Comparative Analysis of Six Different Brands of Date Fruits

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Summary. Flavonoid composition of six different brands of Phoenix dactylifera L. date fruit (Lulu, Khalas, Khenaiz, Al-Medina, Razaiz, and Fardh) was identified, determined, and compared. The separation was done on a Zorbax Eclipse XDB-C18 (150 × 4.6 mm ID) reversed-phase column using gradient elution of 1% aqueous formic acid (A) and acetonitrile (B). Gradient conditions were 00.00 min, 13% B; 20.00 min, 34% B; 25.00 min, 64% B; 29 min, 64% B; 35 min, 13% B; 39 min, 13% B. Monitoring was done using ultraviolet absorbance and MS/MS.

Key Words: dates, HPLC, flavonoids, antioxidants, LC–ESI/MS/MS

Introduction

Date palm trees are native to Northern Africa and the Middle East. Date palm fruits (Phoenix dactylifera L.) are a delicate dessert of the sweet-preferring population. Many Middle Eastern cuisines include dates, as do Mediterranean cuisines such as those of Italy and Greece. In addition to being eaten fresh, the date fruit is dried and eaten whole (with the seed extracted) as a snack or included in an assortment of desserts. Dried dates are usually readily available in many countries, and fresh dates can be found in specialty markets during the harvest season. The ripe date fruits contain carbohydrates, with high energy content, in addition to large quantities of dietary fibers. Date fruits also contain important minerals such as potassium, calcium, iron, magnesium, phosphorous, and zinc [1]. Habib and Ibrahim [2] studied the nutritional quality of various dates cultivated in the United Arab Emirates. Their experimental results showed significant but variable amounts of micro- and macro-nutrients among the pits of date fruits.
A wide variety of pharmacological activities of date fruits have been detected in laboratory animals as well as in human volunteers through clinical studies [1]. Anti-inflammatory activity of date fruits was detected by Doha and Al-Okbi in 2004 [3], while their antihyperlipidemic effect was published by El-Mougi et al. in 1991 [4] and Salah and Al-Maiman in 2005 [5]. The hepato-protective effect was found by Al Quarawi et al. in 2004 [6] and Saafi et al. in 2010 [7]. Many reports have also shown that date fruits have neuroprotective properties [6], anticancer activity [8], immunostimulant effect [9], and gonadotropic actions [4,10]. Rock et al. [11] have published the results of their clinical studies on the glucose and triglycerol levels of healthy human subjects. They observed positive changes in the carbohydrate as well as lipid profiles during the period of date consumption.

HPLC has widely been used to verify organic compounds as components and ingredients in food. When quantitative analysis has been done, UV detection by the individual standards should be used followed by adequate cleanup, which can include several steps of prepurifications. HPLC, combined with either MS or MS/MS, gives both the proper separation and specific detection and is a modern tool for reliable identification of the constituents in food samples.

Plant flavonoids form part of the defense system against oxidative stress of plants, animals, and human beings [7, 12]. Our main objective in this study was to detect and analyze the presence and occurrence of 13 previously reported flavonoids in the extract of six different brands of date fruit using HPLC separation.

**Experimental**

**Materials**

Five types of date palm (*P. dactylifera* L.) fruits ((a) Lulu; (b) Khalas; (c) Khenaiz; (e) Razaiz; (f) Fardh) were kindly donated by the local date factory, Al Saad (Al Ain, United Arab Emirates), and Al-Medina (d) was purchased from commercial sources in Al Ain (United Arab Emirates).

**Instrumentation**

LC-MS-MS analysis for ripe date palm fruits was performed in positive-ion mode on an Agilent 6410 Triple Quad LC-MS system (Wilmington, DE, USA) using electrospray ionization. Flavonoid components were separated on a Zorbax Eclipse XDB-C18 (150 × 4.6 mm ID) reversed-phase column. Gradient elution was applied with 1% formic acid (A) and acetonitrile (B) as
Methods

