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Full Length Article

Chlorophyll and Carotenoid Patterns of Middle Eastern Date (*Phoenix dactylifera*) Fruit at Different Maturity Stages

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Abstract

Lipophilic pigments of the date varieties 'Anbra', 'Megadwel', 'Sfwai' and 'Sacai' were characterized by HPLC-DAD-APCI-MS^n considering the four progressing ripening stages '*Kimri*' (green-ripe), '*Khalal*' (breaker), '*Rutab*' (brown-ripe) and '*Tamr*' (dry-ripe). The prevailing carotenoids in green-ripe and breaker fruits were (all-*E*)-lutein and (all-*E*)-β-carotene, in addition to the minor constituents (all-*E*)-violaxanthin, (all-*E*)-neoxanthin and (all-*E*)-zeaxanthin. Most abundant carotenoids in brown-ripe fruits were β-carotene isomers and (all-*E*)-lutein. Only the early maturity stages contained chlorophylls *a* and *b*, summing up to 820–1,282 and 340–878 *µ*g/100 g of fresh weight (FW) in dates harvested at the '*Kimri*' and '*Khalal*' stage, respectively. Total carotenoids were in the range of 283–411 ('*Kimri*') and dropped to 230–300 ('*Khalal*') and 90–231 *µ*g/100 g of FW ('*Rutab*') with progressing maturation. Among the fully ripe '*Tamr*' fruits, merely those harvested from cv. 'Megadwel' contained 30 *µ*g/100 g total carotenoids on a FW basis. The two edible fruit maturity stages '*Khalal*' and '*Rutab*' represented valuable nutritional sources of the macular carotenoid lutein as well as of β-carotene. At the present concentrations, the latter endows dates with a provitamin A content similar to that of oranges and pineapples. © 2020 Friends Science Publishers

Keywords: Carotenes; Xanthophylls; Chlorophylls; Dates; Vitamin A; Maturation

Introduction

The world production of date palm (*Phoenix dactylifera* L., Arecaceae) fruit amounted to 8.2 million tons in 2017 (FAO 2019). Although the Middle East and North Africa (MENA) region accounts for about 90% of the production of dates, the production of dates has recently begun to expand in many other regions (Ghnimi *et al.* 2017). The robust date palm can be cultivated under harsh climatic conditions and on inferior soils (Hassan *et al.* 2006). Date palms are moderately tolerant to salinity and drought (Aldhebiani *et al.* 2018).

After pollination, a single fruit arises from one of the three carpels (Hadi *et al.* 2015). During subsequent fruit development, four characteristic maturity stages are distinguished. Dates of the first stage termed '*Kimri*' [~19 weeks after pollination (wap)] are green, hard and unpalatable. The '*Khalal*' ripening stage (~25 wap) is characterized by firm, physiologically ripe fruits, as their color changes from green to orange or red tonalities. Such perishable dates are commonly offered on local markets for fresh consumption. During the subsequent four weeks, both the moisture content and astringency of the fruits decreases and their skin color turns brown. Fully ripe fruits of the '*Rutab*' stage (~29 wap) are soft and sweet. The dry, shelf-stable dates of the so-called '*Tamr*' stage are harvested ~31 wap and consumed locally or exported (Aleid 2012).

Depending on the variety, the maturity stage and the analytical method applied, both the qualitative composition and the absolute concentrations of carotenoids in date fruits reported in the literature differ (Al-Farsi and Lee 2008). Lutein, β-carotene and neoxanthin have been reported to be the prevailing pigments in 'Hayani', 'Barhee' and 'Deglet-Nour' date fruits from Israel, thus reflecting a chloroplast-

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specific carotenoid pattern (Gross *et al.* 1983). Lutein and β-carotene were found to be the major carotenoids of '*Khalal*', '*Rhutab*' and '*Tamr*' stages of 'Deglet-Nour', 'Hamraya' and 'Tantebouchte' dates cultivated in Algeria, whereby carotenoid concentrations decreased during ripening (Boudries *et al.* 2007). Despite their significance for daily food supply and agricultural production in the Middle East (Lieb *et al.* 2019), date varieties from Saudi Arabia have not been sufficiently considered in previous studies.

