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The Flavonoid Glycosides and Procyanidin Composition of Deglet Noor Dates (*Phoenix dactylifera*)

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The fruits of the date palm (*Phoenix dactylifera*) are consumed throughout the world and are an important part of the diet in the Middle East. Dates at the rutab and tamar maturity and ripening stages contain a wide array of phenolic antioxidants, but little is known about the composition of phenolic compounds in dates at the khalal stage of ripening. In the current study, the flavonoid glycoside and procyanidin compositions of dates of the cultivar Deglet Noor harvested at the khalal stage of maturity were characterized using liquid chromatography−electrospray ionization−tandem mass spectrometry (LC-ESI/MS/MS). Procyanidin oligomers through decamers were identified in extracts of these dates. Higher molecular weight polymers, undecamers through heptadecamers, were also apparent from mass spectra. Thirteen flavonoid glycosides of luteolin, quercetin, and apigenin, 19 when considering isomeric forms, were also identified. Mass spectra indicate that both methylated and sulfated forms of luteolin and quercetin are present as mono-, di-, and triglycosylated conjugates whereas apigenin is present as only the diglycoside. LC-ESI/MS/MS spectra indicate that quercetin and luteolin formed primarily *O*-glycosidic linkages whereas apigenin is present as the *C*-glycoside.

KEYWORDS: Date palm; *Phoenix dactylifera*; procyanidins; flavonoid glycoside; luteolin; quercetin; apigenin; LC-ESI/MS/MS

INTRODUCTION

The fruit of the date palm (*Phoenix dactylifera* L.) is an important component of the diet in the Middle East and North Africa, as well as some parts of Central and South America and Southern Europe. Although the Middle East is the primary date-growing region in the world, dates are also grown in the United States in southern California, Arizona, and Texas. The Deglet Noor is an important date cultivar that makes up about 90% of California's date crop. Dates are an ideal high-energy food rich in carbohydrates, including dietary fiber and minerals, such as calcium, iron, magnesium, phosphorus, potassium, and zinc. Recent studies indicate that the aqueous extracts of dates have potent antioxidant and antimutagenic activity (*1*−*3*). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including *p*-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins (*1*, *4*−*6*). Growing evidence indicates that diets rich in fruits and vegetables afford protection against chronic disease such as cardiovascular disease. It follows that dates may provide a significant source of daily dietary antioxidants in regions that consume significant quantities through traditional diets.

The sweetness, mouth feel, and texture of date fruit is closely related to the maturity and ripeness stage of the date. Date fruit maturity and ripeness are characterized by four different stages: kimri (green), khalal, rutab, and tamar on the basis of color, softness, moisture, and sugar content. Dates with a sugar content above 30.5% or a water content below 66% are regarded as mature and will ripen properly (*7*). In general, most dates are harvested at the rutab and tamar stages of ripeness, when they are brown and have lower moisture and tannin contents and high concentrations of sugars. Once ripened, dates have a short shelf life, which may reflect lower levels of antioxidants. The composition of the antioxidant phenolics in fruit changes dramatically during ripening, and this can affect shelf life (*7*). For example, during the maturation of grapes, levels of hydroxycinnamic tartrates, flavan-3-ols, procyanidins, and their gallates are known to change (*8*). Of particular interest is the decrease in flavan-3-ols, the basic unit of procyanidins, as well as low molecular weight tannins in grape seeds during fruit ripening (*9*) as these compounds demonstrate potent antioxidant activity.

A small quantity of certain date cultivars, such as Barhee, are harvested and consumed at the khalal stage, when they reach

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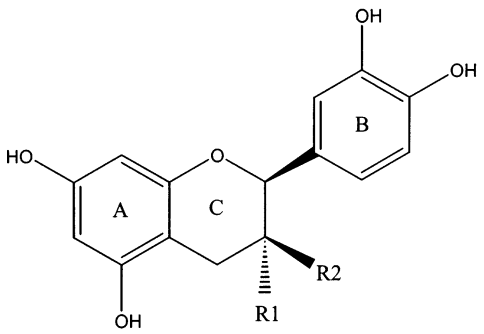
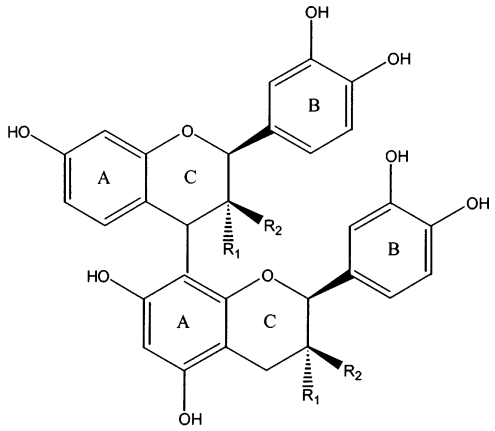
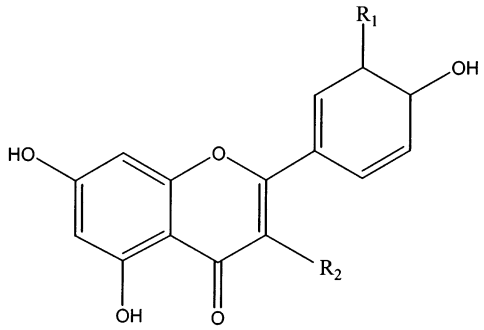


