PAPER

VARIATIONS IN THE SUGARS AND ANTIOXIDANT COMPOUNDS RELATED TO ROOT COLOUR IN TUNISIAN CARROT (*DAUCUS CAROTA* SUBSP. *SATIVUS*) LANDRACES

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ABSTRACT

Carrot (*Daucus carota* L.) is the most widely consumed root vegetable since it is an important source of nutritional compounds, mainly antioxidants and sugars. In Tunisia, despite the genetic diversity observed in carrot germplasm, including landraces and wild relatives, no research has been conducted on the biochemical composition of carrot. Thus, this study aims to analyse carotenoids, soluble sugars, total phenols, total flavonoids and colour properties of 14 carrot landraces, in order to determine the diversity among them and evaluate the relationships among their biochemical contents. The main carotenoids identified were α-carotene, β-carotene and lutein. Orange carrots were richer in β-carotene and α-carotene than yellow carrots. The major sugars were sucrose, glucose, fructose and

galactose. Significant differences were observed among the Tunisian carrot landraces with respect to their biochemical composition and colour characteristics. Total carotenoids and total sugars ranged from 155.74 to 511.44 μg/g of dw and from 368.77 to 546.79 mg/g of dw, respectively. Total phenols and total flavonoids varied from 24.13 to 41.39 mg GAE/100 g of dw and from 16.51 to 24.85 μg CE/100 g of dw, respectively. Significant, positive and negative correlations were found among the measured parameters. A principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed to classify the Tunisian carrot landraces on the basis of colour properties and biochemical compounds. The PCA divided the landraces into four main groups and AHC classified them into two major clusters. The Tunisian carrot landraces were found to be rich in bioactive compounds; they could be good candidates for future breeding programs.

Keywords: antioxidant compounds, carotenoids, carrot, colour properties, sugars

1. INTRODUCTION

Carrot (*Daucus carota* L.) is one of the major vegetable crops grown worldwide (RUBATZKY *et al.*, 1999). This vegetable belongs to the *Apiaceae* family, being the most widely used member. The first carrots, with purple and yellow colours, originated in Central Asia, Asia Minor, Western Europe and England between the 11th and 15th centuries (BANGA, 1963). The orange carrot was domesticated in Europe between the 15th and 16th centuries (BANGA, 1957; STOLARCZYK and JANICK, 2011). Due to its nutritional value, moderate price and consumption modes, carrot is the most consumed root vegetable in the world (CHAUX and FOURY, 1994). Thus, its roots are eaten as crunchy salad; used to prepare juice, sweets and halwa; cooked with mixed vegetables; and preserved by salting, pickling, canning and drying (SINGH *et al.*, 2018).

Compared to other vegetables, carrot possesses the highest amount of carotenoids, defined as organic pigments that naturally occur in the chloroplasts and chromoplasts of plants (ZIELINSKA and MARKOWSKI, 2012). These bioactive compounds have an antioxidant activity, protecting plants against oxidative stress, and have been proven to be beneficial to human health, reducing oxidative stress by scavenging free radicals (TAN and NORHAIZAN, 2019; STAGOS, 2020).

Depending on its carotenoids content, the carrot root can be purple, orange, yellow or white (BARANSKI, 2012). β-carotene constitutes the major portion (60–80%) of the carotenoids, followed by α-carotene (10–40%) and lutein (1–5%), whereas the rest are other minor carotenoids (SUN and TEMELLI, 2006).

In general, carrot has great acceptability among consumers due to its sweetness, which is governed by its sugars content (SIMON *et al.*, 1980a). These compounds represent an important sensory indicator for consumers. Moreover, sweetness represents one of the most important factors in the acceptance of new commercial vegetable cultivars (NOOKARAJU *et al.*, 2010). The most abundant sugar found in carrot is sucrose, followed by glucose and fructose. The total sugars content in fresh carrot varies from 3% to 10%, whereas soluble sugars can represent up to 70% of the dry carrot (DOLORES *et al.*, 1999; CAZOR *et al.*, 2006).

Besides sugars and carotenoids, which determine carrot sweetness and colour, phenolic compounds also contribute to their organoleptic properties (RUBATZKY *et al.*, 1999). The major phenolic acids present in carrot are chlorogenic acid, caffeic acid, *p*-hydroxybenzoic acids, ferulic acid and other isomers of hydroxy cinnamic acid (ALASALVAR *et al.*, 2001). The content of these compounds varies depending on root colour. Chlorogenic acid is the main phenolic acid and represented 52.4, 57.1, 51.4, and 72.5% of the total phenolic compounds in orange, yellow, white, and purple carrots, respectively (SUN *et al.*, 2009). The different carrot tissues have similar composition, but the contents of individual phenolic compounds differ and they decrease from the exterior to the interior (GONÇALVES *et al.*, 2010). Phenolic compounds are involved in the resistance to physiological mechanism of plants, and their accumulation in carrots results from exposure to cold, injury or ethylene (RUBATZKY *et al.*, 1999). Flavonoids are represented mainly by anthocyanins, which are richer in purple carrot. They exert beneficial effects on human health, acting as vasodilators (CHENG et al., 1993) and platelet disaggregators (GRYGLEWSKI *et al.*, 1987), and also as efficient antioxidants with free radical scavenging abilities (BAHORUN *et al.*, 2003).

