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Carotenoid composition of Algerian date varieties (*Phoenix dactylifera*) at different edible maturation stages

Hafid Boudries [a], Panagiotis Kefalas [a], Dámaso Hornero-Méndez [b,*]

[a] *Department of Food Quality Management and Chemistry of Natural Products, Mediterranean Agronomic Institute of Chania (MAICh), P.O. Box 85, 73100, Chania, Crete, Greece*

[b] *Department of Food Biotechnology, Instituto de la Grasa (CSIC), Av. Padre García Tejero 4, 41012-Sevilla, Spain*

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Abstract

The aim of this study was to investigate the carotenoid composition and the provitamin A value of three palm date (*Phoenix dactylifera*) varieties (Deglet-Nour, Hamraya and Tantebouchte) from Algeria at three different ripening stages (khallal, rutab and tamr). Chromatographic analysis showed that the major carotenoid pigment present in dates is lutein followed by β-carotene, with an evident carotenoid disappearance during ripening from the khallal to the tamr stage. The different date fruits present a total carotenoid content in the range of 61.7–167, 32.6–672, and 37.3–773 μg/100 g fresh weight (FW) in Deglet-nour, Tantebouchte and Hamraya varieties, respectively. The rutab stage of Tantebouchte showed the lowest carotenoid content of 32.6 μg/100 g FW, whereas the khallal stage of Hamraya presented the highest value, 773 μg/100 g FW, followed by Tantebouchte with 672 μg/100 g FW. Provitamin A value (due exclusively to β-carotene) increased from 0.4 to 0.5 RE/100 g in Deglet-Nour fruits, but decreased from 11.7 to 1.6 RE/100 g and from 3.9 to 0.5 RE/100 g in Tantebouchte and Hamraya fruits, respectively, during ripening. The lowest value was found at the tamr stage of the Deglet-Nour variety (0.5 RE/100 g) whereas the highest provitamin A content was found at the khallal stage of the Tantebouchte variety (11.7 RE/100 g).

Keywords: *Phoenix dactylifera*; Carotenoid profile; Provitamin A value; Fruit ripening; Algeria date fruit

1. Introduction

Dates, the fruit of the date palm (*Phoenix dactylifera*), can be considered the main staple food in north African countries and the basis of survival for the inhabitants of the Algerian Sahara, especially during the Ramadan period, representing an important source of nutrients and energy. Global production of date fruits exceeds 6 million metric tonnes annually in the world. During 2004, 450,000 metric tons were produced in Algeria which is the first producer in the Arab maghreb union. In 2003, 4.68% of Algerian dates were exported, which represents 23,072 metric tons, ensuring an important income for the population living in the desert, and simultaneously reflecting an important quantity for local consumption (www.FAO.org).

Several studies indicate that consumption of fruits and vegetables is associated with reduced risk of several chronic diseases (Kritchevsky, 1999; Nicoli, Anese, & Parpinel, 1999; Van Duyn & Pivonka, 2000; Willett, 1994). Regarding dates, Vayalil (2002), Mansouri, Embarek, Kokkalou, and Kefalas (2005) indicate that this fruit has important antioxidant activity due to the presence of water-soluble compounds with potent free radical-scavenging effects, such as phenolic compounds (mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones).

Chemical composition of date palm fruits has been reported in various research works (Al-Hooti, Sidhu, & Quabazard, 1995; Al-Shahib & Marshal, 2003; Sawaya, Khatchadourian, Khalil, Safi, & Al-shalhat, 1982), but

* Corresponding author. Tel.: +34 954 691054; fax: +34 954 691262.
E-mail address: hornero@cica.es (D. Hornero-Méndez).

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there is no detailed description of their carotenoid profile. Carotenoids are a group of natural pigments (about 650) that participate in many important nutritional functions. Some of them are converted into vitamin A, and are active antioxidants. Carotenoids are unique tetraterpenoids which are synthesized by plants, bacteria, yeasts and molds. Carotenoids can also be found in the animal kingdom (bird plumage, fish, crustaceans, insects); however, animals (including humans) cannot synthesize carotenoids de novo and therefore food is the only source of these compounds. Consumption of carotenoid-rich foods has been related to prevention of cancer, cardiovascular diseases and other degenerative processes involving oxidative stress (Stahl and Sies, 2003/2005). Regarding palm date fruits, only two works have focussed on the carotenoid composition of palm date fruits (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005; Gross, Haber, & Ikan, 1983).