Catechin R_1 = H, R_2= OH
Epicatechin R_1 = OH, R_2=H



Procyanidin (4 β→ 8) Dimers



Quercetin R_1= OH, R_2= OH
Luteolin R_1=OH, R_2= H
Apigenin R_1 =H, R_2=H

Figure 1. Representative structure of catechin, epicatechin procyanidin B type dimers, quercetin, luteolin, and apigenin.

full maturity and are yellow, pink, or red (depending on the cultivar); these cultivars are less astringent at the khalal stage than other cultivars that are only harvested when fully ripe. Although ripe dates contain significant levels of procyanidins or condensed tannins (*4*), little is known about the distribution of procyanidin oligomers among date cultivars or the effects of ripening on procyanidin composition. Additionally, there is limited and incomplete information available on the flavonoids and flavonoid glycoside composition of dates at various stages of ripeness (*1*, *10*). Characterizing the levels and range of these compounds in foods will help establish the role of specific dietary components (e.g., fruits) in promoting health.

Structurally, procyanidins are polymeric flavan-3-ols composed of monomeric units of (+)-catechin or (−)-epicatechin joined through interflavan linkages. The B type procyanidin oligomers (**Figure 1**) comprise the major polymeric flavan-3-ol fraction in cocoa, grapes, and apples (*4*, *11*), whereas the A type has been identified in only a few foods such as peanuts (*12*), cinnamon (*13*), and cranberries (*14*). Oligomeric procyanidins demonstrate antioxidant activity that increases linearly with the number of reactive catechol and/or pyrogallol groups (*15*, *16*). The content and composition of procyanidins in fruits vary considerably with genetic variation (*17*), maturity at harvest (*18*), agronomic conditions (*19*), and postharvest processing conditions (*20*−*22*). Characterizing the type and distribution of procyanidins in foods is important since B type procyanidins demonstrate the ability to protect endothelial cells from peroxynitrite damage (*23*) and are linked to the inhibition of the synthesis of endothelin-1, a vascular hormone known to play a key role in many of the processes underlying vascular dysfunction and the development of atherosclerosis (*24*).

In this study, we investigated the full range of procyanidin oligomers and flavonol glycosides in Deglet Noor dates harvested at the khalal stage of maturity by liquid chromatography−electrospray ionization/tandem mass spectrometry (LC-ESI/MS/MS).

MATERIALS AND METHODS

Chemicals. Fluorescein was obtained from Sigma (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade acetone, methylene chloride, methanol and acetic acid were obtained from Fisher Scientific (Houston, TX). Reagent-grade, bacteria-free water was generated by a Barnstead *E*-pure 4-module deionization system (Dubuque, IA).

Dates. The dates used for this study were harvested at the khalal stage from five bunches each per date palm tree at the date orchard at the U.S. Department of Agriculture—Agricultural Research Service National Clonal Germplasm Repository for Citrus and Dates, Thermal, California, and shipped via overnight delivery service to the University of California at Davis. Three replicates of 20 dates each were selected (for freedom from defects and color uniformity) from the dates received and were kept at 0 °C and 90% relative humidity until used for the analysis. The dates were pitted so that only the edible portion of the fruit was analyzed.

Extraction of Procyanidins. Procyanidins were extracted from pitted dates using a solvent composed of acetone, water, and acetic acid (70:29.5:0.5, v/v/v) as previously described (*22*). Briefly, a 10 g sample was homogenized for 1 min at maximum speed in a Waring blender in the presence of 100 mL of extraction solvent. Procatechuic acid was

added to the extraction mixture as a recovery standard as it did not coelute with other polyphenolics and demonstrated consistent recoveries of ~78%. Extractions were performed using Sephadex LH-20 (Amersham Biosciences), which was equilibrated overnight in water prior to being packed into chromatography columns (25 cm × 2.5 cm). Samples were loaded onto columns at a rate of 0.5 mL/min. The columns were rinsed with 500 mL of Nanopure water at a rate of 0.5 mL/min. Polyphenols were eluted from the column using a solution (100 mL) of acetone, water, and acetic acid (70:29.5:0.5, v/v/v). Acetone was removed by rotary evaporation, and the aqueous solution was freeze-dried. Dates were extracted in triplicate. The freeze-dried material was dissolved in 500 μL of extracting solvent and filtered through a 0.45 μm PTFE, HPLC membrane filter prior to LC-MS analysis. The injection volume was 20 uL.

Extraction of Flavonoid Glycosides. The pitted fruit (5 g) was homogenized in a Polytron (2 min on ice) with 10 mL of extraction solution (4 mM NaF in methanol to inactivate polyphenoloxidases and prevent phenolic degradation due to browning). Homogenates were kept in ice until centrifuged (15 min, 2−5 °C at 16000*g*). The supernatant was recovered and filtered through 0.45 mm filters (Osmonics/MSI Cameo Nylon Filters, Fisher Scientific, Los Angeles, CA) and directly analyzed by HPLC after a period not exceeding 24 h (*17*).