In Tunisia, carrots are widely cultivated with an annual production of 201.500 tons, representing 5% of the total vegetable production. They are produced on 5.700 ha of land (~94% as a winter crop and 6% as a summer crop) with an average yield of 35.35 tons/ha

(DGPA, 2019). Although these locally produced carrots can be yellow or orange, Tunisians prefer the latter. Tunisia is considered a center of biodiversity for *Daucus* and many other crops because of the diversity of ecosystems and climatic conditions (LE FLOC'H *et al.*, 2010). This diversity has been the subject of several studies in order to identify and enhance this genetic heritage. Thus, MEZGHANI *et al.* (2014; 2017; 2018) studied the genetic diversity of wild carrot, based on morphological and molecular data. This research revealed a great genetic variability among the accessions. Moreover, BEN AMOR *et al.* (2019) characterized Tunisian carrot (*Daucus carota* subsp. *sativus*) landraces collected from different regions by using agro-morphological descriptors related to roots and leaves. The study showed a high phenotypic variability, particularly in root colour, which could be reflected by the biochemical composition and content. Thus, the aim of this work was to determine the contents of individual carotenoids and sugars, as well as the total phenols and flavonoids, in different Tunisian carrot landraces, and to classify these landraces on the basis of their biochemical compounds and colour properties.

2. MATERIALS AND METHODS

2.1. Plant material

The study material consisted of 14 carrot (*Daucus carota* subsp. *sativus*) landraces, derived from a collection of 33 carrot landraces originating from the main regions of carrot cultivation in Tunisia and conserved at the National Gene Bank of Tunisia. The landraces were selected to maximize the phenotypic diversity, based on our previous study (BEN AMOR *et al.*, 2019). Seeds obtained from self-pollinated landraces were sown in the experimental site of the High Agronomic Institute of Chott Mariem in Tunisia (35.1182 N; 10.7297 E), in November 2016. At the maturity stage, the carrots were manually harvested. Uniform roots (five per landrace) were selected, and washed with tap water to remove soil and other dirt. Representative samples (500 g) of each set of five roots were taken for subsequent analysis.

2.2. Colour measurements

The colour measurements were performed on the skin of the carrot roots using a Minolta chromameter (CR-410). The measurements obtained are reported in the L*, a*, b* systems. The L* value varies from 0 to 100, representing the darkness or lightness of colour. The a* value ranges from green (-a*) to red (+a*). The b* value varies from blue (-b*) to yellow (+b*). The chroma C* and hue angle H^0 were also calculated, using the following equations: $C^* = (a^{*2} + b^{*2})^{1/2}$; $H^0 = \tan^{-1}(b^*/a^*)$. The C* value shows the saturation of colour and it is proportional to the colour intensity. The H^0 is the basic unit of colour and varies from 0^0 to 90^0, indicating red and yellow colour, respectively.
After the colour measurements, the roots were cut into slices and lyophilized. Dry samples were ground and the powders were stored in a refrigerator until analysis.

2.3. Extraction and analysis of carotenoids

Carotenoids were extracted from the lyophilized carrot (0.5 g) using 35 ml of methanol/tetrahydrofuran (1:1, v/v) containing 0.1% butylated hydroxytoluene. The mixture was blended for 5 min and then vacuum filtered through Whatman™ no.5 filter

paper (Whatman, England). The extraction was performed three times, leaving an uncoloured residue. The combined extracts were dried under vacuum at 37 °C in a rotary evaporator. The residue was re-dissolved in a methanol/*tert*-butyl methyl ether mixture (1:1, v/v) until the solution reached a final volume of 10 ml. The solution was centrifuged for 10 min at 14 000 rpm (at 4 °C) and then analyzed (BÖHM, 2001). The experiment was conducted under dark conditions in order to avoid carotenoids degradation. The quantification of carotenoids was carried out using high-performance liquid chromatography (HPLC) with diode array detection (DAD). The HPLC analysis was performed with methanol (solvent A) and methyl *tert*-butyl ether (solvent B), using a gradient procedure on a C_{30} column (250 mm x 4.6 mm, 5 μm, Trentec, Gerlingen, Germany) at 17 °C and a flow rate of 1.3 ml min^{-1}. Carotenoids were quantified at 450 nm and were identified on the basis of the retention time, as described by CHEN and TANG (1998), and according to the DAD spectra. Standard solutions of the main carotenoids were used to prepare calibration lines, in order to determine the concentrations corresponding to the different peaks of the chromatograms. The concentrations of individual carotenoids were expressed as μg/g of dry weight (μg/g of dw).

2.4. Extraction and analysis of soluble sugars

The measurement of the soluble sugars content was performed using HPLC, according to the method described by NOMURA *et al.* (1995). A 0.5 g sample of lyophilized carrot powder was extracted with 5 ml of 80% ethanol (v/v). The mixture was homogenized and then left in an ultrasound bath for 30 min at 60 °C, before centrifugation at 4 500 rpm for 15 min. The supernatant was recovered and the extraction was repeated twice more. The combined extracts were dried under vacuum at 80 °C in a rotary evaporator. The residue was re-dissolved in 2 ml of water, filtered and finally analyzed with HPLC. The chromatographic separation of the sugars was carried out with a Carbo Sep CHO-682 column (7.8 × 300 mm) held at 80 °C, with distilled water as the mobile phase at a flow rate of 0.4 ml min^{-1}. The temperature for the refractive index detector was set at 45 °C.
Standard solutions of sucrose, glucose, fructose and galactose were prepared and calibration lines were made for each one in order to determine the concentrations corresponding to the different peaks in the chromatograms. The concentrations of sugars were expressed as mg/g of dry weight (mg/g of dw).

2.5. Extraction and analysis of total phenols and flavonoids

2.5.1 Samples preparation

Total phenols and total flavonoids were extracted from 0.5 g of lyophilized carrots with 4 ml of an acidified methanolic solution (80% MetOH + 1% formic acid). The mixture was homogenized, left in an ultrasound bath for 5 min and finally centrifuged for 10 min at 5 000 rpm. The supernatant was collected for the analysis of total phenols and total flavonoids.