The aim of the present work is to describe the carotenoid profiles of three varieties of Algerian date fruits at three edible maturation stages to find, from a nutritional point of view, which stage represents the highest provitamin A value. Data derived from this work could be of important, due to the fact that vitamin A deficiency is a common cause of blindness and infant mortality in southeast Asia, Africa, and parts of south and central America.

2. Materials and methods

2.1. Plant material

Three different Algerian date palm fruit varieties (Hamraya variety (H), Deglet-Nour variety (DN) and Tantebouchte variety (T)) were harvested at three edible maturation stages according to the pollination date: Khallal stage: 20 weeks after pollination, 10 September, 2004 (stage 1); Rutab stage: 22 weeks after pollination, 20 September, 2004 (stage 2); Tamr stage: 24 weeks after pollination, completely mature, 5 October, 2004 (stage 3). For Hamraya and Tantebouchte varieties, fruits were collected from the EL-Oued region while, for Deglet-Nour, fruits were collected from the Biskra region.

The harvesting was carried out in the traditional way, with only the appreciation and experience of the farmer. It should be mentioned that Deglet-Nour fruits were exposed to high temperatures due to the weather conditions at the time of harvesting, which accelerates their maturation, and therefore the brown colour was deeper than that of the normal one. Four hundred grammes of fruits from each variety and each maturity stage were collected and stored at −20 °C prior to analysis.

2.2. Chemicals and standards

Acetone, methanol, diethyl ether, anhydrous sodium sulphate and sodium chloride of analytical grade were purchased from Riedel-de Haën; potassium hydroxide, petroleum ether and HPLC grade solvents, acetonitrile (ACN) and ethyl acetate (EtOAc), were from Merck; deionized water was produced with a MilliQ system (Millipore). Commercial carotenoid standards (lutein and β-carotene) used for identification and detector calibration were purchased from Sigma–Aldrich.

2.3. Carotenoid extraction

Carotenoid pigments were extracted by following general procedures (Mínguez-Mosquera & Hornero-Méndez, 1993; Rodriguez-Amaya, 2001). A homogenous representative sample (5–30 g) was ground with a mortar and pestle with enough cold acetone (4 °C) for 15 min to soften the cell wall, which was subsequently filtered through filter paper. The residue was returned to the mortar and macerated again with fresh acetone. The extraction was repeated until exhaustion of colour was achieved (usually three times were enough). Finally, the mortar and filter were washed with a small amount of acetone which was collected with the rest of the extract. A concentration of 0.1% BHT was in acetone, which helped to protect the analytes from eventual oxidation. All operations were carried out under dimmed light to prevent isomerization and photodegradation of carotenoids. The acetone solution was partitioned, using diethyl ether, by adding 10% NaCl. The organic (upper) phase was washed with water, filtered over anhydrous sodium sulphate and evaporated under vacuum ($T < 30$ °C). For saponification, 50 ml of petroleum ether containing 0.1% BHT were added to dissolve the residue, followed by an equal volume of 10% potassium hydroxide in methanol. The mixture was stirred overnight in the dark and under N_2. The mixture was partitioned with water, and the upper phase washed with distilled water (four to five times) up to neutral pH of washings, then dried over anhydrous sodium sulphate. The organic phase was concentrated under vacuum ($T < 30$ °C) and redissolved in 1 ml of HPLC grade acetone, and stored at −20 °C prior to analysis. The analyses were carried out in triplicate.