Procyanidin Analysis by LC-ESI/MS. The procyanidin extract was separated using normal phase HPLC on a 250 mm × 2.0 mm i.d., 5 μm Phenomenex (Torrance, CA) Luna silica column monitoring 280 nm (*22*). The HPLC system (Shimadzu Scientific, Columbia, MD) was equipped with a SIL-10A autoinjector, binary LC 10AD pumps, and a SPD-10A UV/vis detector. The binary mobile phase consisted of methylene chloride, methanol, water, and acetic acid (82:14:2:2, v/v/v/v) (solvent A) and methanol, water, and acetic acid (96:2:2 v/v/v) (solvent B). Separations were performed by a series of linear gradients using a flow rate of 0.2 mL/min as follows: 0−17.6% B, 0−30 min; 17.6−30.7% B, 30−45 min; and 30.7−87.8% B, 45−50 min. In all cases, the columns were reequilibrated between injections with the equivalent of 10 mL of the initial mobile phase. The HPLC system was interfaced through an ESI to a ZSPRAY Micromass Quattro LC (Beverly, MA). For LC-MS, a capillary voltage of −3.2 kV and a cone voltage of −30 V were applied using a source temperature of 145 °C and a desolvation gas temperature of 300 °C. The nebulizer gas and desolvation gas were 65 and 450 L/h. Ammonium acetate (10 mM) was introduced at 0.03 mL/min via a tee in the eluant stream as an ionization reagent. MS data were collected from *m*/*z* 100 to 3500 and processed using MassLynx v 3.5. Identification of the procyanidin oligomers in Deglet Noor dates was made by filtering the total ion chromatogram (TIC) at *m*/*z* values corresponding to singly and multiply charged ions produced from each oligomer and by comparing LC retention times with a procyanidin standard extracted from cocoa according to previously published methods (*22*).

Flavonoid Glycoside Analysis by LC-ESI/MS/MS. Methanolic extracts were used for the analysis of the flavonoid glycosides using the same LC-ESI/MS/MS system described above. Extracts were separated using reversed phase HPLC on a 250 mm × 2.0 mm i.d., 5 μm Phenomenex Prodigy, ODS column monitoring at 370 nm. The mobile phase consisted of 5% formic acid (solvent A) in water and 5% (v/v) formic acid in methanol (solvent B). Separations were effected by a series of linear gradients using a flow rate of 0.2 mL/min as follows: 10−35% B, 0−40 min; 35−80% B, 40−60 min. LC-ESI/MS/MS conditions were optimized using the flavonol glycoside rutin (quercetin 3-rhamnosyl-glucoside). A capillary voltage of 3.2 kV and a cone voltage of 35 V were applied using a source temperature of 145 °C and a desolvation gas temperature of 300 °C. TICs were recorded over a mass range of *m*/*z* 100−1000 using a scan duration of 1 amu. Peaks giving *m*/*z* values corresponding to possible flavonoid glycosides in TICs were further investigated by LC-ESI/MS/MS. Argon gas was used as a collision gas, and the potential change defining the collision energy was optimized in the range of 30−60 V as appropriate for optimizing the production of both parent and daughter ions.

RESULTS AND DISCUSSION

Procyanidin Analysis. At the khalal stage of maturity, the Delget Noor dates appear redish-brown in color and have a firm texture. Negative mode LC-ESI/MS conditions were optimized for the analysis of procyanidins using a composite procyanidin standard extracted from cocoa (*11*). To assist the ionization of procyanidins in normal phase solvents, a tertiary pump was added in-line for the postcolumn addition of ammonium acetate prior to the ESI interface. LC-ESI/MS is an ideal analytical method for procyanidin analysis (*25*−*27*) as it can distinguish between monomeric units and between A type and B type procyanidin oligomers (*4*, *26*). The procyanidin composition of Deglet Noor dates was determined by comparing retention times of date drupe procyanidins with the procyanidin standard (**Figure 2**) and by extracting ions corresponding to each oligomeric group from the TIC and generating reconstructed ion chromatograms (RICs; **Figure 3**). RICs were obtained by filtering data for *m*/*z* ratios corresponding to singly charged $[M - H]^-$ and multiply charged $[M - nH]^{n-}$ ($n \geq 2$) ions correlating to each procyanidin oligomer.

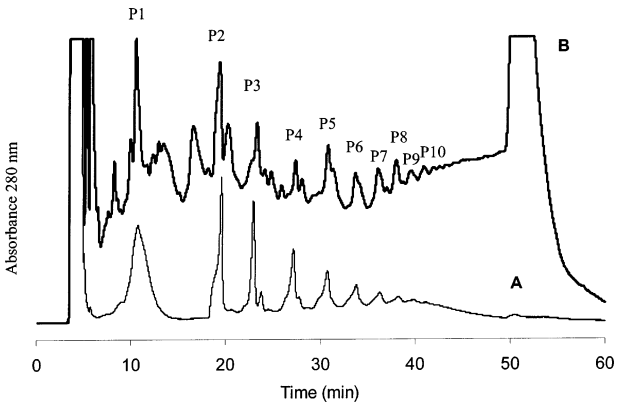


Figure 2. HPLC chromatogram of (**A**) cocoa and (**B**) Deglet Noor extract monitoring at 280 nm. P1–P10 represent increasing oligomers.