2.5.2 Analysis of total phenols

The quantification of total phenols was performed using a spectrophotometric technique based on the Folin-Ciocalteu method described by SINGLETON and ROSSI (1965). In microcuvettes, 1 ml of the sample, 500 μl of diluted Folin-Ciocalteu reagent (1:10, v/v) and

400 µl of Na_2CO_3 were mixed. The absorbance was measured at 750 nm after 2 h at room temperature. Gallic acid was used as the standard and the data were expressed as mg of gallic acid equivalents/100 g of dry weight (mg GAE/100 g of dw).

2.5.3 Analysis of total flavonoids

The quantification of total flavonoids was conducted spectrophotometric method described by DEWANTO *et al.* (2002). An aliquot of 100 µl of each methanolic sample was mixed with 625 µl of distilled water and 37.5 µl of $NaNO_2$ (5%). After 6 min, 37 µl of $AlCl_3$ were added and the mixture was left for 5 min. Finally, 250 µl of 1M NaOH and 162.5 µl of distilled water were added to the mixture. The absorbance was measured at 510 nm. Catechin was used as the standard and the data were expressed as µg of catechin equivalents/100 g of dry weight (µg CE/100 g of dw).

2.6. Statistical analysis

Statistical analyses were performed using appropriate packages in R software, available from the Comprehensive R Archive Network (CRAN) at http://CRAN.R-project.org/. For all parameters an analysis of variance (ANOVA) was carried out using the rcmdr package, to determine differences among cultivars. The data are expressed as the mean and standard deviation for each parameter. A Pearson correlation analysis was carried out to estimate the relationships among the studied parameters. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed using the FactoMineR package, to determine relationships among the biochemical parameters and to group landraces into homogenous classes.

3. RESULTS

3.1. Colour parameters

The colour characteristics measured showed significant variability among the carrot landraces (Table 1). The a* value, indicating the intensity of red colour, ranged from 3.62 for NGBTUN560 to 27.73 for NGBTUN539. For yellowness b*, NGBTUN520 and NGBTUN556 showed the highest and lowest values, respectively. All carrot landraces had high values of C* (> 50), which could be due to the high intensity and saturation of the colour. Also, all landraces had high values of the lightness parameter L* and the hue angle H^0; in general, these values were higher in yellow landraces than in orange landraces.

3.2. Carotenoids content

The analysis of carotenoids with HPLC enabled the identification of three major carotenoids: α-carotene, β-carotene (the most abundant one in all samples) and lutein. In addition, the total carotenoids content was estimated as the sum of the individual carotenoids. The ANOVA showed that the individual and total contents of carotenoids depended to a significant extent on the carrot landrace (Table 2). The α-carotene content varied from 71.36 µg/g of dw for NGBTUN558 to 159.34 µg/g of dw for NGBTUN572. The content of β-carotene was highest for NGBTUN539 (418.58 µg/g of dw) and lowest for NGBTUN560 (71.74 µg/g of dw). For lutein, NGBTUN520 had the highest content

(68.99 μg/g of dw). The total carotenoids content ranged between 155.74 μg/g of dw (NGBTUN560) and 511.44 μg/g of dw (NGBTUN539), showing significant and marked differences among landraces.

Table 1. Colour measurements of 14 Tunisian carrot landraces[1].

Landrace	Skin colour	a*	b*	C*	H⁰	L*
NGBTUN512	Yellow	11.94±0.38 [f]	58.97±1.04 [e]	60.16±1.09 [ef]	78.55±0.15 [d]	81.38±0.04 [b]
NGBTUN514	Yellow	7.14±0.1 [gh]	54.98±0.32 [g]	55.44±0.33 [g]	82.59±0.06 [c]	74.32±0.59 [e]
NGBTUN520	Yellow	7.56±0.18 [g]	66.44±0.32 [a]	66.86±0.33 [a]	83.5±0.12 [b]	83.85±0.15 [a]
NGBTUN522	Yellow	6.15±0.03 [i]	60.74±0.17 [cd]	61.05±0.18 [e]	84.21±0.01 [b]	83.99±0.01 [a]
NGBTUN523	Yellow	6.95±0.5 [ghi]	62.37±1.44 [b]	62.75±1.48 [d]	83.64±0.31 [b]	80.51±1.04 [bc]
NGBTUN527	Orange	14.67±0.02 [e]	51.07±0.09 [h]	53.13±0.09 [h]	73.97±0 [g]	79.36±1.01 [c]
NGBTUN539	Orange	27.73±0.43 [a]	59.27±0.39 [e]	65.43±0.53 [b]	64.92±0.20 [j]	74.06±1.17 [e]
NGBTUN540	Orange	19±0.81 [c]	61.66±0.68 [bc]	64.52±0.40 [bc]	72.86±0.87 [h]	79.31±1.19 [c]
NGBTUN541	Orange	16.16±0.75 [d]	61.38±0.3 [bcd]	63.47±0.48 [cd]	75.25±0.58 [f]	79.52±0.63 [c]
NGBTUN556	Orange	18.6±1.09 [c]	50.81±0.66 [h]	54.1±1.00 [h]	69.9±0.84 [i]	76.78±1.15 [d]
NGBTUN558	Yellow	6.43±0.09 [hi]	60.48±0.1 [d]	60.82±0.90 [e]	83.93±0.09 [b]	83.73±0.61 [a]
NGBTUN560	Yellow	3.62±0.51 [j]	59.1±0.31 [e]	59.21±0.34 [f]	86.49±0.47 [a]	84.84±0.19 [a]
NGBTUN567	Yellow	14.73±0.09 [e]	59.19±0.2 [e]	60.99±0.17 [e]	76.02±0.13 [e]	75.87±0.28 [d]
NGBTUN572	Orange	21.34±0.28 [b]	56.66±0.4 [f]	60.54±0.47 [e]	69.36±0.12 [i]	76.72±1.22 [d]
p value	-	<0.0001	<0.0001	<0.0001	<0.0001	<0,0001
F value	-	613.53	157.14	122.88	844.81	62.86

[1]Data are expressed as the mean and standard deviation. [a-j]Different letters in the same column indicate significant differences for $p<0.05$.