2.4. Pigment isolation and identification

Routine procedures for the isolation and identification of carotenoid pigments, already described in detail in previous publications, were used (Mínguez-Mosquera & Hornero-Méndez, 1993). Briefly then consisted of: separation and isolation of the pigments by TLC on silicagel 60 GF plates and co-chromatography with standard pigments, acquisition of UV–visible spectra (Hewlett–Packard UV–visible diode array spectrophotometer model 8452A) in different solvents and comparison with the values reported in the literature (Britton, 1991/1995; Davies, 1976/1988; Foppen, 1971), as well as chemical derivatization microscale tests for the examination of 5,6-epoxide groups investigated by addition of 2% HCl in EtOH, acetylation with Ac_2O/Py to test for hydroxyl groups and

reduction with $NaBH_4$ in EtOH to test for carbonyl groups (Eugster, 1995).

2.5. Chromatographic conditions

Separation and analysis of carotenoid pigments were carried out by high performance liquid chromatography (HPLC), using a Hewlett–Packard 1090 Series II chromatograph, with autoinjector and UV–visible diode array detector, on a LiChrospher 100 RP18 column (250 × 4 mm, 5 μm particle size, Merck, Germany), which was protected by a guard column containing the same stationary phase. The mobile phase consisted of mixtures of acetonitrile: water (9:1) containing 0.1% triethylamine (A) and ethyl acetate (B). The gradient started with 100% A and changed linearly to 100% B in 25 min. The flow rate was 1 ml/min, the chromatogram was monitored at 450 nm, and UV–visible spectra were recorded in the range 250–600 nm. Quantification was performed by using external calibration curves for β-carotene, and lutein, respectively. The extract, dissolved in 1 ml of acetone, was filtered through 0.45 μm Gelman filters and 25 μl of sample were injected. Column temperature was set at 40 °C.

2.6. Provitamin A value

The provitamin A values, expressed as retinol equivalents (RE), were calculated according to NAS-NRC (1989), for which 6 μg of β-carotene corresponds to 1 μg of retinol equivalents (RE). Considering that the only provitamin A precursor carotenoid present in palm date fruit is β-carotene, the following expression was used:

$$RE = \mu g\ \beta\text{-carotene}/6$$

2.7. Data processing

All measurements were performed at least in triplicate and values were averaged. Results are given as means ± standard deviation (SD). The analyses were processed using Excel 2000 software.

3. Results and discussion

3.1. Carotenoid composition

The carotenoid pigments occurring in the palm date fruit (*P. dactylifera*) have only been scarcely studied; which may be due to the assumption that fruit with so dark a colour do not contain carotenoids, since these pigments usually impart a bright yellow, orange or red colour (Gross et al., 1983). The present investigation focussed on the changes occurring in the carotenoid pattern during ripening of date fruits and for this purpose, three date fruit varieties were chosen from different representative regions of Algeria.

The carotenoid composition was investigated at three edible maturation stages (khallal, rutab and tamr) for each variety (Hamraya, Tantebouchte and Deglet-Nour). Analyses were carried out on saponified extracts and therefore information about the presence and nature of xanthophyll esterification was not included in the present work. Two major carotenoids, lutein and β-carotene, were identified in *P. dactylifera* mature fruits by means of their chromatographic behaviour (TLC and HPLC) and by comparison of the UV–visible spectra and the retention times with those of authentic standards and data reported in the literature (see Section 2). Identification was aided with derivatization microscale tests, after TLC isolation, for the examination of 5,6-epoxide, hydroxyl and carbonyl groups. Traces of neoxanthin, violaxanthin and antheraxanthin were tentatively identified (data not shown) according to their HPLC behaviour, UV–visible spectra recorded with the DAD detector and by performing the epoxide test in the whole pigment extract and analyzing its results by HPLC before and after addition of HCl. These minor pigments will not be considered in the present study. Tables 1 and 2, summarize the chromatographic and spectroscopic properties of the carotenoids found in date fruits. Fig. 1 shows the HPLC chromatogram obtained from a saponified carotenoid extract of ripe date fruits. The three analyzed date fruit varieties presented similar chromatographic profiles for the major carotenoids at all maturation stages.

