quantification of (poly)phenols in different date cultivars at the four main ripening stages (black column, kimri; light gray column, khalal; white column, rutab; dark gray column, tamr): (A) total polyphenol content; (B) hydroxybenzoic and hydroxycinnamic acids; (C) flavonoids; (D) anthocyanidins. Statistical analysis was performed using a univariate analysis of variance with Tukey's post hoc test. Data are the mean of three independent analysis of each representative cultivar and ripening stage (mean ± SD; $n = 3$). "a" indicates a significant difference between similar ripening stages of different date cultivars; "***" indicates significant differences in levels between ripening stages ($P < 0.001$).

according to the Folin–Ciocalteu test.[39] The antioxidant activity of the column methanol eluent was assessed using the ferric-reducing antioxidant potential (FRAP) assay.[40] Ninety-six-micro-well plates were analyzed using a GENios Pro microplate reader set with an absorbance of 650 nm for total polyphenols and of 600 nm for antioxidant capacity. The total (poly)phenol content was calculated using a calibration curve constructed using gallic acid as a standard, and results were expressed as gallic acid equivalent (GAE) mg/L/100 g FW (mean ± SD; $n = 3$). Antioxidant capacity was calculated using ascorbic acid as standard and expressed as ($\mu$mol)/100 g dry weight (mean ± SD; $n = 3$).

**Statistical Analysis.** A one-way ANOVA was used to assess significant differences in the polyphenol contents between different date varieties. Univariate analysis of variance was used to analyze for significant differences in polyphenol levels between the four main ripening stages of three different cultivars. Tukey's post hoc test was used to test for individual variations in variety and ripening stages. A linear regression test was used to determine the correlation between antioxidant capacity and total polyphenols. Data were expressed as the mean of three individual analyses of each cultivar and ripening stage performed in triplicate (mean ± SD; $n = 3$). Significant differences between different varieties of date fruits was represented by "*", $p < 0.05$, and "***", $p < 0.001$, whereas the presence of significant differences between the same ripening stages within the date fruit varieties was represented by "a". All statistics were performed using SPSS 18.0.

## RESULTS

All cultivars contained the hydroxybenzoic acids gallic acid, protocatechuic acid, hydroxybenzoic acid, vanillic acid, isovanillic acid, and syringic acid and the hydroxycinnamic acids chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, and isoferulic acid as well asthe flavanol catechin (Figure 1). A number of flavonoid glycosides were also identified (Figure 2), which, following acid hydrolysis, gave rise to the flavonoid aglycones quercetin, naringenin, myricetin, apigenin, kaempferol, and luteolin (Figure 2). The Ajwa variety was the only cultivar that contained significant quantities of anthocyanidins, with petunidin detected following hydrolysis, particularly in the kimri stage (Figure 3), and was confirmed using LC-MS/MS. The total measurable levels of (poly)-phenols were observed to fall significantly with increasing degree of ripeness (Figure 4). Specifically, the kimri stage contained significantly higher levels of total polyphenols (Figure 4A), total phenolic acids (Figure 4B), and total flavonoids (Figure 4C) ($P < 0.001$) compared to all other ripening stages. With regard to variety, Ajwa contained the highest amounts of polyphenols compared to the others ($P < 0.001$), which was predominately due to its significantly higher levels of polyphenols present at the khalal stage (Figure 4).

The total phenolic data, as assessed using the Folin–Ciocalteu assay, paralleled measures of date (poly)phenols, indicating that the kimri stage possessed significantly higher total polyphenol levels than the other ripening stages ($P < 0.001$) (Figure 5A) and that the levels of phenolics in the khalal stage of the Ajwa date were significantly higher than those in other date varieties at the same ripening state. In line with this, there was also a significant reduction in the antioxidant capacity of fruit extracts with increasing degree of ripeness (Figure 5B),