3.3. Soluble sugars content

The main soluble sugars detected in the Tunisian carrot landraces were glucose, fructose, sucrose and galactose, with significant differences among the landraces (Table 3). Glucose and fructose were the most abundant soluble sugars, followed by sucrose and galactose. In general, the individual sugar contents were: for glucose >96.9 mg/g of dw, for fructose >119 mg/g of dw, for sucrose >46.2 mg/g of dw and for galactose >1.13 mg/g of dw, except for NGBTUN558 and NGBTUN560 in which galactose was not detected. The total sugars content, represented as the sum of the individual sugars, varied from 368.77 to 546.79 mg/g of dw for NGBTUN522 and NGBTUN540, respectively.

3.4. Total phenols and total flavonoids contents

The total phenols and total flavonoids were also analyzed and, as for the carotenoids and sugars, their contents varied significantly among the carrot landraces (Table 4). For total phenols, the highest content was recorded for NGBTUN520 (41.39 mg GAE/100 g of dw), whereas NGBTUN527 showed the lowest content (24.13 mg GAE/100 g of dw). The total flavonoids content ranged from 16.51 to 24.85 μg CE/100 g of dw for NGBTUN539 and

NGBTUN572, respectively. It is noteworthy that the flavonoids represented a small proportion of the total phenolic compounds in carrots.

Table 2. Content of individual and total carotenoids, expressed as μg/g of dry weight, in 14 Tunisian carrot landraces[1].

Landrace	α-carotene	β-carotene	Lutein	Total carotenoids
NGBTUN512	76.28±0.27 ^{ef}	116.77±1.46 ^{f}	61.36±1.93 ^{b}	254.41±0.19 ^{e}
NGBTUN514	79.00±1.48 ^{e}	113.31±1.66 ^{f}	50.10±2.66 ^{c}	242.41±2.83 ^{e}
NGBTUN520	77.12±0.19 ^{ef}	106.77±1.73 ^{fg}	68.99±2.94 ^{a}	252.88±4.86 ^{e}
NGBTUN522	73.64±0.06 ^{fg}	100.32±0.54 ^{g}	33.28±0.91 ^{de}	207.25±1.52 ^{f}
NGBTUN523	71.49±0.09 ^{g}	85.44±1.29 ^{h}	29.65±3.51 ^{e}	186.58±4.89 ^{g}
NGBTUN527	109.73±2.07 ^{d}	235.68±4.31 ^{c}	6.07±0.05 ^{i}	351.48±6.43 ^{c}
NGBTUN539	79.76±2.06 ^{e}	418.58±8.42 ^{a}	13.1±0.07 ^{h}	511.44±10.55 ^{a}
NGBTUN540	76.94±0.60 ^{ef}	210.98±7.33 ^{d}	13.17±2.38 ^{h}	301.09±10.31 ^{d}
NGBTUN541	122.72±3.21 ^{b}	203.29±13.91 ^{d}	22.81±8.29 ^{f}	348.82±18.99 ^{c}
NGBTUN556	121.72±0.86 ^{b}	168.73±5.8 ^{e}	5.17±0.84 ^{i}	295.62±7.5 ^{d}
NGBTUN558	71.36±0.1 ^{g}	74.99±0.39 ^{hi}	13.91±2.61 ^{gh}	160.26±3.11 ^{h}
NGBTUN560	72.11±0.19 ^{g}	71.74±0.44 ^{i}	11.89±1.69 ^{h}	155.74±2.32 ^{h}
NGBTUN567	115.38±3.78 ^{c}	201.31±2.06 ^{d}	35.79±1.76 ^{d}	352.49±4.08 ^{c}
NGBTUN572	159.34±4.99 ^{a}	292.09±17.21 ^{b}	18.77±3.67 ^{fg}	470.21±25.88 ^{b}
p value	<0.0001	<0.0001	<0.0001	<0.0001
F value	514.05	595.30	129.76	337.71

[1]Data are expressed as the mean and standard deviation. [a-i]Different letters in the same column indicate significant differences for $p<0.05$.

3.5. Correlation analysis

Pearson correlation coefficients (r) were calculated to determine the relationships among the studied parameters. A total of 61 features were correlated at the 0.05 or 0.01 significance level (Fig. 1). Total carotenoids were significantly and positively correlated with β-carotene (r=0.96) and the redness value a* (r=0.91), but were negatively correlated with the hue angle H^0 (r=-0.90) and the lightness parameter L* (r=-0.70). The hue angle H^0 was positively and significantly correlated with the lightness parameter L* (r=0.71), but negatively correlated with the redness value a* (r=-0.98), β-carotene (r=-0.90) and galactose (r=-0.71). Significant and positive correlations were also observed between β-carotene and the redness value a* (r=0.93); the yellowness value b* and the chroma C* (r=0.91). The total sugars showed a significant and positive correlation with fructose and glucose (r=0.79 and r=0.73, respectively) whereas glucose had a significant and negative correlation with galactose (r=-0.80).

Table 3. Content of individual and total sugars, expressed as mg/g of dry weight, in 14 Tunisian carrot landraces[1].