The present contribution targeted the characterization of chlorophylls and carotenoids in date fruit cultivated in Saudi Arabia and harvested at four progressing maturity stages. The individual constituents were characterized in-depth by HPLC-DAD-APCI-MS^n following quantitation by HPLC-DAD to reveal varietal differences and to unravel the effect of maturation on the bioactive carotenoids of dates.

Materials and Methods

Reagents

Authentic reference standards of (all-*E*)-β-carotene, chlorophylls *a* and *b* were from Sigma Aldrich (Taufkirchen, Germany), while (all-*E*)-violaxanthin, (all-*E*)-neoxanthin and (all-*E*)-lutein were from CaroteNature (Ostermundingen, Switzerland). Butylated hydroxytoluene/hydroxyanisole (BHT/BHA) was from Fluka Chemie (Buchs, Switzerland). Calcium carbonate, acetone and *tert*-butyl methyl ether (*t*BME) were purchased from Merck (Darmstadt, Germany), methanol and light petroleum (boiling point 40–60°C) from VWR International (Darmstadt, Germany). Ultrapure water was used throughout the study (arium® 611 UV, Sartorius, Göttingen, Germany).

Date samples

Between the end of May and the beginning of August 2018, fruits of four different Saudi Arabian date (*Phoenix dactylifera* L.) varieties 'Anbra', 'Megadwel', 'Sfwai' and 'Sacai' were harvested in Medina (Saudi Arabia) from commercial cultivation sites. According to local harvest procedures, samples were collected at four progressing maturity stages termed '*Kimri*' (1), '*Khalal*' (2), '*Rutab*' (3) and '*Tamr*' (4) that were harvested at the end of May (25.05.2018), the beginning of July (05.07.2018), the end of July (27.07.2018) and the beginning of August (07.08.2018), respectively. The entire fruits were immediately frozen after harvest using dry ice and stored at -20°C until processed further. Subsequently, the seeds were separated manually and the pooled edible fraction (~270 g per replicate) was lyophilized. The dried samples were homogenized under liquid nitrogen to obtain a fine, homogeneous powder, vacuum-sealed into aluminum pouches, and kept frozen (-20°C) until further use.

Carotenoids analysis

Sample preparation: Freeze-dried date samples were worked up as reported previously (Schex *et al.* 2018). Briefly, after the admixture of 50 mg calcium carbonate ($CaCO_3$), 100 mg of the sample was extracted with 3 × 2.0 mL cold acetone enriched with both 0.1 g/L BHT and 0.1 g/L BHA. Using a probe sonicator (Sonopuls UW 3100 with MS 72 microtip, Bandelin Electronics, Berlin, Germany), the extraction was performed at 70% amplitude for 15 s. The sample was centrifuged for 3 min at 4,500 rpm, equalling 2,173 × *g* and the acetone phase was recovered. The combined acetone extract was evaporated with N_2 to dryness, re-dissolved in 0.3 mL *t*BME/methanol (1/1, *v*/*v*) and membrane-filtered using a 0.45 µm polytetrafluoroethylene (PTFE) filter prior to HPLC analysis.

Quantitation by HPLC-DAD and calculation of retinol activity equivalents (RAE): Quantitative carotenoid and chlorophyll analyses were conducted with a Waters (Eschborn, Germany) HPLC system (type 2695 with DAD type 2996). The system was operated and data evaluated applying Millenium®32 Chromatography Manager Software (Waters). Further details regarding the HPLC parameters for the analyses of chlorophylls and carotenoids have been reported previously (Hempel *et al.* 2017).

Linear calibration curves of (all-*E*)-β-carotene (452 nm) and chlorophyll *b* (647 nm) were established. Concentrations of stock solutions were determined by spectrophotometry (Britton *et al.* 1995; Jeffrey *et al.* 1997). The response factors for chlorophyll *a* recorded at 664 nm, (all-*E*)-violaxanthin (440 nm), (all-*E*)-lutein (445 nm) and (all-*E*)-zeaxanthin (450 nm) were calculated from those of the aforementioned two standards using the respective molar extinction coefficients (Britton *et al.* 1995; Jeffrey *et al.* 1997). Geometrical isomers of β-carotene were quantitated applying the (all-*E*)-β-carotene calibration. The limit of quantification (LOQ) and the limit of detection (LOD) were estimated based on the signal-to-noise (S/N) ratios of 10:1 and 3:1, respectively.