3.2. Changes in the carotenoid composition at late-stages of fruit ripening

The carotenoid composition of palm date fruits was analyzed at three consecutive late ripening stages, khallal (stage 1), rutab (stage 2) and tamr (stage 3). Tables 3–5 summarize the changes in the carotenoid composition during ripening. In general terms, the presence of lutein and β-carotene as the major pigments suggests that these fruits retain some of the chloroplastic pigments originally present

Table 1
Chromatographic and spectroscopic characteristics used for carotenoid identification in palm date fruits

Carotenoid	TLC Rf value	Spectral data, λ_{max} (nm)[a]		Epoxide test hypsocromic shift (nm) after HCl treatment	Acetylation	Reduction
		Light petroleum ether 40–60 °C	Ethanol			
Lutein	0.41	418, 442, 470	418, 442, 472	0	+	–
β-Carotene	1.00	(426), 444, 470	(426), 448, 476	0	–	–

Chromatographic adsorbent for TLC: Silicagel 60 GF_{254}. TLC solvent system: light petroleum ether (40–60 °C)–acetone–diethylamine (10:4:1).
[a] Parentheses indicate shoulder.

Table 2
Main HPLC properties of carotenoid pigments extracted from palm date fruits

Peak no.[a]	Carotenoid	t_R(min)	λ_{max}(nm)[b,c]	%III/II[d]
1	Lutein	10.46	(426), 450, 476	56
2	*cis*-Lutein	11.10	(422), 444, 470	50
3	β-Carotene	19.18	(430), 452, 479	21
4	*cis*-β-Carotene	19.35	(424), 450, 474	15

[a] Numbered according to the chromatogram shown in Fig. 1.
[b] Obtained with the diode array detector.
[c] Parentheses indicate shoulder.
[d] %III/II. Calculated according to Britton (1995).

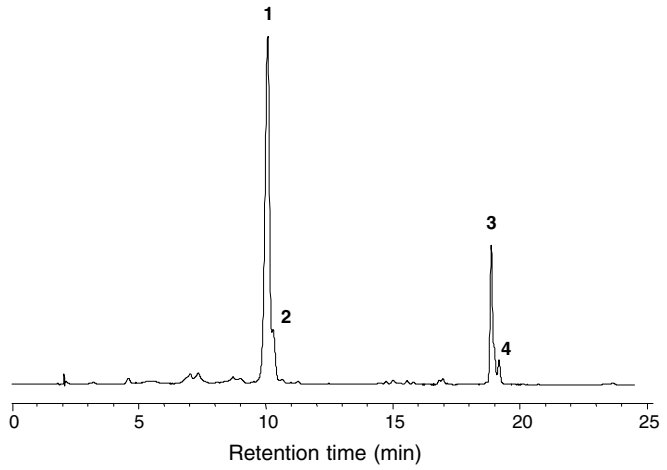

Fig. 1. HPLC chromatogram obtained for a saponified carotenoid extract of ripe palm dates fruits (Deglet-Nour variety). Peak identities: 1. lutein; 2. *cis*-lutein; 3. β-carotene; 4. *cis*-β-carotene.

Table 3
Carotenoid composition (μg/100 g FW) in Deglet-Nour variety at three different edible maturation stages

Carotenoid	Maturation stages		
	Stage 1: khallal	Stage 2: rutab	Stage 3: tamr
Lutein	57.7 ± 12.1 (93.5%)[a]	156 ± 24.3 (93.2%)	59.6 ± 26.6 (92.7%)
β-Carotene	2.6 ± 0.4 (4.2%)	6.4 ± 0.6 (3.8%)	3.00 ± 1.4 (4.6%)
Minor carotenoids	1.4 ± 0.2 (2.3%)	5.0 ± 2.2 (3.0%)	1.7 ± 2.0 (2.7%)
Total carotenoids	61.7 ± 12.2	167.3 ± 24.7	64.3 ± 26.9

[a] Data between brackets represent relative (%) composition with respect to total content.