Landrace	Sucrose	Glucose	Fructose	Galactose	Total sugars
NGBTUN512	85.93±0.31 [d]	125.19±8.64 [ef]	200.15±36.94 [bc]	5.99±1.33 [d]	417.27±26.66 [def]
NGBTUN514	89.57±4.49 [d]	137.28±1.11 [de]	157.35±18.44 [cd]	4.88±0.56 [e]	388.90±13.40 [ef]
NGBTUN520	73.73±1.28 [e]	173.28±2.18 [c]	183.04±13.93 [bc]	1.43±0.08 [h]	431.49±17.48 [cde]
NGBTUN522	60.27±1.58 [f]	133.57±2.18 [e]	170.38±6.9 [bc]	4.54±0.18 [e]	368.77±10.85 [f]
NGBTUN523	46.20±3.18 [g]	188.18±13.96 [b]	194.43±4.93 [bc]	1.13±0.10 [h]	429.95±12.31 [cde]
NGBTUN527	137.7±14.29 [a]	124.02±1.22 [ef]	119.07±9.00 [d]	8.04±0.51 [c]	388.84±18.39 [ef]
NGBTUN539	123.95±1.27 [b]	118.18±5.19 [f]	156.79±20.15 [cd]	10.61±0.10 [b]	409.54±23.98 [def]
NGBTUN540	120.66±3.30 [b]	171.17±1.02 [c]	252.03±75.64 [a]	2.92±0.09 [g]	546.79±73.27 [a]
NGBTUN541	85.33±4.15 [d]	147.07±11.16 [d]	190.80±17.55 [bc]	3.47±0.72 [fg]	426.67±2.96 [cde]
NGBTUN556	100.22±8.05 [c]	96.90±5.84 [g]	159.55±14.61 [cd]	14.98±1.47 [a]	371.65±27.03 [f]
NGBTUN558	68.37±3.23 [ef]	245.47±5.84 [a]	219.52±17.68 [ab]	0.00 [i]	533.36±26.75 [a]
NGBTUN560	64.91±0.63 [ef]	240.00±0.37 [a]	193.28±22.68 [bc]	0.00 [i]	498.19±22.42 [ab]
NGBTUN567	121.13±2.25 [b]	171.63±5.02 [c]	171.80±18.42 [bc]	7.47±0.25 [c]	472.04±15.90 [bc]
NGBTUN572	50.49±2.19 [g]	188.56±15.47 [b]	206.12±11.71 [abc]	4.18±0.04 [ef]	449.35±29.41 [bcd]
p value	<0.0001	<0.0001	0.0005	<0.0001	<0.0001
F value	101.53	106.69	4.35	145.43	12.33

[1]Data are expressed as the mean and standard deviation. [a-i]Different letters in the same column indicate significant differences for $p<0.05$.

Table 4. Contents of total phenols and total flavonoids, expressed as mg of gallic acid equivalents/100 g of dry weight and μg of catechin equivalents/100 g of dry weight, respectively, in 14 Tunisian carrot landraces[1].

Landrace	Total phenols	Total flavonoids
NGBTUN512	33.29±0.24 [cd]	20.95±2.19 [c]
NGBTUN514	32.38±0.98 [d]	23.06±0.34 [b]
NGBTUN520	41.39±0.65 [a]	24.57±0.35 [a]
NGBTUN522	33.10±0.26 [cd]	20.56±0.78 [cd]
NGBTUN523	27.19±0.64 [gh]	17.56±0.01 [fg]
NGBTUN527	24.13±0.34 [i]	19.36±1.39 [cde]
NGBTUN539	36.70±1.55 [b]	16.51±0.08 [g]
NGBTUN540	27.01±0.17 [gh]	20.79±0.26 [c]
NGBTUN541	28.43±0.37 [f]	19.53±1.55 [cde]
NGBTUN556	33.98±0.17 [c]	22.91±0.30 [b]
NGBTUN558	29.70±0.17 [e]	19.06±0.34 [def]
NGBTUN560	24.60±0.83 [i]	18.41±0.88 [ef]
NGBTUN567	26.36±0.30 [h]	19.64±0.20 [cde]
NGBTUN572	28.07±0.08 [fg]	24.85±0.04 [a]
p value	<0.0001	<0.0001
F value	184.39	23.86

[1]Data are expressed as the mean and standard deviation. [a-i]Different letters in the same column indicate significant differences for $p<0.05$.