HPLC chromatograms and mass spectra indicate that the Deglet Noor dates are homogeneous in B type oligomers and contain oligomers through decamers (**Figure 2**). In a previous study of Delget Noor dates, Gu et al. (*4*) reported B type procyanidin oligomers through pentamers. The discrepancy in the range of procyandins identified in this cultivar of dates likely arises from its stage of maturity. Our studies examine dates in the khalal stage of maturity, whereas Gu et al. (*4*) examined the tamar stage of maturity. Widening peaks and an upward baseline shift in the chromatogram were observed in all UV chromatograms of date drupe extracts. This phenomenon was also reported earlier (*4*) and is thought to arise from an increase in the number of isomers associated with increasing degrees of polymerization (DP). Waterhouse et al. (*28*) demonstrated that in grapes, this large unresolved peak corresponds to a mixture of high molecular mass procyanidin polymers. In other studies, the area of this peak has been used to indiscriminately quantify higher mass polymers (*25*).

RICs demonstrate the presence of procyanidin oligomers through decamers (**Figure 3**). The $[M - 2H]^{2-}$ ions of oligomers > pentamers formed more readily than the $[M - H]^-$ ions. Similar reports have been noted in previous studies employing LC-ESI/MS for the analysis of procyanidin oligomers (*29*, *30*). Accordingly, the $[M - 2H]^{2-}$ ions were used to create the RIC of pentamers through decamers (**Figure 3F**−**J**). The charge state of the individual procyanidin oligomers was verified by monitoring the distance between isotope peaks. For example, the distance between an isotopic peak for a $[M - H]^-$ ion is expected to be one mass unit, while it would be 0.5 units for $[M - 2H]^{2-}$ and 0.3 units for $[M - 3H]^{3-}$. Mass spectra of peaks corresponding to the trimers, hexamers, and nonamers, *m*/*z* 865, 864, and 863.6, respectively, are given in **Figure 4**.

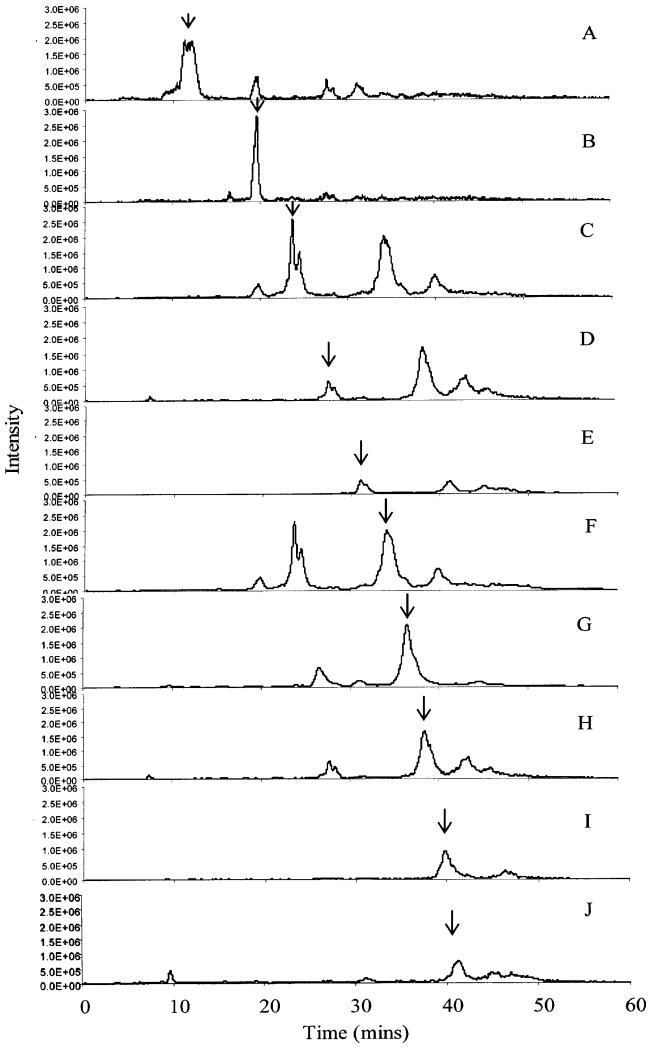


Figure 3. RICs of procyanidin oligomers through decamers in Deglet Noor date extracts. RICs were generated by filtering the most abundant ions corresponding to (**A**) monomers, (**B**) dimers, (**C**) trimers, (**D**) tetramers, (**E**) pentamers, (**F**) hexamers, (**G**) hepatmers, (**H**) octamers, (**I**) nonamers, and (**J**) decamers.