About 5 g samples of six different types palm fruits were subjected to extractions to determine the flavonoid components. The exact quantities of the samples were as follows: Lulu: 5.0775 g; Khalas: 5.0627 g; Khenaiz: 4.9660 g; Al-Medina: 4.9510 g; Razaiz: 5.0674 g; Fardh: 5.1255 g. The extractions were done using a solution of 4 mM sodium fluoride in methanol (to inactivate polyphenoloxidases and prevent phenolic degradation due to browning) [14]. Extraction with $2 \times 25$ mL of extraction fluid (5 and 4 min extractions) and further extraction with $1 \times 20$ mL for 4 min were done using an ultrasound device (Braun Labsonic U, Meßungen, Germany). After centrifugation (6000 rpm for 10 min, 2500 g), the extracts were combined and evaporated to dryness under reduced pressure (Rotavapor R-200, Buchi, Flawil, Switzerland) below 60°C. The dry residue was dissolved in 5 mL of 10% methanol and subjected to 500 mg of Supelclean solid-phase extraction C18 cartridges (500 mg, 3 mL, supplied by Supelco, Bellefonte, PA, USA) previously activated using 5 mL of methanol and 2.5 mL of water. The contaminants were washed out using 5 mL of methanol and 2.5 mL of water. The glycosides were eluted with 2 mL of 70% aqueous methanol. The tentatively occurring aglycons were eluted with 3 mL methanol.

Results

The HPLC separations of six different palm date fruits are presented in Fig. 1. The chromatograms were monitored at 360 nm. The mass spectral data for flavonoid glycosides are shown in Table I.

On performing product ion scanning in the positive-ion mode, five different peaks (peak nos. 3, 4, 5, 6, and 8) were identified as luteolin derivatives. Among them, peak no. 8 was the most abundant with a molecular ion at 595 AMU. It also had two fragment ions at $m/z$ 449 and 287. A similar fragmentation pattern was observed for peak no. 6, indicating that pentose
Fig. 1. The HPLC separations of six different palm date fruits were monitored at 360 nm. Partial chromatograms marked (a), (b), (c), (d), (e), and (f) are those of Lulu, Khalas, Khenaiz, Al-Medina, Razaiz, and Fardh samples, respectively.

Table I. Summary of peaks and MS information by HPLC-UV-MS/MS

<table>
<thead>
<tr>
<th>Peak</th>
<th>Assignment</th>
<th>( t_R ) (min)</th>
<th>([M-H]^+) (AMU)</th>
<th>MS/MS main fragments (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rhamnosyl dihexosyl methyl quercetin</td>
<td>4.70</td>
<td>787</td>
<td>479, 317</td>
</tr>
<tr>
<td>2</td>
<td>Apigenin di-C-hexoside</td>
<td>4.61</td>
<td>595</td>
<td>577, 559, 523, 457, 427, 379, 325</td>
</tr>
<tr>
<td>3</td>
<td>Rhamnosyl dihexosyl luteolin</td>
<td>5.01</td>
<td>757</td>
<td>449, 287</td>
</tr>
<tr>
<td>4</td>
<td>Rhamnosyl dihexosyl luteolin</td>
<td>5.56</td>
<td>757</td>
<td>449, 287</td>
</tr>
<tr>
<td>5</td>
<td>Rhamnosyl dihexosyl luteolin</td>
<td>7.01</td>
<td>757</td>
<td>449, 287</td>
</tr>
<tr>
<td>6</td>
<td>Rhamnosyl hexosyl luteolin</td>
<td>8.31</td>
<td>595</td>
<td>449, 287</td>
</tr>
<tr>
<td>7</td>
<td>Rhamnosyl hexosyl quercetin (rutin)</td>
<td>8.30</td>
<td>611</td>
<td>465, 303</td>
</tr>
<tr>
<td>8</td>
<td>Rhamnosyl hexosyl luteolin</td>
<td>8.98</td>
<td>595</td>
<td>449, 287</td>
</tr>
<tr>
<td>9</td>
<td>Rhamnosyl hexosyl methyl quercetin</td>
<td>10.49</td>
<td>625</td>
<td>479, 317</td>
</tr>
<tr>
<td>10</td>
<td>Hexosyl methyl luteolin sulfate</td>
<td>10.73</td>
<td>543</td>
<td>463, 301</td>
</tr>
<tr>
<td>11</td>
<td>Rhamnosyl hexosyl methyl luteolin</td>
<td>10.77</td>
<td>609</td>
<td>463, 301</td>
</tr>
<tr>
<td>12</td>
<td>Hexosyl methyl luteolin sulfate</td>
<td>11.19</td>
<td>543</td>
<td>463, 301</td>
</tr>
<tr>
<td>13</td>
<td>Rhamnosyl hexosyl methyl luteolin</td>
<td>11.46</td>
<td>609</td>
<td>463, 301</td>
</tr>
</tbody>
</table>
(146 Da) as well as hexose (162 Da) units were attached to the aglycon. Therefore, peaks nos. 6 and 8 can be identified as due to rhamnosyl hexosyl luteolin isomers. Peak no. 3 has the same mass data as peaks nos. 4 and 5. The molecular ion at 757 AMU fragmented to yield ions at both m/z 449 and 287. This fragmentation indicated also that a rhamnose and two hexose units contribute to its chemical structure. Peaks nos. 3, 4, and 5 were identified as due to rhamnosyl dihexosyl luteolin isomers.

