Carotenoid and Tocopherol Composition of an Orange-colored Carrot as Affected by Water Supply

Attila Ombodi¹
Institute of Horticulture, Faculty of Agriculture and Environmental Sciences, Szent István University; Páter Károly u. 1, Gödöllő, H-2100, Hungary

Hussein Gehad Daood¹
Central Food Research Institute, Herman Ottó u. 15, Budapest, H-1022, Hungary

Lajos Helyes
Institute of Horticulture, Faculty of Agriculture and Environmental Sciences, Szent István University; Páter Károly u. 1, Gödöllő, H-2100, Hungary

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Abstract. Carotenoids and tocopherols are important phytonutrients of orange-colored carrots. The main goal of this work was to investigate the effects of irrigation on the content and composition of carotenoids and tocopherols in an orange-colored carrot cultivar (Bangor) compared with a rain-fed control. The experiment was conducted for 2 years with a considerably different amount of precipitation during the growing season (576 mm in 2010 and 190 mm in 2011). Six carotenoids and four tocopherols were detected and quantitatively determined. Significant negative correlations were found between water supply and content of total carotenoids and total tocopherols. Irrigation significantly decreased the concentrations of these phytonutrients during the arid year of 2011. Water supply did not affect the carotenoid and the tocopherol composition, which can be an important factor for functional food manufacturers. A significant positive correlation was found between total carotenoid and total tocopherol concentrations, which is very favorable from a nutritional point of view.

Materials and Methods

Experimental conditions and plant material. The present work was conducted in 2010 and in 2011 at the Experimental Farm of the Institute of Horticulture, Szent István University, Gödöllő, Hungary (lat. 47°61’ N, long. 19°32’ E). The soil of the experimental site was loamy sand classified as Cambisol, having a pH of 7.20 and 7.13, electric conductivity of 0.25 and 0.26 dS·m⁻¹, and organic matter of 1.61% and 0.91% in 2010 and in 2011, respectively. Climatic parameters were recorded six times per hour by a Campbell CR21X meteorological instrument (Campbell Scientific Inc., Loughborough, U.K.). Daily potential evapotranspiration was calculated by FAO CROPWAT 8.0 software (FAO, Rome, Italy).

Seeds of the orange-colored, storage-type carrot cultivar Bangor (Bejo Zaden, Warmenhuizen, The Netherlands) were sown on 8 Apr. 2010 and on 7 Apr. 2011 into ridges having 20 cm height and 20 cm crown width. Center ridge to center ridge distance was 70 cm. Seeds were sown in twin rows at a distance of 60 cm and 10 cm between the rows and at 2 cm between the seeds in the rows, resulting in ~1.4 million seeds per hectare. Fertilizers were applied 1 d before the sowing and twice during the growing period at the total rate of 80N–15P–125K kg·ha⁻¹. Top-dressed fertilizers were spread on the top of the ridges when soil surface was wet and shallowly incorporated into the soil.

A rain-fed control and an irrigated treatment were compared. A randomized experimental design was used with four replications. Every plot comprised of five 12-m long ridges, resulting in a 42-m² area. The separating distance between rain-fed and irrigated plots was 3 m. To ensure adequate stand establishment, plants for both treatments were irrigated during the emergence period (the first 21 d of the growing period) with a total amount of 15 mm and 20 mm of water in 2010 and in 2011, respectively. Later, the rain-fed control plots were not irrigated at all. Irrigation was carried out with sectorial overhead sprinklers (Pegazus 3413/0480, 480 L·h⁻¹ flow rate at 200 kPa; Palaplast s.a., Thessaloniki, Greece) when wind speed was less than 2 m·s⁻¹. Irrigations were started when the soil water tension reached 30 kPa according to tensiometer readings (Tensiometer Classic 8060; Stelzner GmbH, Nürnberg, Germany). The exact amount of the supplied irrigation water was checked by using catch cans. Six catch cans (with an 85-mm inner diameter) were placed out in three × two grids, slightly above the canopy, in the middle part of every irrigated plot. An irrigation cutoff period was planned for the last 3 weeks of the growing period.

Harvests were done on 15 Sept. 2010 and 20 Sept. 2011. Sampling was done on the middle 8 m of the central ridge of each plot. The foliage was removed by hand. Immediately after soil residue removal, marketable roots (over 20 mm in diameter) were selected, counted, and weighed. Yield and average root weight were determined.