The isotope peak distribution for each of these *m*/*z* values is embedded in this figure, demonstrating the charge state of each peak.

An estimation of the range of procyanidin polymers (DP > 10) eluting at the end of the chromatographic run was obtained by generating a mass spectrum of the accumulated mass scans from 45 to 60 min (**Figure 5**). Multiply charged ions corresponding to the $[M - 2H]^{2-}$, $[M - 3H]^{3-}$, and $[M - 4H]^{4-}$ were observed for undecamers through heptadecamers (**Table 1**), indicating that these polymers likely exist in dates but are unresolved due to the increasing number of isomeric forms of the higher oligomers. Again, isotopic patterns were used to verify charge states. Although this approach is a good estimate of the range of procyanidin polymers present in a food, further separations are required for absolute identity of procyanidin polymers. To date, there are no analytical HPLC methods capable of resolving higher mass procyanidin polymers.

Flavonoid Glycosides Analysis. Flavonoid glycosides were identified by reverse phase LC-ESI/MS/MS in positive ion mode. The HPLC chromatogram (**Figure 6**) demonstrates the presence of 19 peaks absorbing at 370 nm, which are possible conjugates arising from either quercetin, luteolin, and apigenin (**Figure 1**) based upon calculated masses. Previously published

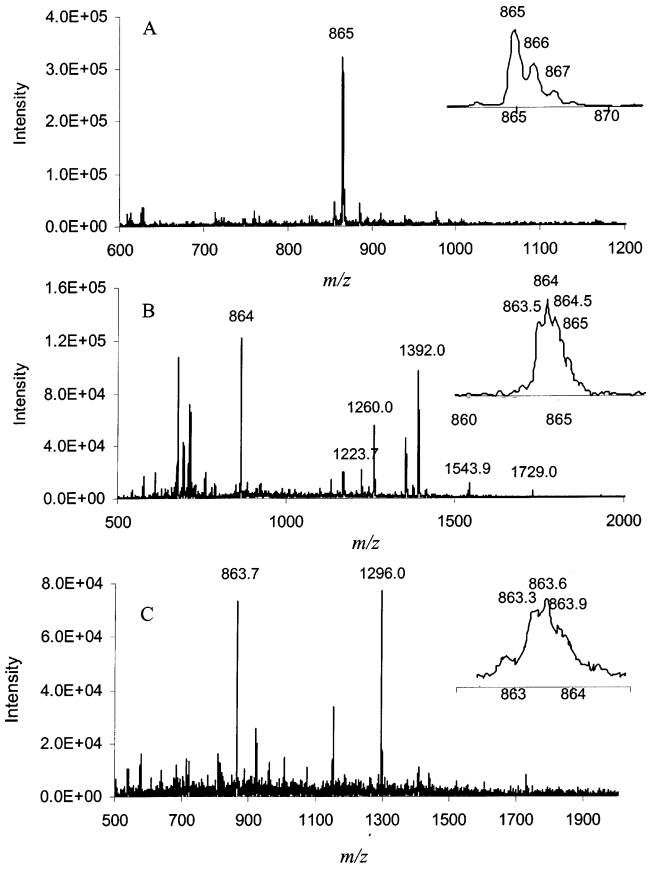


Figure 4. Mass spectra of (**A**) trimers, (**B**) hexamers, and (**C**) nonamers.

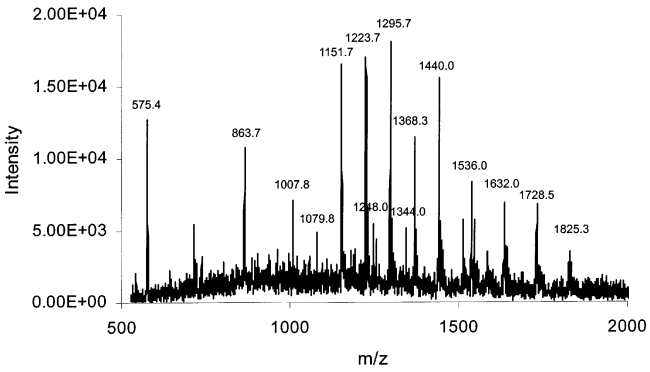


Figure 5. Accumulated mass spectra of procyanidin polymers from 45 to 60 min.

work (*10*) demonstrates that luteolin and chrysoeriol (luteolin 3′-methyl ether) glycosides present in dates are linked at position 7 of the flavone molecule, the most common linkage for these flavone glycosides. The UV spectrum of the compounds present in dates in this study agrees with this kind of substitution; therefore, we tentatively suggest that the luteolin and chrysoeriol (methyl luteolin) glycosides characterized in the present work are 7-glycosides. In the case of the flavonol glycosides of both quercetin and isorhamnetin (quercetin 3′-methyl ether), the UV spectrum indicates that the sugar residues are linked to the hydroxyl at the 3-position of the flavonol molecule, as the UV maximum for band I is always below 355 nm, which is indicative of substitution of the hydroxyl at the 3-position. Tomas-Lorente and Ferrerres (*10*) also described nonsulfated and sulfated flavonoid glycoside conjugates of luteolin, quercetin, chrysoeriol, and isorhamnetin. The 19 peaks corresponding to possible conjugates were further identified by LC-ESI/MS/MS as described below.