RAEs were calculated assuming that 12 µg dietary (all-*E*)-β-carotene and 24 µg of other dietary provitamin A carotenoids, here (13*Z*)- and (9*Z*)-β-carotene, correspond to 1 µg RAE (US Institute of Medicine 2010).

Compound identification by HPLC-DAD-APCI-MS^n: For HPLC-DAD-APCI-MS^n analyses, a series 1100 HPLC system with a G1315B diode array detector (both from Agilent, Waldbronn, Germany) was interfaced with an ion trap mass spectrometer (Esquire 3000+, Bruker Daltonik, Bremen, Germany). HPLC parameters were as detailed above. Detection wavelengths for LC-MS analysis were set to 450 and 660 nm. Mass spectra in the scan range *m/z* 100–1,000 (scan speed 13,000 (*m*/*z*)/s) were recorded in the alternate polarity mode. Settings of the used APCI ion source and further MS settings were as follows. Nebulizing gas was N_2 at 65 psi. Dry gas was N_2 at 5 L/min. Nebulizer and vaporizer temperatures were 350 and 400°C, respectively.

Corona current was 3,000 nA and capillary potential was ± 2,800 V. For collision induced dissociation (CID), collision gas was He at 4.6×10^{-6} mbar and fragmentation amplitude was set at 1.0 V. System control and data evaluation was done with ChemStation for LC version A.00.03 (Agilent) and Esquire version 5.1 software (Bruker), respectively.

Statistical analysis

Each harvest date, between 40 to 60 single fruit per variety were randomly collected from the same palm tree. Subsequently, the fruit were separated into two biological replicates ($n = 2$) that were analyzed in analytical duplicates. From a completely randomized design, significant differences of means were determined by analysis of variance and Tukey´s post-hoc test ($p < 0.05$), except for the means of total carotenoid levels, where Duncan's test was applied ($p < 0.05$). Statistical data evaluation was carried out with SAS (v.9.4, SAS Institute, Cary, NC, USA). Box plots were constructed using MS Excel 2016 for Mac (Microsoft, Redmont, WA, USA).

Results

Compound identification

Representative HPLC-DAD chromatograms recorded at 660 and 450 nm of an acetone-extract from a date sample harvested at the '*Kimri*' stage are depicted in Fig. 1. A total of eight compounds was assigned by a comparison of their retention times, UV/Vis absorption and mass spectra with those of authentic reference standards or with data reported earlier (Table 1).

Chlorophylls: The two most abundant compounds detected at 660 nm were characterized as chlorophylls *b* (4) and *a* (5) as follows. CID of their protonated molecules $[M + H]^+$ at *m/z* 907 and 893 resulted in fragment ions from the neutral loss of phytadiene $[M + H - 278]^+$ at *m/z* 629 and 615, respectively. Further product ions were detected at *m/z* 597 and 583 ($[M + H - 278 - 32]^+$) as well as at *m/z* 569 and 555 ($[M + H - 278 - 60]^+$). These ions may result from the subsequent elimination of methanol (32 amu) and the entire carboxymethoxy group concomitantly with the loss of an H atom (60 amu).

Carotenoids: Although (all-*E*)-violaxanthin (1a) was found elute close and not baseline resolved to (all-*E*)-neoxanthin (1b), they were identified using authentic reference standards. Eluting at 11.1 min, protonated molecules of (all-*E*)-violaxanthin were observed at *m/z* 601 ($[M + H]^+$) in the APCI(+)-MS^1 spectrum. CID resulted in product ions at *m/z* 583 ($[M + H - H_2O]^+$), 565 ($[M + H - 2\ H_2O]^+$), 509 ($[M + H - 92]^+$) and 491 ($[M + H - 92 - H_2O]^+$) that can be attributed to eliminations of water (18 amu) and the neutral loss of toluene (92 amu). A distinctive fragment ion was earlier found to be related to the cleavage between carbons