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¹Present address: Regional Science Center, Szent István University, Páter K. u. 1, Gödöllő, H-2100, Hungary.
²To whom reprint requests should be addressed; e-mail ombodi.attila@nikk.szie.hu.
Analytical measurements. From each replication a 1-kg sample with representative average root fresh weight (81 g for the 2010 rain-fed, 116 g for the 2010 irrigated, 45 g for the 2011 rain-fed, and 85 g for the 2011 irrigated treatment) was taken for chemical analysis. Dry matter content was determined after freeze-drying of homogenized carrot roots.

Fat-soluble pigments and tocopherols were extracted, in triplicate, from 3 g of well-homogenized carrot roots (raw materials) first by methanol and then by 1:5 methanol-1,2-dichloroethane after crushing in a crucible mortar according to a previously described procedure (Daood et al., 1987). Pigment-containing phase was dried over anhydrous Na2SO4 and evaporated to dryness under vacuum by a rotary evaporator at not higher than 40 °C. The residues were either redissolved in the high-performance liquid chromatography (HPLC) eluent (acetone) for analysis of carotenoids or saponified by refluxing with methanolic KOH at boiling point of methanol for 35 min for the analysis of tocopherols. After saponification, the tocopherol fraction was extracted by n-hexane, which was separated, evaporated to dryness under vacuum by a rotary evaporator at not higher than 40 °C. The residues were then redissolved in HPLC-grade n-hexane and filtered through a 0.45-μm Teflon filter before injection onto HPLC.

A Waters Alliance (Waters Co., Milford, MA) liquid chromatographic instrument consisting of a Model 2696 Separation Module and a Model 2695 photodiode-array detector was used for carotenoid analysis. Operation and data processing were performed by Empower Chromatography Data software (Empower Software Solutions, Orlando, FL). For tocopherol analysis, a combination of a Beckman 114M isocratic pump (Beckman Co., Kyoto, Japan), and a Waters-740 Data Module (Waters Co.) integrator was used. Separation of carotenoids was performed on crosslinked Nucleodur ISIS C-18 150 × 4.6 mm (Macherey-Nagel GmbH, Düren, Germany) with gradient elution of water and acetone according to Daood et al. (in press). The column effluents were detected between 190 and 600 nm. For quantification, peaks were integrated at the maximum absorption wavelength of each compound detected on chromatogram. Peak identification was based on comparison of retention time and spectral characteristics of compound from the samples with those of available standards such as β-carotene, zeaxanthin (Sigma-Aldrich, Budapest, Hungary), or tentatively on comparison of spectral properties with literature data.

In the analysis of tocopherols, the separation was performed on Nucleosil-100, 5 μm, 250 × 4.6 mm (Macherey-Nagel GmbH, Düren, Germany) with a mobile phase consisting of 99.5:0.5 n-hexane-ethanol. The fluorescent detector was set at 295 and 320 nm as the excitation and emission wavelengths, respectively. The peak of every measured tocopherol was identified on the basis of standard materials (Sigma-Aldrich, Budapest, Hungary) used as external standards and cochromatographed with the samples.

Statistical analysis. For statistical analysis, yield and carotenoid and tocopherol concentrations were calculated on a dry weight basis for assuring better comparability and expressed as the mean of four replications in tables and in Figure 1. Data were compared by two-way analysis of variance with water supply treatment, year, and treatment × year as main effects. Mean separations were performed using Fisher’s protected least significant difference test at P ≤ 0.05. Correlation and regression analyses were performed using SPSS 22 software (IBM Co., New York, NY).

Results and Discussion

Weather and irrigation conditions. Average air temperature was higher considerably, by 0.7 °C, in 2011 than in 2010 (Table 1). Except for July, monthly averages of daily maximum air temperatures were higher in 2011 than in 2010; the biggest difference, more than 8 °C, occurred in September. Daily fluctuation of air temperature was bigger in 2011. The sum of the calculated daily potential evapotranspiration values was 569 mm (3.5 mm·d−1) for the 2010 and 660 mm (4.0 mm·d−1) for the 2011 season.

There was a 3-fold difference in the precipitation of the two growth periods, being 576 mm in 2010 and 190 mm in 2011. Because of the high amount of precipitation, in 2010, only three irrigations were necessary to apply during a rainless period in July with a total amount of 69 mm. In 2011, 11 irrigations were applied with a total amount of 238 mm.

Dry matter yield. In both seasons, irrigation has significantly increased the dry matter yield (t·ha−1) of marketable carrots (Fig. 1). For the irrigated treatment, the season did not affect significantly the level of this parameter. However, in the case of the rain-fed control, the result was significantly lower in 2011 compared with 2010, because of the small amount of precipitation. A significant positive correlation was found between water supply (sum of precipitation and amount of irrigation water) and dry matter yield (y = 0.021x−1.16, R² = 0.558, N = 16, P ≤ 0.001).