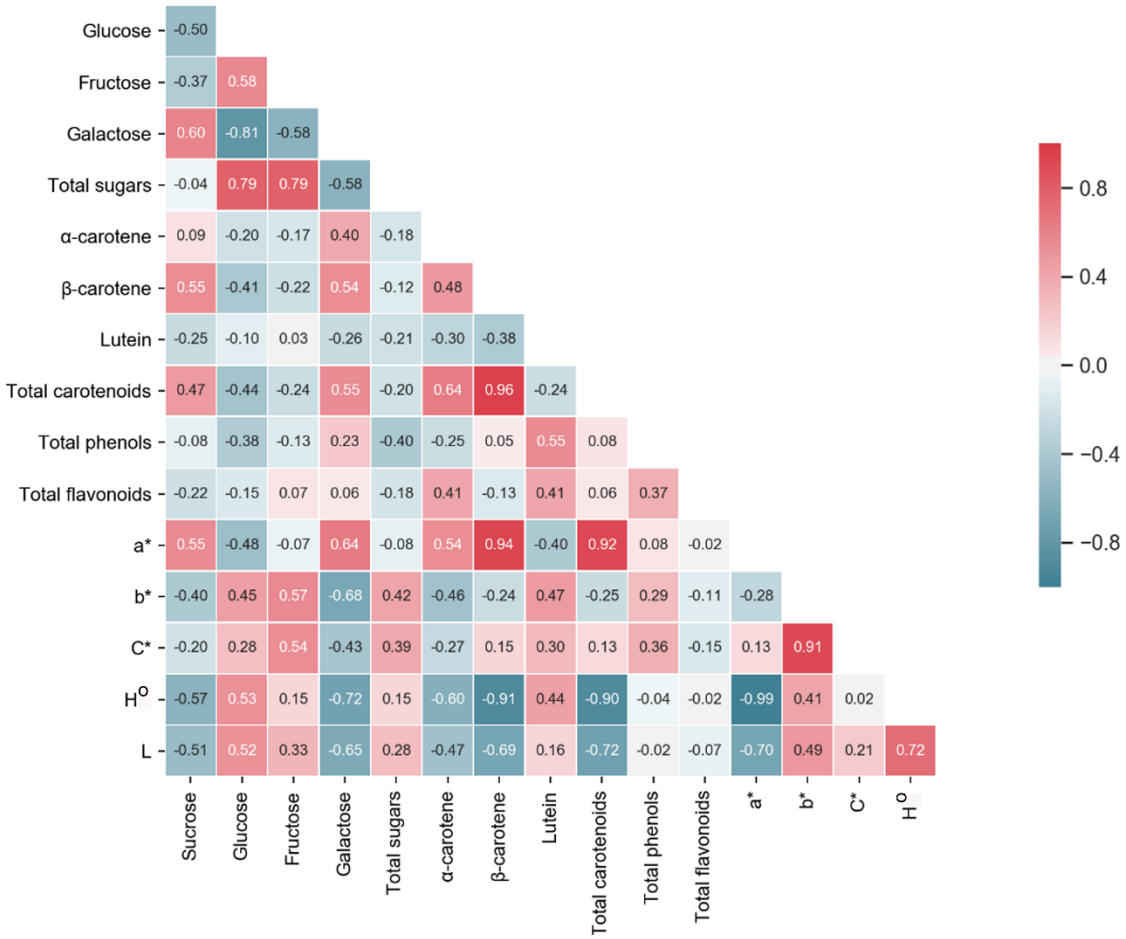

Figure 1. Pearson correlation coefficients among biochemical and colour parameters of 14 Tunisian carrot landraces. The intensity of the pink and blue colours indicates the significance of the correlation existing between each pair of studied parameters. The more the colour turns towards dark pink, the more significant is the positive correlation. The more the colour turns toward dark blue, the more significant is the negative correlation.

3.6. Multivariate analysis

A principal component analysis (PCA) was conducted to determine the differences in the studied parameters among Tunisian carrot landraces. This analysis generated 10 axes with distinct percentage contributions to the total variance (Fig. 2). The contribution of each parameter to the first five principal components is shown in Fig. 3. The first three principal components accounted for 76.1% of the total variance. The first axis explained 42.8% and was associated with the hue angle H^0, galactose, the redness value a* and total carotenoids. The second axis explained 18.2% and was associated with total sugars, the chroma C* and fructose. The third axis represented 15.1% of the total variance and was correlated with total phenols. The PCA scatter plot defined by the two principal components (Fig. 4) separated the carrot landraces into four distinct groups. The first group (G1) included NGBTUN539, 541, 567 and 572, originated from Kairouan, Sfax, Slimane and Siliana, respectively, and having similar contents of α-carotene, β-carotene, total carotenoids and sucrose and similar redness values a*, which were positively

correlated among themselves (Fig. 1). NGBTUN540, cultivated in Sfax, diverged from all the other landraces and formed the second group (G2) due to its high contents of total sugars and fructose (Table 3). The third group (G3) was formed by NGBTUN527 and 556, from Sidi-Bouzid and Gabes, respectively, which had higher contents of galactose than the other landraces (Table 3). NGBTUN512, 514, 520, 522 and 523, from Monastir (Moknine, Teboulba), and NGBTUN558 and 560, from Nabeul (Manzel-Temime), formed the fourth group (G4). These landraces are characterized by a yellow colour, related to the presence of lutein.