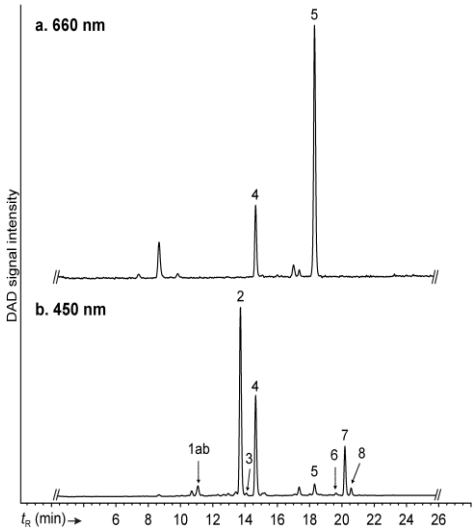

Fig. 1: HPLC-DAD chromatograms of chlorophylls (a) and carotenoids (b) from a representative date sample recorded at 660 and 450 nm, respectively. For compound assignment, see Table 1

C10-C11 (or C10´-C11´), or cyclic oxonium ions after epoxy-oxepinoid rearrangements of the protonated molecules of 3-hydroxy-5,6-epoxy xanthophylls; both resulting in a signal at *m/z* 221 (Table 1). Noteworthy, the MS^1 experiment of (all-*E*)-neoxanthin displayed prevailing precursor ions at *m/z* 583 ($[M + H - H_2O]^+$) from the in-source elimination of water, whereas protonated molecules at *m/z* 601 were only detected at low abundance. CID of the $[M + H]^+$ precursors resulted in the fragment ions at *m/z* 583, 565, 509, 491 and 221, as also observed for (all-*E*)-violaxanthin. In addition, the distinctive fragments observed at *m/z* 547 ($[M + H - 3\ H_2O]^+$) and 393, that may be attributed to the threefold elimination of water and the cleavage of the double bond in allylic position to the allenic carbon, respectively, supported the assignment of compound 1b as (all-*E*)-neoxanthin. CID of the $[M + H - H_2O]^+$ precursor ions at *m/z* 583 again resulted in fragments ions at *m/z* 565, 547, 509, 491 and 221, in addition to the distinctive fragment ions at *m/z* 375. The latter ions differed by 18 amu from the *m/z* 393 ions detected in the MS^2 experiment of protonated molecules and thus may be attributed to the aforementioned double bond-cleavage of the dehydrated precursor ion. To the best of our knowledge, the distinctive mass fragment at *m/z* 375 to differentiate neoxanthin (CID of $[M + H - H_2O]^+$) from violaxanthin has not been previously reported.

Similarly, compound 2 was assigned to (all-*E*)-lutein using an authentic reference standard, which displayed an abundant, characteristic signal of an in-source fragment ($[M + H - H_2O]^+$) at *m/z* 551 in the MS^1 experiment. In contrast to compounds 1a and 1b that were not detected in the negative ion mode, abundant molecular ions ($M^{-\bullet}$) were observed at *m/z* 568 in the MS^1 spectrum. As reported earlier, CID of the dehydrated species of (all-*E*)-lutein at *m/z*

Table 1: HPLC-DAD-APCI-MS^n data of carotenoids and chlorophylls from date fruit maturity stages