Table 1. Identification of Flavonol Glycosides (Luteolin, Methyl Luteolin, Quercetin, and Methyl Quercetin) in Delget Noor Dates

peak	compound	$[M + H^+]^+$ (*m*/*z*)	major fragments (*m*/*z*)
1	rhamnosyl dihexosyl quercetin sulfate	853	773 ($[M + H]^+$-Sul), 627 ($[M + H]^3$-Rham-Sul), 465 ($[M + H]^3$-Rham-Glc-Sul), 303 ($[M + H]^+$-Rham-Glc-Glc-Sul)
2	rhamnosyl dihexosyl methyl quercetin	787	641 ($[M + H]^+$-Rham), 479 ($[M + H]^+$-Rham-Glc), 317 ($[M + H]^+$-Rham-Glc-Glc)
3	apigenin di-C-hexoside	595	557, 559, 541, 523, 457, 427, 355, 325 (refer **Figure 7**)
4	rhamnosyl dihexosyl luteolin	757	611 ($[M + H]^+$-rham), 449 ($[M + H]^+$-Glc-Rham), 287 ($[M + H]^+$-Rham-Glc-Glc)
5	rhamnosyl dihexosyl luteolin	757	611 ($[M + H]^+$-Rham, 449 ($[M + H]^+$-Glc-Rham, 287 ($[M + H]^+$-Rham-Glc-Glc)
6	dihexosyl luteolin sulfate	691	611 ($[M + H]^+$-Sul), 529 ($[M + H]^+$-Glc), 287 ($[M + H]^+$-Glc-Glc-Sul)
7	rhamnosyl dihexosyl methyl luteolin	771	625 ($[M + H]^+$-Rham), 608 ($[M + H]^+$-Glc), 301 ($[M + H]^+$-Rham-Glc-Glc)
8	rhamnosyl dihexosyl luteolin	757	611 ($[M + H]^+$-Rham), 449 ($[M + H]^+$-Glc-Rham), 287 ($[M + H]^+$-Rham-Glc-Glc)
9	dihexosyl luteolin sulfate	691	611 ($[M + H]^+$-Sul), 529 ($[M + H]^+$-Glc), 287 ($[M + H]^+$-Glc-Glc-Sul)
10	rhamnosyl hexosyl luteolin	595	449 ($[M + H]^+$-Rham), 287 ($[M + H]^+$-Glc-Rham)
11	rhamnosyl hexosyl quercetin	611	465 ($[M + H]^+$-Rham), 303 ($[M + H]^+$-Glc-Rham)
12	rhamnosyl hexosyl luteolin	595	449 ($[M + H]^+$-Rham), 287 ($[M + H]^+$-Glc-Rham)
13	dihexosyl quercetin	627	303 ($[M + H]^+$-Glc-Glc)
14	rhamnosyl hexosyl methyl quercetin	625	479 ($[M + H]^+$-Rham), 317 ($[M + H]^+$-Glc-Rham)
15	rhamnosyl hexosyl methyl luteolin	609	463 ($[M + H]^+$-Rham), 301 ($[M + H]^+$-Glc-Rham)
16	rhamnosyl hexosyl methyl luteolin	609	463 ($[M + H]^+$-Rham), 301 ($[M + H]^+$-Glc-Rham)
17	hexosyl luteolin sulfate	529	449 ($[M + H]^+$-Sul), 287 ($[M + H]^+$-Glc-Sul)
18	hexosyl methyl luteolin sulfate	543	463 ($[M + H]^+$-Sul), 301 ($[M + H]^+$-Glc-Sul)
19	hexosyl methyl luteolin sulfate	543	463 ($[M + H]^+$-Sul), 301 ($[M + H]^+$-Glc-Sul)

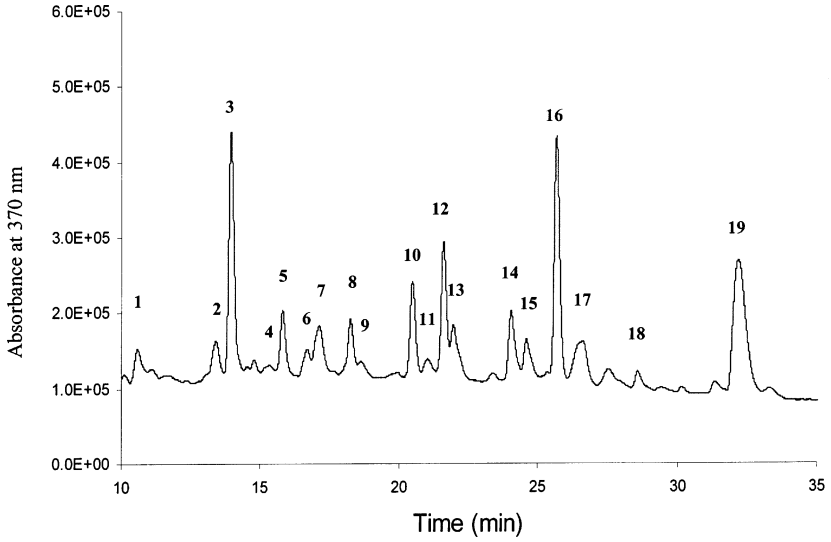


Figure 6. HPLC chromatogram of Deglet Noor date extracts monitored at 370 nm.