Carotenoid concentration and composition. Six carotenoids were identified from the orange-fleshed carrot root examined in the present work (Table 2). In their work, Nicolle et al. (2004) and Tsukakoshi et al. (2009) dealt with less carotenoid compounds. Total carotenoid values [from 93 to 151 mg·kg−1 on a fresh weight (FW) basis] were found to be either close to (Baranski et al., 2012) or lower (Fikselová et al., 2010) than those reported for other orange-colored carrot cultivars under central European climatic conditions. Results for α- and β-carotenes (from 22.6 to 36.9 mg·kg−1 FW and from 47.2 to 76.2 mg·kg−1 FW, respectively) were lower, whereas those for zeaxanthin (from 2.5 to 3.3 mg·kg−1 FW) were higher than data reported for carrots in the National Nutrient Database for Standard Reference (U.S. Dept. Agr., Agr. Res. Serv., 2011).

![Fig. 1. Effect of irrigation on the dry matter yield of marketable carrot (cv. Bangor) roots.](image-url)
The highest level of total carotenoid content was recorded for the 2011 rain-fed treatment (Table 2), the treatment with the lowest water supply and consequently with the lowest dry matter yield (Fig. 1). Although statistically significant differences did not arise among the total carotenoid concentrations of the other three cases, a significant negative correlation was found between water supply and total carotenoid concentration ($y = –0.065x + 1214, R^2 = 0.653, N = 16, P ≤ 0.001$). Total carotenoid concentration also showed a strong negative correlation with dry matter yield of marketable roots ($y = –23.1x + 1111, R^2 = 0.657, N = 16, P ≤ 0.001$). It is presumable that the insufficient water supply resulted in too high plant temperature and as a result in lower biomass production and in higher carotenoid accumulation. It is well documented that elevated temperature and greater exposure to sunlight increase carotenogenesis (Dutta et al., 2005). Concentrations of α- (Tsukakoshi et al., 2009) and β-carotenes (Kaack et al., 2001) in carrots were found to be higher during warmer and sunnier years. Thus, decreased carotenoid concentration in carrot roots as a result of irrigation seems to be inevitable. Based on studies conducted with other vegetables having high carotenoid content, like tomatoes and watermelons, Leskovar et al. (2009) concluded that deficit irrigation can increase carotenoid levels, but at the risk of lowered yield. In the present study, during the arid year of 2011, concentration of α- and β-carotenes, the major and nutritionally the most important carotenoids of orange-colored carrots, and as a consequence the total carotenoid concentration, was significantly lower in the irrigation treatment compared with the rain-fed control (Table 2). On the other hand, water supply did not affect the concentrations of phytoene, ζ-carotene, and zeaxanthin within a given season, indicating that the concentrations of these carotenoids are more stable under different water supply conditions.

Because the different carotenoids have different biological functions in the human body (Dutta et al., 2005), their ratios are of special importance. Being the abundant carotene in orange-colored carrots, β-carotene accounted for 51% of the total carotenoid content (Table 2). This value is lower than the usual proportion reported for β-carotene in orange-colored carrots (Arscott and Tanumihardjo, 2010). The lower ratio is presumably the consequence of the wider range of carotenoids investigated in this work. The ratio of α-carotene was from 23% to 25%, and it was followed by the two ζ-carotene isomers, which together accounted for a 14% to 19% ratio. It was found that water supply did not affect significantly the ratio of any of the six measured carotenoids (Table 2). Hence, carotenoid composition of carrot root proved to be rather irrigation-independent.

Carotenoid composition also remained quite stable during the two seasons with the exception of the ratios of cis-ζ-carotene and zeaxanthin, which were significantly affected by the seasonal variations (Table 2). The lowest cis-ζ-carotene ratio was recorded for the 2010 irrigation treatment, which presented the most favorable conditions for carrots. The fact that heat promotes isomerization of trans carotenoids to their cis form (Dutta et al., 2005) can be the reason for the higher cis-ζ-carotene ratios in 2011 and in the 2010 rain-fed control. Based on these data, it seems the ratio of cis-ζ-carotene could be a good stress indicator for orange-colored carrots.

Based on results of HPLC analysis of the six individual carotenoids, it could be stated that water supply and even the seasonal variations have no pronounced effect on carotenoid composition in such a type of carrot (Table 2). This result is of special interest for the manufacture of functional food products, where the relatively stable ratio of bioactive compounds is of more importance than in the case of conventional food products.