No.	t_R (min)	λ_{max} (nm)	D_B/D_{II} [a] (%)	D_{III}/D_{II} [b] (%)	$[M]^{-\bullet}$ (*m/z*)	$[M + H]^+$ (*m/z*)	APCI(+)-MS^n experiment (*m/z*)	Proposed structure
1a	11.1	266, 328, 417/441/470	6	87	n. d.	601	[601]: 583, 565, 509, 491, 221	(all-*E*)-Violaxanthin [d]
1b	11.1	266, 328, 413/438/466	8	87	n. d.	601 583 [c]	[601]: 583, 565, 547, 509, 491, 393, 221 [583]: 565, 547, 509, 491, 375, 221	(all-*E*)-Neoxanthin [d]
2	13.7	268, 332, sh423/446/474	11	47	568	551 [c]	[551]: 533, 495, 477, 459, 429	(all-*E*)-Lutein [d]
3	14.1	275, 343, sh427/452/478	6	24	568	569	[569]: 551, 533, 477, 459	(all-*E*)-Zeaxanthin [d]
4	14.7	463/600/648	-	-	n. d.	907	[907]: 629, 597, 569, 541	Chlorophyll *b* [d]
5	18.3	432/619/665	-	-	n. d.	893	[893]: 615, 583, 555	Chlorophyll *a* [d]
6	19.6	276, 339, sh424/447/470	44	4	536	537	[537]: 481, 457, 413, 401, 399, 387, 347, 321, 281, 177	(13*Z*)-β-Carotene
7	20.2	275, 344, sh429/453/478	5	21	536	537	[537]: 481, 457, 413, 401, 399, 387, 347, 321, 281, 177	(all-*E*)-β-Carotene [d]
8	20.6	258, 341, sh426/447/473	10	24	536	537	[537]: 481, 457, 413, 401, 399, 387, 347, 321, 281, 177	(9*Z*)-β-Carotene

t_R: retention time, λ_{max}: UV/Vis absorption maxima, sh: shoulder, n. d.: not detected
[a] D_B/D_{II}: ratio of absorption intensity at '*cis*-band' near UV maximum (D_B) to intensity at main absorption maximum (D_{II})
[b] D_{III}/D_{II}: ratio of absorption intensity at longest wavelength maximum (D_{III}) to D_{II}
[c] In-source elimination of water ($[M + H - H_2O]^+$)
[d] Verified by an authentic reference standard

551 resulted in product ions at *m/z* 533 ($[M + H - 2\ H_2O]^+$), 495 ($[M + H - H_2O - 56]^+$) and 477 ($[M + H - 2\ H_2O - 56]^+$). The aforementioned fragment ions may be attributed to additional water eliminations (18 amu) and a retro-Diels-Alder cleavage (56 amu) specific for carotenoid ε-rings. The carotenoid nature of the analyte was substantiated by a signal at *m/z* 459, representing a typical fragment ($[M + H - H_2O - 92]^+$) resulting from toluene elimination (92 amu) from the carotenoid-specific polyene chain. Another characteristic CID product ion at *m/z* 429 ($[M + H - 122]^+$) that has been related to the elimination of hydroxylated β-rings confirmed the assignment of compound 2 as (all-*E*)-lutein.

Compound 3 was identified as (all-*E*)-zeaxanthin by the aid of an authentic reference compound and its mass spectrometric behavior as follows. Molecular ions $M^{-\bullet}$ were observed at *m/z* 568 in the negative ion mode. In contrast to (all-*E*)-lutein, its isomer (all-*E*)-zeaxanthin displayed protonated molecules at *m/z* 569 in the APCI(+)-MS^1 spectrum. CID resulted in MS^2 fragment ions at *m/z* 551 ($[M + H - H_2O]^+$) and 533 ($[M + H - 2\ H_2O]^+$), indicating a xanthophyll carrying two hydroxyl-groups. Additional product ions were detected at *m/z* 477 ($[M + H - 92]^+$) and 459 ($[M + H - H_2O - 92]^+$), as described earlier. Furthermore, retention times and spectral data of compound 3 were identical to those of authentic (all-*E*)-zeaxanthin.