Identification of Luteolin Glycosides. Eight luteolin glycosides were identified using precursor ion scanning of *m*/*z* 287 for the $[M + H]^+$ corresponding to the aglycone of luteolin in positive ion mode (**Figure 7A**). Peaks 4, 5, and 8 of the UV chromatogram (**Figure 6**) were identified as rhamnosyl and dihexosyl luteolin isomers (**Figure 7B**; peaks 1a−c). LC-ESI/MS/MS spectra demonstrate major fragment ions at *m*/*z* 611, 449, and 287 corresponding to the loss of a rhamnose unit and hexosyl unit and another hexose residue to give the aglycone, respectively. Peaks 6 and 9 of the UVchromatogram (**Figure 6**) gave rise to two sulfated forms of dihexosyl luteolin isomers (**Figure 7C**; peaks 2a,b). LC-ESI/MS/MS spectra show major fragment ions at *m*/*z* 611, 529, and 287 corresponding to the loss of sulfate, hexose, and the second hexose to give the aglycone, respectively. Peaks 10 and 12 (**Figure 6**) were identified as rhamnosyl hexosyl luteolin isomer (**Figure 7D**; peaks 3a,b). LC-ESI/MS/MS spectra demonstrate major fragment ions at *m*/*z* 449 and 287, which correspond to the loss of the rhamnose unit and then the hexose residue to give the aglycone. Peak 17 (**Figure 6**) was characterized as hexosyl luteolin sulfate (**Figure 7E**; peak 4) yielding fragment ions at *m*/*z* 449 and 287, accounting for the loss of the sulfate residue and the hexose residue, respectively. A description of all luteolin glycosides and corresponding fragment ions obtained by tandem MS is given in **Table 1**.

Identification of Methyl Luteolin Glycosides. The same approach of using precursor ion scanning monitoring *m*/*z* 301 for the aglycone of methyl luteolin in conjunction with MS/MS was used to identify five methyl luteolin glycosides. Peak 7 of the TIC (**Figure 6**) was identified as rhamnosyl dihexosyl methyl luteolin, peaks 15 and 16 were identified as rhamnosyl hexosyl methyl luteolin, and peaks 18 and 19 corresponded to two isomers of sulfated hexosyl luteolin methyl luteolin. A description of all methyl luteolin glycosides and corresponding fragment ions obtained by LC-ESI/MS/MS is given in **Table 1**.

Identification of Quercetin Glycosides. Three quercetin-related derivatives were characterized using precursor ion scanning of *m*/*z* 303 for the aglycone of quercetin in conjunction with LC-ESI/MS/MS. Briefly, peaks 1, 11, and 13 of the UV chromatogram (**Figure 6**) were identified as a rhamnosyl dihexosyl quercetin sulfate, rhamnosyl hexosyl quercetin, and dihexosyl quercetin, respectively (**Table 1**).

Identification of Methyl Quercetin Glycosides. Several isorhamnetin derivatives were also characterized using precursor ion scanning of *m*/*z* 317 in conjunction with LC-ESI/MS/MS.