Tocopherol concentration and composition. Tocopherols are very efficient lipid-soluble antioxidants, possessing a wide range of biological functions with no characteristic differences reported among the four (α, β, γ, and δ) homologs (DellaPenna and Méne-Safranne, 2011). Three tocopherols (α-, β-, and γ-tocopherol) and β-tocotrienol were detected and quantitatively determined in carrot root in this study (Table 3). Less tocopherol homologs and no tocotrienol have been reported in other carrot studies, probably as a result of different analytical procedures as well as different cultivars used (Koch and Goldman, 2005; Metzger and Barnes, 2009; Nicolle et al., 2004). Among the tocopherols, δ-tocopherol, which has the lowest antioxidant and vitamin E activity (DellaPenna and Méne-Safranne, 2011), could not be detected in carrot. Seeds and plant-derived oils are the major dietary sources of δ-tocopherol, whereas it is usually not present in vegetables, except for spinach (DellaPenna and Méne-Safranne, 2011; U.S. Dept. Agr., Agr. Res. Serv., 2011). Because tocotrienols have neuroprotective and anticancer properties not exhibited by tocopherols (Patel et al., 2011), it can be of nutritional importance that β-tocotrienol is present in carrot, although in small quantities. Based on the gathered information, we could not explain that of the four tocotrienol homologues why just the β form has accumulated.

Measured total tocopherol values (from 3.31 to 4.72 mg kg⁻¹ FW) are close to or lower than those reported for other carrot cultivars (Metzger and Barnes, 2009; Nicolle et al., 2004). Results for α-tocopherol (from 2.49 to 3.27 mg kg⁻¹ FW) were considerably lower, whereas those for β- and γ-tocopherols (from 0.12 to 0.67 mg kg⁻¹ FW and from 0.36 to 0.62 mg kg⁻¹ FW, respectively) were higher than data reported in the National Nutrient Database for Standard Reference (U.S. Dept. Agr., Agr. Res. Serv., 2011).

Tocopherol concentrations were significantly affected by the water supply, but it was not the case for β-tocotrienol, which has a slightly different biosynthesis pathway than tocopherols (DellaPenna and Méne-Safranne, 2011). Irrigation resulted in lower tocopherol concentrations as compared with the rain-fed control. The season also had a significant effect on contents of tocopherols, except the γ homolog. The season vs. water supply interaction also influenced the concentrations of α- and β-tocopherol (Table 3). Differences between the tocopherol concentrations of the rain-fed control and the irrigation treatment became higher during the arid year of 2011 than during the humid year of 2010.

Like in the case of carotenoids, the highest level of total tocopherol was found in the 2011 rain-fed treatment, and there was no significant difference between the total tocopherol concentrations of the rain-fed control and the irrigated treatment in 2010 (Table 3). DellaPenna and Méne-Safranne (2011) concluded that the functions of tocopherols in regard with plant stress are more limited than it had long been assumed. However, a weak but statistically significant negative correlation.
Different cultivars were cultivated under the same environmental conditions. Our results, derived from a single cultivar raised under different water supply conditions, support this theory and indicate that this correlation is also valid for different water supply conditions. Based on the available literature information, the significant negative correlations between \( \alpha \)-tocopherol and \( \beta \)-carotene is of the greatest importance in the case of orange-colored carrots. On the other hand, based on our data, the relationships between the less significant tocopherols and carotenoids were ambiguous. Beta-tocopherol had a strong correlation with the total tocopherol concentration as a result of its significant positive correlations with \( \beta \)-carotene, \( \gamma \)-carotene, and phytoene (Table 4). Neither a relationship between \( \gamma \)-tocopherol and carotenoids nor significant correlation between \( \beta \)-tocotrienol and total carotenoids could be found. Based on the available literature information, the significant negative correlations between \( \beta \)-tocopherol and \( \beta \)-carotene and between \( \beta \)-tocotrienol and phytoene could not be explained. Koch and Goldman (2005) also found unexplained significant negative correlations between \( \alpha \)-tocopherol and both phytoene and lycopene in roots of colored carrots.

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Tocopherol homologs have different antioxidant and vitamin E activities (\( \alpha > \beta > \gamma > \delta \)) (DellaPenna and Méne-Safranée, 2011); thus, their ratio is of importance in terms of antioxidant response and nutritional value. Water supply did not affect significantly the ratio of the individual tocopherols with the exception of \( \beta \)-tocopherol (Table 3). Unlike carotenoids, the seasonal variation had a pronounced effect on the tocopherol composition. The arid year of 2011 resulted in significantly lower \( \alpha \)-tocopherol and \( \beta \) -tocotrienol ratios and higher \( \beta \)-tocopherol ratio compared with the colder and rainy year of 2010. Based on the obtained results, we can state that tocopherol composition of carrot root was less stable than that of carotenoids.