Furthermore, three β-carotene isomers (6, 7 and 8) were assigned. Regarding all three compounds, molecular ions ($M^{-\bullet}$) were observed at *m/z* 536 in negative ionization mode, while, in positive ion mode, protonated molecules ($[M + H]^+$) were found at *m/z* 537. CID of the $[M + H]^+$ yielded product ions at *m/z* 481, 401 and 399 that have previously been reported. The product ions at *m/z* 401 may have been released by the fission of the double bond between C7-C8. Further discriminative product ions were detected at *m/z* 457 ($[M + H - 80]^+$), being proposed to arise from the neutral loss methyl-cyclopentadiene. The product ion series at *m/z* 413, 387, 347, 321 and 281 may result from the cleavage of the single bonds between C6-C7, C8-C9, C10-C11, C12-C13 and C14-C15, respectively. The abundant product ions at *m/z* 177 have previously been reported to arise from the fragmentation of the C9-C10 double bond. These distinctive ions at *m/z* 177 may be stabilized *via* a cyclic structure. Besides their mass spectral data, the characteristic absorption spectrum displaying a shoulder at 429 nm in addition to the maxima at 453 and 478 nm led to the identification of compound 7 as (all-*E*)-β-carotene. In addition, retention times and spectral properties to an authentic (all-*E*)-β-carotene standard were identical to those of compound 7. Compounds 6 and 8 displayed identical mass fragmentations and ~4 nm shorter Vis λ_{max} compared to (all-*E*)-β-carotene (7) and thus, were assigned to corresponding mono-*cis*-isomers. Based on their D_B/D_{II} ratios of 44 and 10% as well as their elution order on a C30 stationary phase, compounds 6 and 8 were tentatively identified as (13*Z*)- and (9*Z*)-β-carotene, respectively. Such a detailed identification of chlorophylls and carotenoids in date fruit by multistage mass spectrometry (MS^n) has hitherto been unprecedented.

Quantitation of chlorophylls and carotenoids by HPLC-DAD

Total concentrations of chlorophylls, carotenoids and RAEs are compiled in Table 2. Dates harvested at the green-ripe and breaker maturity stage still contained chlorophylls *a* and *b*, summing up to 820–1,282 and 340–878 μg/100 g of fresh weight (FW) in dates harvested at the '*Kimri*' and '*Khalal*' stage, respectively. Chlorophylls were absent in the progressed maturity stages '*Rutab*' and '*Tamr*' (Fig. 2a).

Similarly, total carotenoids ranging between 283–411 μg/100 g of FW in dates of the '*Kimri*' stage dropped to 230–300 ('*Khalal*') and 90–231 μg/100 g of FW ('*Rutab*') with progressing maturation. Among the fully ripe '*Tamr*' fruits, merely those harvested from cv. 'Megadwel' contained low carotenoid concentrations of 30 μg/100 g of

Table 2: Quantitation of chlorophylls, carotenoids and RAEs (*µ*g/100 g of fresh weight, FW) in four date (*Phoenix dactylifera* L.) fruit varieties of four different maturity stages