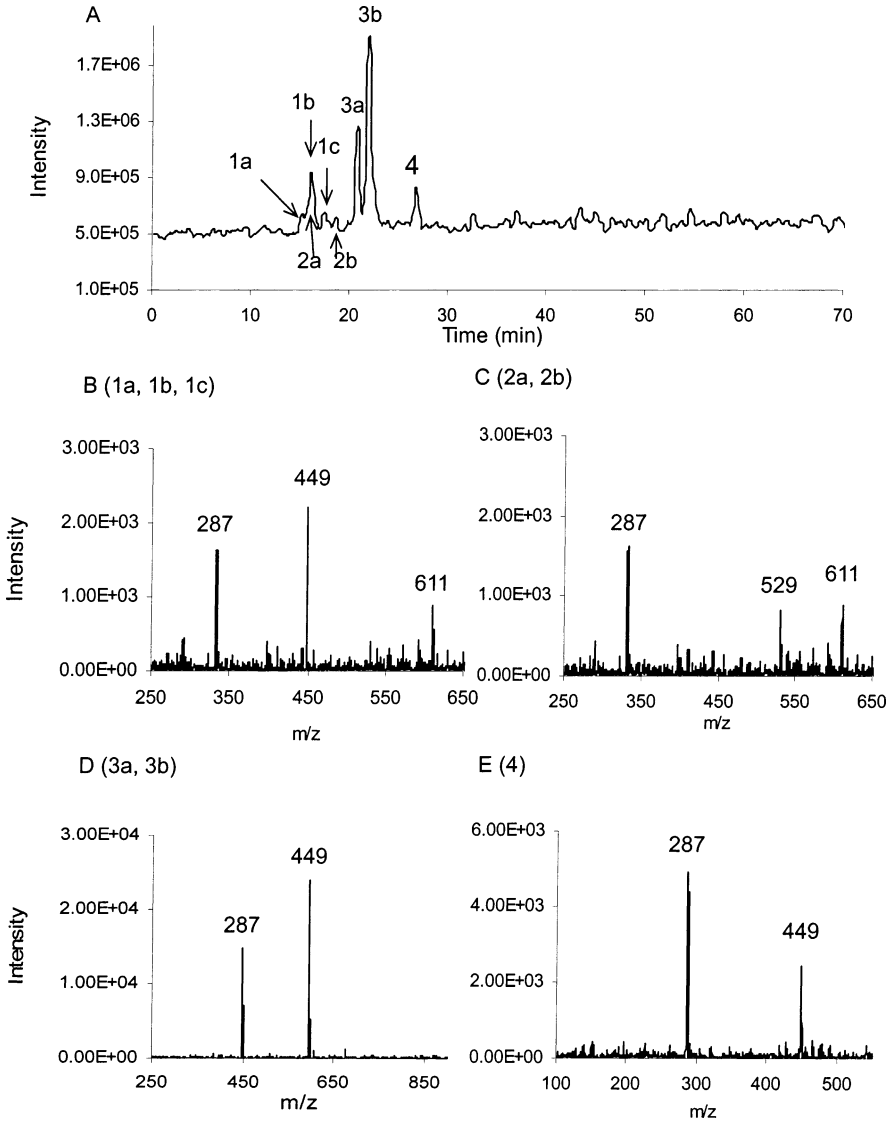


Figure 7. (**A**) Precursor ion scan of *m*/*z* 287 ([M + H]$^+$ for luteolin) and CID-MS/MS spectra of peaks **1**–**4**; (**B**) peaks 1a–c, rhamnosyl dihexosyl luteolin; (**C**) peaks 2a,b, dihexosyl luteolin sulfate; (**D**) peaks 3a,b, rhamnosyl hexosyl luteolin; and (**E**) peak 4, hexosyl luteolin sulfate.

Peak 2 (**Figure 6**) was identified as a rhamnosyl dihexosyl methyl quercetin as it gave a [M + H]$^+$ at *m*/*z* 786 and yielded fragment ions at *m*/*z* 641, 479, and 317 corresponding to the loss of respective glycoside units (rhamnose, hexose, and hexose residues). Peak 14 (**Figure 6**) was identified as a rhamnosyl hexosyl isorhamnetin. A description of these methyl quercetin glycosides and corresponding fragment ions is given in **Table 1**.

Identification of Apigenin Di-*C*-glycoside. Peak 3 (**Figure 6**) was identified as apigenin di-*C*-hexoside by LC-ESI/MS/MS. UV spectra of this peak demonstrates λ max at 267 and 338 nm, which are characteristic of apigenin. LC-ESI/MS/MS spectra of this peak suggest that it is a *C*-glycoside instead of *O*-glycoside as no aglycone was observed. Fragmentation of *O*-glycosides results in the loss of the glycosyl units and produces a predominate fragment ion corresponding to the aglycone. In contrast, *C*-glycosides produce ions that correspond to the fragmentation of the *C*-glycoside unit itself (*31*). The precursor ion and major fragments ions corresponding to peak 3 indicate that this peak is apigenin di-*C*-hexoside (vicenin-2). LC-ESI/MS/MS of ions corresponding to this peak result in fragment ions at *m*/*z* 457, 427, 355, and 325; these fragment ions have been described in previous studies (*31*−*33*). In addition, the short retention time of peak 3 as compared to the *O*-glycosides also suggests its *C*-glycoside nature.

Mature (khalal stage) Deglet Noor dates contain 13 flavonoid glycosides of luteolin, quercetin, and apigenin, 19 when considering isomeric forms. Mass spectra indicate that both methylated and sulfated forms of luteolin and quercetin are present as *O*-glycosides combined with up to three sugar residues, whereas apigenin is present as the di-*C*-hexoside (most likely the di-*C*-glucoside also known as vicenin-2). LC-ESI/MS/MS spectra indicate that quercetin and luteolin form primarily *O*-glycosidic linkages, whereas apigenin is present as the *C*-glycoside. The combination with sulfate residues is also observed in some of the flavonol glycosides but not in the procyanidins. This is a rather uncommon feature for food flavonoids, as dates are, to the best of our knowledge, the only food in which flavonoid sulfates have been reported. The MS analysis suggests that the sulfates are linked to the sugars of flavonol glycosides and not to the phenolic hydroxyls in agreement with previous reports (*10*). Procyanidin oligomers were identified through decamers. Higher molecular weigh polymers were also present at the end of the chromatographic run, and accumulated mass spectra indicate that this peak is composed of undecamers through heptadecamers.

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