Table 4
Carotenoid composition (μg/100 g FW) in Tantebouchte variety at three different edible maturation stages

Carotenoid	Maturation stages		
	Stage 1: khallal	Stage 2: rutab	Stage 3: tamr
Lutein	544 ± 31.5 (81.0%)[a]	27.6 ± 23.5 (84.8%)	129 ± 11.1 (89.3%)
β-Carotene	70.0 ± 55.2 (10.4%)	3.3 ± 2.1 (10.0%)	9.5 ± 0.7 (6.4%)
Minor carotenoids	57.8 ± 8.2 (8.6%)	1.7 ± 1.5 (5.2%)	6.2 ± 1.8 (4.3%)
Total carotenoids	672 ± 129.3	32.6 ± 25.4	145 ± 13.6

[a] Data between brackets represent relative (%) composition respect total content.

Table 5
Carotenoid composition (μg/100 g FW) in Hamraya variety at three different edible maturation stages

Carotenoid	Maturation stages		
	Stage 1: khallal	Stage 2: rutab	Stage 3: tamr
Lutein	702 ± 58.6 (90.8%)[a]	33.6 ± 6.4 (90.2%)	45.7 ± 18.3 (89.2%)
β-Carotene	23.2 ± 1.8 (3.0%)	2.5 ± 1.1 (6.7%)	3.0 ± 1.2 (5.8%)
Minor carotenoids	47.9 ± 19.1 (6.2%)	1.1 ± 0.6 (3.1%)	2.6 ± 1.6 (5.0%)
Total carotenoids	773 ± 42.8	37.3 ± 7.3	51.3 ± 20.5

[a] Data between brackets represent relative (%) composition respect total content.

in the green immature fruit, which become externally evident by the yellow to orange colour of ripening fruits once the chlorophylls disappear.

To our knowledge, this is the first time that the entire carotenoid composition of Algerian date fruit varieties has been studied in detail, including the provitamin A value. No qualitative changes were observed in the carotenoid composition of the three date fruit cultivars studied during maturation from the khallal to the tamr stage. The different date fruits present a total carotenoid content in the range of 61.7–167, 32.6–672 and 37.3–773 μg/100 g fresh weight (FW) for Deglet-nour, Tantebouchte and Hamraya varieties, respectively. These results are much lower than previous estimations with Israeli dates (Gross et al., 1983), and also lower than some date varieties from Oman (Al-Farsi et al., 2005). According to our results the rutab stage of Tantebouchte showed the lowest carotenoid content of 32.6 μg/100 g FW, whereas the khallal stage of Hamraya presented the highest value 773 followed by Tantebouchte, with 672 μg/100 g FW.

The lutein content ranged from 92% to 94%, from 81% to 89% and from 89% to 91% of the total carotenoid content in Deglet-Nour, Tantebouchte and Hamraya varieties, respectively, followed by β-carotene, ranging from 3.8% to 4.6%, 6.5% to 10.4% and 3.0% to 6.7% of the total carotenoid content, respectively. The rest of the minor unidentified carotenoids (in traces) were expressed as β-carotene and they represent about 2–3%, 4–8% and 3–6% of the total carotenoid content in Deglet-Nour, Tantebouchte and Hamraya varieties, respectively.

As a result of over-ripening, the total carotenoid content remains almost constant in Deglet-Nour variety (from 61.7 to 64.3 μg/100 g FW), whereas it decreases from 672 to 145 μg/100 g FW in Tantebouchte and 773 to 51.3 μg/100 g FW in Hamraya from the khallal to the tamr stage.

In Deglet-Nour variety, lutein and β-carotene experience a smooth increase from 57.7 and 2.6 μg/100 g FW at the khallal stage to 59.6 and 3.0 μg/100 g at the tamr stage, respectively, while in the Tantebouchte variety, these carotenoids dramatically decreased from 544 and 70.0 μg/100 g FW to 129 and 9.5 μg/100 g FW, respectively. Finally in Hamraya, values fall from 702 and 23.2 μg/100 g FW to 45.7 and 3.0 μg/100 g FW, respectively. The decrease in carotenoid concentration is important from the khallal to the rutab stage whereas, from the rutab to tamr stage, we notice a slight increase, which should not be due to de novo biosynthetic activity, but to the decrease in the water content in the fruit at this stage.