Variety	Stage[a]	(all-*E*)-Violaxanthin + (all-*E*)-Neoxanthin	(all-*E*)-Lutein	(all-*E*)-Zeaxanthin	Chlorophyll *b*	Chlorophyll *a*	(13*Z*)-β-Carotene	(all-*E*)-β-Carotene	(9*Z*)-β-Carotene	Total chlorophylls	Total carotenoids	RAEs
'Anbra'	1	19 ± 2 bcd	228 ± 2 b	5 ± 0 a	385 ± 28 a	711 ± 9 ab	6 ± 0 e	36 ± 11 cdef	9 ± 1 f	1095 ± 37 a	304 ± 15 b	4 ± 1 efg
	2	14 ± 1 cde	197 ± 23 bc	4 ± 5 a	251 ± 30 b	627 ± 71 bc	8 ± 0 de	50 ± 4 bcdef	14 ± 1 def	878 ± 100 b	288 ± 35 bc	5 ± 0 cde
	3	n. d.	30 ± 2 e	n. d.	n. d.	n. d.	13 ± 1 bc	27 ± 5 f	19 ± 2 c	n. d.	90 ± 11 d	4 ± 1 efg
	4	n. d.	tr.	n. d.	n. d.	n. d.	n. d.	tr.	tr.	n. d.	n. d.	n. d.
'Megadwel'	1	20 ± 1 abc	182 ± 10 c	7 ± 0 a	276 ± 23 b	543 ± 35 cd	8 ± 0 de	53 ± 2 bcde	12 ± 0 ef	820 ± 58 b	283 ± 14 bc	5 ± 0 cde
	2	14 ± 0 de	123 ± 4 d	9 ± 1 a	114 ± 2 c	225 ± 0 e	15 ± 0 bc	108 ± 9 a	31 ± 0 b	338 ± 2 c	300 ± 4 b	11 ± 1 b
	3	n. d.	31 ± 2 e	tr.	n. d.	n. d.	35 ± 2 a	121 ± 11 a	44 ± 4 a	n. d.	231 ± 19 c	13 ± 1 a
	4	n. d.	n. d.	n. d.	n. d.	n. d.	tr.	30 ± 0 ef	tr.	n. d.	30 ± 0 d	2 ± 0 g
'Sfwai'	1	22 ± 1 ab	302 ± 4 a	6 ± 1 a	457 ± 0 a	850 ± 5	8 ± 0 de	60 ± 3 b	13 ± 0 def	1307 ± 6 a	411 ± 3 a	6 ± 0 c
	2	10 ± 0 e	126 ± 8 d	tr.	102 ± 4 c	238 ± 22 e	11 ± 0 cd	54 ± 1 bcd	28 ± 0 b	340 ± 26 c	230 ± 8 c	6 ± 0 c
	3	n. d.	8 ± 11 e	n. d.	n. d.	n. d.	16 ± 1 b	30 ± 1 def	26 ± 1 b	n. d.	80 ± 10 d	4 ± 0 def
	4	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	tr.	n. d.	n. d.	n. d.	n. d.
'Sacai'	1	26 ± 0 a	287 ± 8 a	6 ± 1 a	436 ± 7 a	847 ± 16 a	7 ± 1 de	67 ± 7 b	13 ± 1 def	1282 ± 23 a	406 ± 17 a	6 ± 1 c
	2	17 ± 3 bcd	202 ± 14 bc	tr.	214 ± 28 b	472 ± 59 d	7 ± 0 de	58 ± 5 bc	15 ± 1 cde	686 ± 87 b	300 ± 23 b	6 ± 0 cd
	3	n. d.	29 ± 4 e	n. d.	n. d.	n. d.	tr.	27 ± 3 f	18 ± 2 cd	n. d.	74 ± 9 d	3 ± 0 fg
	4	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.

[a]Maturity stages: '*Kimri*' (1), '*Khalal*' (2), '*Rutab*' (3), '*Tamr*' (4)
n. d., not detected (<LOD). tr.: trace (<LOQ), RAEs: retinol activity equivalents
Values represent means ± standard deviations ($n = 2$). Different letters in one column indicate significant ($p < 0.05$) differences of means

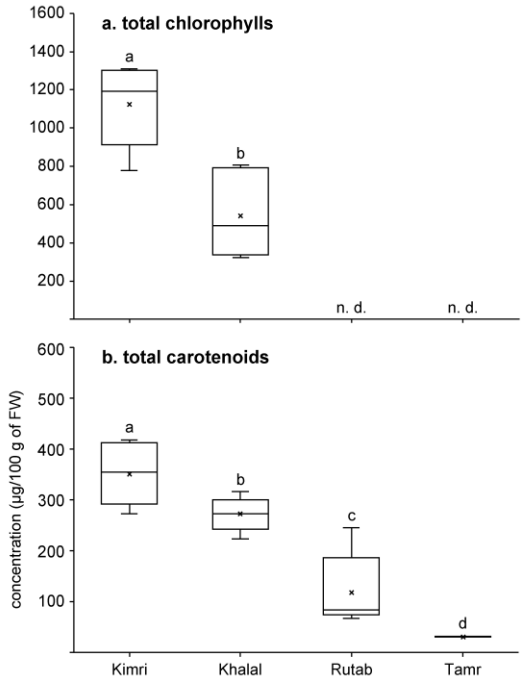

Fig. 2: Box plots illustrating the total chlorophylls and carotenoids (*µ*g/100 g of fresh weight, FW) in date (*Phoenix dactylifera* L.) fruit of ripening stages '*Kimri*' (green-ripe), '*Khalal*' (breaker), '*Rutab*' (brown-ripe), '*Tamr*' (dry-ripe) of the Middle Eastern varieties 'Anbra', 'Megadwel', 'Sfwai' and 'Sacai'. The boxes represent the 25 and 75% quartiles, the band inside the boxes the median (50% quartile). Whiskers indicate minimum and maximum, cross symbols the mean values. For concentrations of the individual pigments, see Table 2

FW (Fig. 2b). In general, this variety stood out by elevated concentrations of the provitamin A precursor (all-*E*)-β-carotene, amounting to 108, 121 and 30 *µ*g/100 g of FW in the three edible maturity stages '*Khalal*', '*Rutab*' and '*Tamr*', respectively.