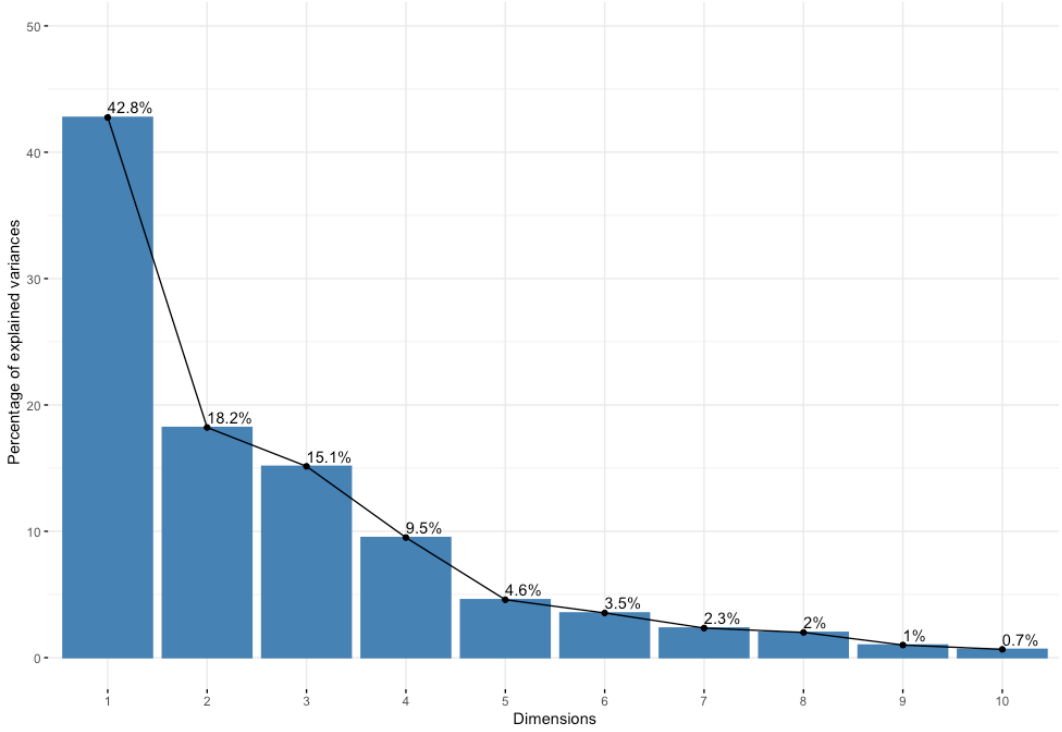

Figure 2. Decomposition of the total variation among the components of the PCA based on biochemical parameters of Tunisian carrot landraces.

Hierarchical clustering (AHC) was also performed and permitted the grouping of landraces on the basis of similarities (Fig. 5). Total carotenoids, β-carotene, sucrose and *α*-carotene were correlated and allowed the grouping of NGBTUN527, 539, 541, 556, 567 and 572 into cluster I. These landraces have similar contents of these compounds. Total sugars and glucose were strongly correlated, as were fructose and the lightness parameter L*. Landraces NGBTUN512, 514, 520, 522, 523, 540, 558 and 560 with similar values for these parameters were grouped together into cluster II. This grouping is in accordance with the PCA results.

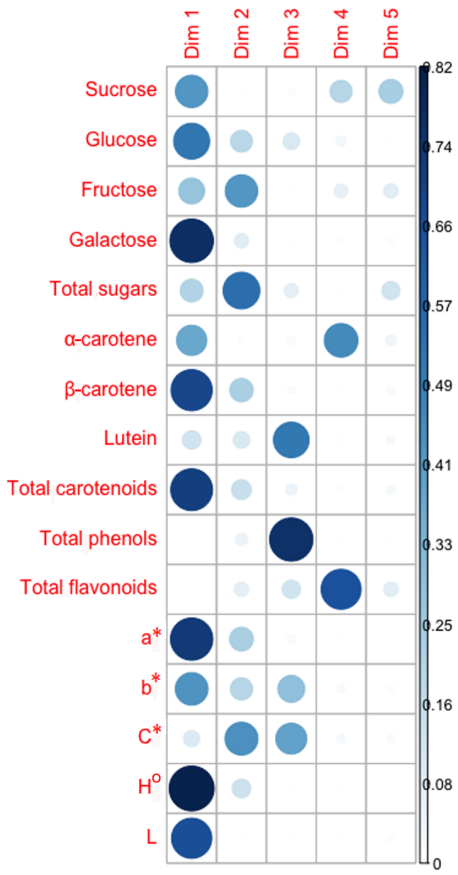

Figure 3. Contribution of biochemical parameters to the variability on the first five components of the PCA. The most significant correlations are indicated by a big, dark-blue circle.

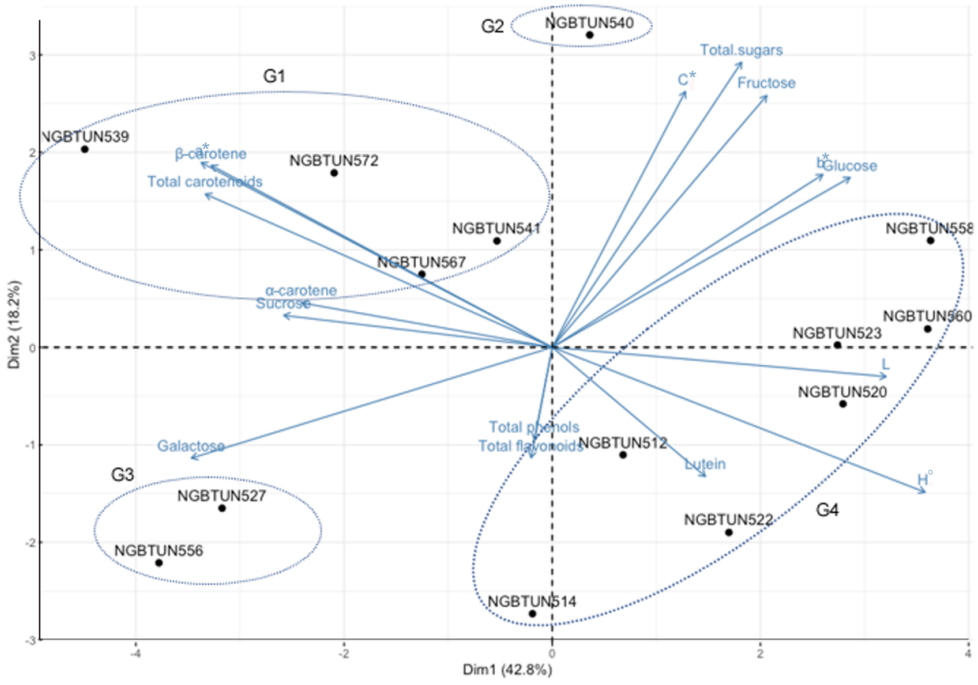

Figure 4. Scatter plot grouping of 14 Tunisian carrot landraces based on the first two principal components of the PCA.