According to several studies in other fruits and vegetables (Hornero-Mendez, Gómez-Ladrón de Guevara, & Mínguez-Mosquera, 2000; Mercadante & Rodriguez-amaya, 1998; Roca & Minguez-Mosquera, 2001), the changes in the carotenoid content during ripening are markedly dependent on the varieties while, in our study, the difference noted in the pigment content in quantitative terms between Tantebouchte and Hamraya varieties, at all stages of maturation, from the same region, was not significant, which means that the variety in this case does not have a big effect on the carotenoid composition.

3.3. Geographic effects

The difference between our results and the results obtained by Gross et al. (1983), Al-Farsi et al. (2005), in their studies on carotenoids content and profile of Israeli and Omani date fruits, respectively, may be attributed to different factors, such as varieties, weather, fertilizer, soil type, season, the amount of sunlight irradiation and geographical origin, among others.

In the Deglet-Nour variety, there are certain differences with respect to the carotenoid content compared to the two other varieties, which might be explained by climatic conditions of the origin region. Comparing the carotenoid composition at the khallal stage of Deglet-Nour variety from the Biskara, situated north east of sahara (hot climate), with Tantebouchte and Hamraya from El-Oued, situated south east of Biskara (less hot), the latter at the khallal stage had more than 12 times as much lutein and eight times as much β-carotene than had the Deglet-Nour variety from Biskara. Sunshine and temperature are well above average in Biskara; such climatic characteristics have, of course, an effect on the physiology of fruit ripening.

Markus, Daood, Kapitany, and Biacs (1999), in their study on red peppers, noticed that the fruits harvested during long sunshine periods, low rainfall, and high temperature had less carotenoids than those harvested in seasons with poor sunshine, high rainfall and low temperatures. Lima et al. (2005) also found that carotenoid content was higher in mature acerola fruits harvested in the rainy season than in those harvested in the dry season.

Table 6
Provitamin A value (RE/100 g FW) in three Algerian palm date fruits (Hamraya, Deglet-Nour and Tantebouchte) at three edible maturation stages

Variety	Maturation stages		
	Stage 1: khallal	Stage 2: rutab	Stage 3: tamr
Deglet-Nour	0.4 ± 0.1	1.1 ± 0.1	0.5 ± 0.2
Hamraya	3.9 ± 0.3	0.4 ± 0.2	0.5 ± 0.2
Tantebouchte	11.7 ± 9.2	0.5 ± 0.3	1.6 ± 0.1

3.4. Provitamin A

β-Carotene is the only pigment contributing to the provitamin A activity in date fruits, and therefore variations in the provitamin A value during ripening will reflect the changes that occur in β-carotene. Table 6 summarizes provitamin A content in the three studied varieties. Provitamin A value remained almost constant (from 0.4 to 0.5 RE/100 g) in Deglet-Nour, but decreased from 11.7 to 1.6 RE and 3.9 to 0.5 RE in Tantebouchte and Hamraya, respectively, during ripening from the khallal to the tamr stage. From the khallal to the tamr stage in the Tantebouchte variety, there was a decrease of sevenfold in the provitamin A value. The lowest level was found at the tamr stage of the Deglet-Nour variety (0.5 RE/100 g) whereas the highest level was found at the khallal stage of the Tantebouchte variety (11.7 RE/100 g). The provitamin A value is lower at the tamr stage compared with other fruits such as mango, apricot, and pepper, but it is relatively high at the khallal stage, thus being a very rich source of vitamin A for the desert inhabitants.

4. Conclusion

This study represents an overview of the carotenoid profile of some Algerian date fruit varieties. The HPLC analysis showed that the major carotenoid pigment is lutein, followed by β-carotene. From the results obtained, we can conclude that there is degradation of carotenoids during ripening from the khallal to the tamr stage and, as a result, the ripe date fruit has a low carotenoid content, compared with other fruits. The provitamin A value of date fruit varieties derives essentially from the β-carotene content, and both of them decrease during maturation from the khallal to the tamr stage. In that sense it would be better from the nutritional point of view, to choose the khallal stage instead of the tamr stage.

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