Peaks nos. 11 and 13 showed the same molecular ion (609 AMU) and MS/MS fragmentation pattern, with the two main fragment ions at m/z 463 and 301 revealing the loss of both a rhamnose and a hexose moiety. These two peaks were identified as due to rhamnosyl hexosyl methyl luteolin. Both peaks nos. 10 and 12 showed a molecular ion at 543 AMU and mass fragments at m/z 465 and 305. The compounds presenting these two peaks were characterized as due to hexosyl methyl luteolin sulfate isomers.

Fragmentation of the molecular ion at 611 AMU (peak no. 7) led to product ions at m/z 463 and 301. These fragment ions can be formed by a concomitant loss of a rhamnose and a hexose unit, resulting in quercetin. By comparing these mass data and also its HPLC retention time to those of standard sample, peak no. 7 was identified as due to rutin (rhamnosyl hexosyl quercetin).

Peak no. 1 was characterized as due to rhamnosyl dihexosyl methyl quercetin. Fragmentation of its molecular ion at 787 AMU resulted in three fragment ions at m/z 641, 479, and 317, which corresponded to the concomitant loss of one rhamnose unit and two hexose residues to give its aglycon. Peak no. 9 had a molecular ion at 625 AMU and a fragment ion at m/z 317, corresponding to the loss of a rhamnosyl hexosyl unit to give the aglycon. Peak no. 9 was thereby identified as due to rhamnosyl hexosyl methyl quercetin.

Peak no. 2 was identified as due to apigenin di-C-hexoside by comparing its molecular ion (595 AMU) and m/z values of its MS/MS fragments (523, 457, 427, 325) with those of the data in the literature [13, 14].

The area under the HPLC peaks detected at the molecular ion signal is proportional to the flavonoid composition. These values are presented in Fig. 2. Among them, Khenaiz, Khalas, and Lulu cultivars contain rhamnosyl hexosyl methyl luteolin (peak no. 13) and hexosyl methyl luteolin sulfate (peak no. 10) as their major constituents. Khenaiz contains apigenin di-C-hexoside as the third major component also, and its concentration is more than double in this cultivar than in the Khalas. Lulu can be differentiated from the other cultivars by the relatively low flavonoid content. The main components of the Al-Medina fruit are rhamnosyl hexosyl methyl luteolin (peak no. 13) and, as a distinct mark, rhamnosyl hexosyl luteolin (peak no. 8). Razaiz and Fardh contain only one major flavonoid component, rhamno-
syl hexosyl methyl luteolin (peak no. 13), but the content of the other minor flavonoid glycosids is higher in Fardh than in Razaiz.

![Flavonoid content comparison](image_url)

**Fig. 2.** Semiquantitative comparison of flavonoid content of dates with various brand names (Lulu, Khalas, Khenaiz, Al-Medina, Razaiz, and Fardh). Area × 1000 versus flavonoid peak are plotted.

**Discussion**

There are five stages of ripening of date fruits lasting approximately 7 months. The stages include the Hababouk (first stage), the Khimri (green stage), Khalal (color stage), Rutab (soft ripe stage), and Tamar (full ripe
Analysis of Six Different Brands of Date Fruits

Fruits of date trees are commercially available at departmental stores in many parts of the world. Date fruit extracts contain a wide variety of organic and inorganic compounds, as recently reviewed by Baliga et al. [1].

HPLC separation with ultraviolet absorbance monitoring (at 290 and 340 nm) is an excellent method to monitor organic acids in ripe date fruits [16]. However, qualitative and quantitative evaluation using MS/MS may be preferred to photometric detection, especially when structural elucidation of plant flavonoids is intended [16–20]. Vukics et al. [16–19] separated and identified the main flavonoids in heartsease (Viola tricolor L.) using HPLC with both UV–vis and MS/MS detection methods. Mansouri et al. [15] indicated that many of the detected compounds of the Algerian ripe date palm fruits belong to the group of flavones glycosides. Hong et al. [20] identified 19 flavonol glycosides (various isomers of luteolin, methyl luteolin, quercetin, methyl quercetin) in the extract of Deglet Nor (P. dactylifera, L.) dates. The results and precise information given by Hong et al. [20], supported by the other references [15–20], made reliable identification of flavonoids possible without the requirement of the standard chemical compounds. The major conditions of identification of the flavonoid glycosides were adequate with the use of HPLC separations and the HPLC–MS/MS.

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References


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