Discussion

The early date fruit maturity stages displayed a chloroplast-specific pigment profile, thus being consistent with a previous study (Gross *et al.* 1983). In addition to chlorophylls *a* and *b*, chloroplast-bearing tissues contain (all-*E*)-violaxanthin, (all-*E*)-neoxanthin, (all-*E*)-lutein and (all-*E*)-β-carotene as the most abundant carotenoids (Schweiggert and Carle 2017). With progressing fruit development, in particular the concentrations of the prevailing carotenoid (all-*E*)-lutein dropped, contributing to 64–75 and 41–68% of the total carotenoids in the '*Kimri*' and '*Khalal*' samples, respectively. Lutein has been previously shown to selectively accumulate in the human retina and brain, mediating potential health benefits for human vision and cognitive functions (Johnson 2014). The prevailing pigment in the fruits harvested at the '*Rutab*' stage was (all-*E*)-β-carotene, together with its geometrical isomers, *i.e.*, (13*Z*)- and (9*Z*)-β-carotene accounting for 61–90% of the total carotenoids. According to the levels specified by Britton *et al.* (2009), date fruits are nutritional sources containing low to moderate carotenoid concentrations (low: 0–100 *µ*g/100 g of FW; moderate: 100–500 *µ*g/100 g FW). In agreement with our results, total carotenoid contents previously reported in the literature for dates harvested at '*Khalal*', '*Rhutab*' and '*Tamr*' stages ranged from 62–773, 33–167 and 51–145 *µ*g/100 g of FW, respectively, as determined earlier in 'Deglet-Nour', 'Hamraya' and 'Tantebouchte' varieties from Algeria (Boudries *et al.* 2007).

The nutritional value of date fruits regarding their provitamin A content may be estimated by RAE concentrations, exclusively contributed by β-carotene in case of the studied dates. The RAEs calculated from the concentrations of (13*Z*)-, (all-*E*)- and (9*Z*)-β-carotene of 4–6, 5–11 and 3–13 *µ*g RAE/100 g of FW as determined

across all '*Kimri*', '*Khalal*' and '*Rutab*' samples assessed were comparatively low. Among the '*Tamr*' fruits, merely 'Megadwel' contained low *i.e.*, 2 ± 0 µg RAE/100 g of FW. Thus, the RAEs determined herein were comparable to the 0.3–7.3 and 4 µg RAE/100 g of FW reported for pineapples and oranges, respectively (Solomons and Orozco 2003; Steingass *et al.* 2020). The aforementioned RAEs (1000 µg RAE/100 g of FW) are clearly exceeded by those reported for carrot roots, leafy vegetables (343 µg RAE/100 g of FW) or apricots (125 µg RAE/100 g of FW) (Solomons and Orozco 2003). However, as dates are consumed frequently in certain regions, they still may considerably contribute to the dietary supply with lutein and β-carotene.

Conclusion

The present contribution provides new insights into the pigment composition and development of ripening dates by liquid chromatography and mass spectrometry. From a nutritional point of view, '*Khalal*' and '*Rutab*' fruits represent valuable dietary sources of provitamin A and the potentially health-promoting carotenoid lutein. Date palms are adapted to very hot and dry habitats such as the deserts in Africa and the Arabian Peninsula, and thus, especially the early maturity stages may contribute to supply the local population with dietary carotenoids, providing RAEs similar to those of oranges and pineapples. Future studies may further explore the diversity of date chemotypes permitting the recommendation of certain cultivars for cultivation or the selection of promising accessions for breeding date palms bearing fruits with elevated provitamin A levels.

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