**Correlations between carotenoids and tocopherols.** There was a significant positive correlation between the concentration of \( \alpha \)-tocopherol and the concentrations of zeaxanthin, \( \alpha \)-, and \( \beta \)-carotenes (Table 4). Because \( \alpha \)-tocopherol was the most abundant tocopherol, and \( \alpha \)- and \( \beta \)-carotenes were the most abundant carotenoids in our study; total carotenoid and total tocopherol concentrations also correlated. Parallel accumulation of \( \alpha \)-tocopherol and both \( \alpha \)- and \( \beta \)-carotenes was found in previous studies too (Koch and Goldman 2005; Nicolle et al., 2004). The authors explained this relationship by the facts that these two groups of phytochemicals share a common precursor (GGPP), play similar antioxidant roles in the photosynthetic process, and probably have a common regulating mechanism in their biosynthesis pathways. In these studies, the statement was based on data obtained from several

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**Table 3. Effect of water supply on the tocopherol and tocotrienol concentration (mg·kg\(^{-1}\) on a dry weight basis) and composition of carrot (cv. Bangor) root.**

| Yr  | Water supply | \( \alpha \)-tocopherol (mg·kg\(^{-1}\)) | \( \beta \)-tocopherol (mg·kg\(^{-1}\)) | \( \gamma \)-tocopherol (mg·kg\(^{-1}\)) | \( \beta \)-tocotrienol (mg·kg\(^{-1}\)) | Total tocopherols (mg·kg\(^{-1}\)) |
|-----|--------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|----------------------------------|
| 2010 | Rain-fed     | 23.6 a\(^1\) 74.5 a | 1.3 c 4.1 c | 4.6 a 14.5 | 2.2 a 6.8 a | 31.6 b |
|      | Irrigated    | 21.1 b 75.3 a | 1.0 c 3.7 c | 4.1 a 14.7 | 1.8 a 6.3 a | 28.0 b |
| 2011 | Rain-fed     | 25.2 a 68.9 b | 5.2 a 14.2 b | 5.0 a 13.4 | 1.2 b 3.3 b | 36.6 a |
|      | Irrigated    | 15.0 c 69.0 b | 3.8 b 17.7 a | 2.0 b 9.3 | 0.9 b 4.0 b | 21.7 c |

**Significance**

- **Yr Water supply**
- **Year**
- **Water supply**
- **Year \times water supply**

\(^{a}\)Mean separation in columns by Fisher’s protected least significant difference test at \( P \leq 0.05 \).

\(^{b}\)NS, *, **, *** Nonsignificant or significant differences at \( P \leq 0.05, 0.01, \) or 0.001, respectively (\( N = 16 \)).

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**Table 4. Correlation matrix of carotenoid and tocopherol components of carrot (cv. Bangor) root grown under different water supplies.**

| Zeaxanthin | \( \alpha \)-carotene | \( \beta \)-carotene | \( \gamma \)-carotene | cis-\( \gamma \)-carotene | Phytoene | Total carotenoids |
|-----------|----------------------|----------------------|----------------------|------------------------|----------|-------------------|
| \( \alpha \)-tocopherol | 0.508** | 0.580* | 0.502* | \(-0.059\) NS | 0.131 NS | \(-0.190\) NS | 0.505* |
| \( \beta \)-tocopherol | \(-0.559\)* | 0.376 NS | 0.567* | 0.668** | 0.735** | 0.616* | 0.678** |
| \( \gamma \)-tocopherol | 0.300 NS | 0.044 NS | 0.216 NS | \(-0.160\) NS | \(-0.074\) NS | \(-0.118\) NS | 0.115 NS |
| \( \beta \)-tocotrienol | 0.701** | 0.027 NS | \(-0.029\) NS | \(-0.356\) NS | \(-0.400\) NS | \(-0.566\)* | \(-0.118\) NS |
| Total tocopherols | 0.243 NS | 0.473 NS | 0.515* | 0.142 NS | 0.317 NS | \(-0.095\) NS | 0.537* |

\(^{ns, *, **, ***}\)Nonsignificant or significant at \( P \leq 0.05 \) or 0.01, respectively (\( N = 16 \)).
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