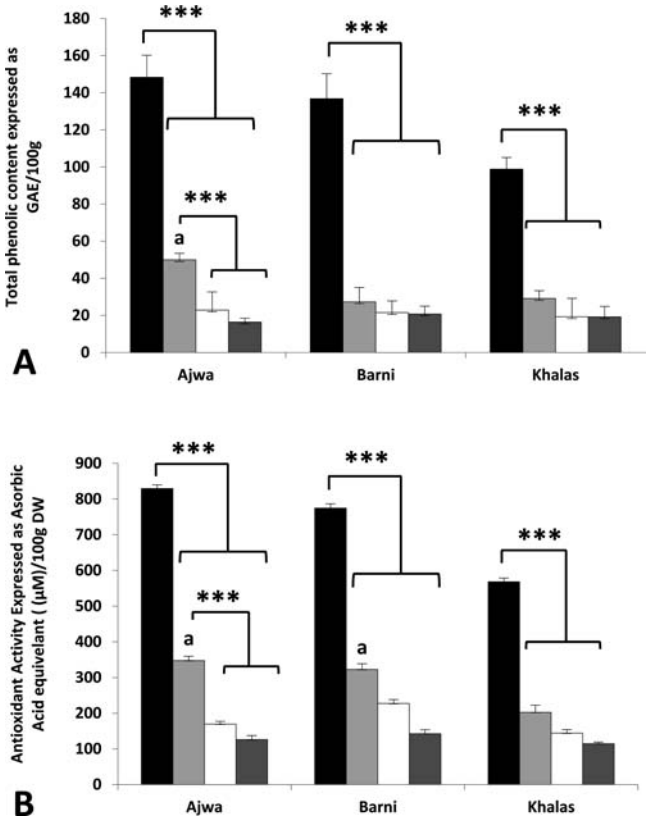


**Figure 5.** Total polyphenol content and antioxidant activity of date cultivars and ripening stages (black column, kimri; light gray column, khalal; white column, rutab; dark gray column, tamr): (A) total phenols determined by using the Folin−Ciocalteu assay; (B) antioxidant activity determined by using the FRAP assay. Statistical analysis was done using a univariate analysis of variance with Tukey's post hoc test. Data are the mean of three independent analyses of each representative cultivar and ripening stage (mean ± SD; $n = 3$). "a" indicates a significant difference between similar ripening stages of different date cultivars; "***" indicates significant differences in levels between ripening stages ($P < 0.001$).

and there was a significant correlation between these two parameters ($R = 67\%$; $P < 0.001$).

## DISCUSSION

Date palm (*P. dactylifera*) fruits are a relatively rich source of polyphenols, containing an array of phenolics, hydroxycinnamates, and flavonoids. In the present study we detail for the first time how the (poly)phenol content of dates alters with ripening and with cultivar. Our data are in agreement with previous studies, indicating that dates contain phenolic and hydroxycinnamic acids[3,4,6] gallic acid, protocatechuic acid, hydroxybenzoic acid, caffeic acid, *p*-coumaric, and ferulic acid[6,18,19] and various flavonoid glycosides.[3,14] The data emanating from this study also agree with previous studies reporting the presence of flavonoid glycosides in dates,[3,4,41] which were observed as aglycones following acid hydrolysis and add to the limited evidence for the presence of anthocyanidins in dates.[6] However, the majority of these previous analyses have been conducted on the tamr ripening stage and to a lesser extent on the khalal stage.[3] We show that dates in all major stages of ripening contain a large profile of polyphenols and that the levels of measured polyphenols decrease as ripening progresses, with the kimri stage containing significantly higher levels than all other stages of ripening. Furthermore, we indicate that the levels of polyphenols in the Ajwa cultivar were significantly higher than those in the other cultivars at the khalal stage, in particular the levels of the anthocyanin petunidin, which was present at levels similar to that measurable in blueberry, a fruit known to be high in this class of flavonoid.[42,43] In addition, the levels of date polyphenols in the earlier stages of ripening are similar to that found in apple[44] but lower than that found in citrus[45] and strawberries[46] and higher than that detected in different cultivars of honey.[47]

In agreement with previous work, our results indicate that higher levels of antioxidant activity are present in date extracts of the kimri and khalal stages relative to the later stages rutab and tamr.[32,34] In comparison with different types of onions, the antioxidant ability and polyphenol content of date palm fruits are considered to be significantly higher, which could be affected with different cultivation practices.[48] A large degree of variability with respect to polyphenol content may exist with respect to variety, geographic location, environmental conditions, and ripening status.[49,50] During maturation, a loss of cell wall structure and accumulation of carbohydrates have been reported.[51] In many fruits, proanthocyanidins that are accumulated prior to ripening are subject to the Bate−Smith reaction,[52] leading to the release of anthocyanins,[53] which in turn increase in concentration with maturation and during postharvest storage.[35,54] In contrast, other polyphenols tend to decrease due to oxidation and enzymatic browning.[55] In our studies, we observed that petunidin, in addition to the other polyphenols measured, reduced in concentration with increasing maturation. The differences observed with regard to date fruit may result from differences in ripening conditions, with dates subjected to high ultraviolet exposure and relatively high temperatures during maturation leading to a dominance of the oxidation reactions in fruit.[2] In comparison, many berries, including blueberry, are cultivated in significantly cooler climates, allowing anthocyanins to accumulate at much higher levels throughout ripening.

Our data reveal for the first time how the polyphenol content in dates varies as a function of cultivar type and maturation. We show that the Ajwa varietal was the richest in polyphenols between the varieties measured and was unique in that it contained significant quantities of anthocyanins. We also report that the khalal stage was the most polyphenol-rich edible stage, although levels of all polyphenols were higher in the kimri stage. Although there are other compounds, such as proanthocyanidins, carotenoids, and glucosinolates, present in dates, which were not assessed in the present study, we believe that our data provide important information regarding changes in polyphenol levels that occur during ripening. Many data have suggested that polyphenol-rich fruits and vegetables may contribute to potential human health benefits.[56−59] We propose that palm date fruit may also contribute to such health benefits through delivering relatively high amounts of polyphenols, especially if novel foods are designed containing dates at early stages of ripening.

## AUTHOR INFORMATION

### Corresponding Author

*Phone: 01183788724. Fax: 01189310080. E-mail: j.p.e.spencer@reading.ac.uk.

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### Notes

The authors declare no competing financial interest.

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