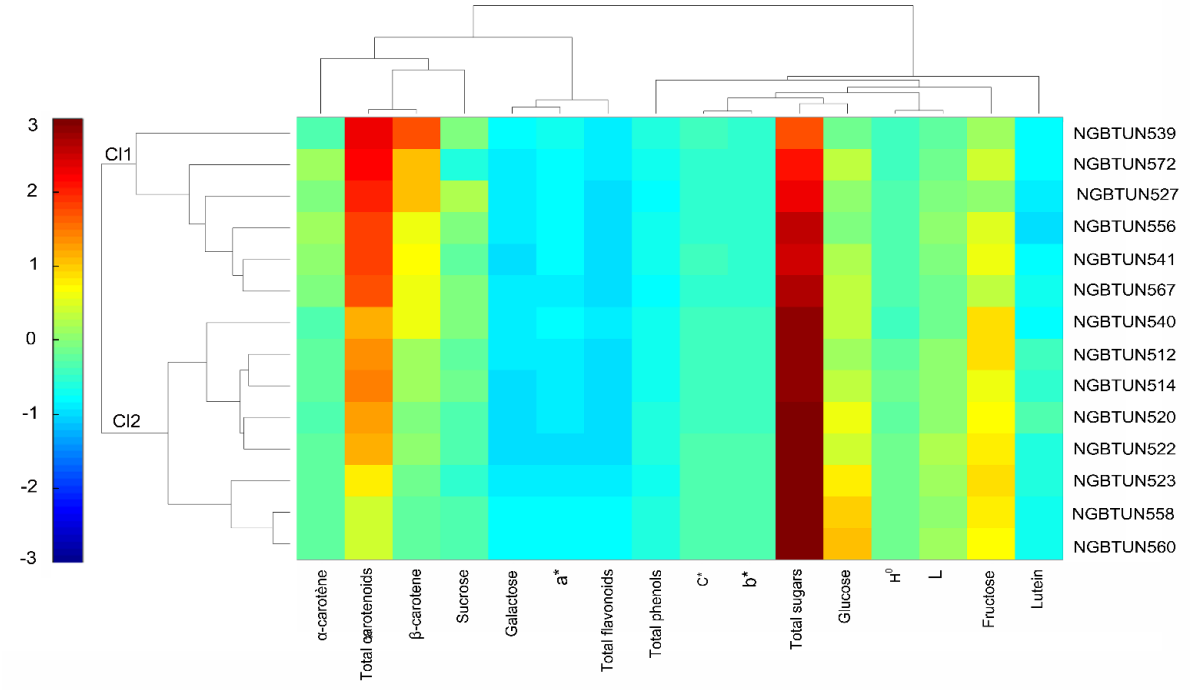

Figure 5. Dendrogram obtained from the cluster analysis of 14 Tunisian carrot landraces. The intensity and variation of colour (from dark red to blue) indicate the correlation existing between the parameters that allowed the discrimination of landraces.

4. DISCUSSION

In the present study, colour parameters and components of the biochemical composition such as carotenoids, sugars and phenolic compounds were identified in various carrot landraces in order to determine their genetic variability. The results prove that there is a large degree of variation among these landraces with respect to their biochemical composition and its relationship with root colour.

Food colour is a very important quality attribute influencing the consumer's choice and preferences (MELENDEZ-MARTINEZ *et al.*, 2005). It is widely used, together with measurements of pigments contents and flavor, because of its correlation with biochemical properties in vegetables. It is also used to assess food quality in post-harvest and processing conditions (PATHARE, 2013). Our correlation results highlight the association between colour and carotenoids accumulation. Thus, the redness value a* was highly correlated with β-carotene, α-carotene and total carotenoids, which give the orange colour to carrot landraces. The yellowness value b* was correlated with lutein and the hue angle H^0, which had greater values for yellow carrots than for orange ones. The high correlation between the hue angle H^0 and the lightness parameter L* was accompanied by carotenoids accumulation. The purity of carrot colour for all the landraces was indicated by the high chroma value C*. These results confirm that carotenoids contents can be determined by using a chroma meter (RUIZ *et al.*, 2005). The variation of colour in carrot is due mainly to the genotype, development of the plant, temperature and fertilization (BAJAJ *et al.*, 1980). The most suitable temperature for a better colour, accompanied by the highest content of carotenoids, is considered to be around 16 °C (BANGA *et al.*, 1955). The correlation results

are consistent with those of a previous study on pumpkin and squash conducted by ITLE and KABELKA (2009), who reported a positive correlation between a* and total carotenoids (r=0.91) and β-carotene (r=0.77); and a negative correlation between total carotenoids and H^0 (r=-0.83) and L* (r=-0.66), and between H^0 and β-carotene (r=-0.69). According to REEVES (1987), the best parameter to predict the total carotenoid content in pepper is a negative correlation with L*. Unlike previous studies (ALASALVAR *et al.*, 2001), we noted significant correlations between sugars and colour parameters. Sucrose was positively correlated with colour parameters, while glucose and fructose were negatively correlated with them. This could be explained by the fact that fructose and glucose accumulation is inversely proportional to sucrose accumulation (SUOJALA, 2000; SEKOLI *et al.*, 2016). KOROLEV *et al.* (2000 a, b) confirmed that the fructose and glucose contents increased in carrot root until 50 days after germination, while sucrose increased beyond this date until harvest (90 days). Similar to our results, correlations (positive or negative) between individual and total sugars contents have been reported in watermelon genotypes (YOO *et al.*, 2012). Glucose and fructose were negatively correlated with the total sugars, while sucrose had a positive correlation. These results show that each sugar contributes to the total sugars content to a different degree and that an increased sucrose content is accompanied by decreased fructose and glucose contents.

In the current work, carotenoids were quantified due to their contribution to carrot colour and the general quality of the roots. The type and amount of carotenoids differ among distinct parts of the plant (SAINI *et al.*, 2015). In carrot, the cortex (flesh) is richer in carotenoids than the core (KARABACAK and KARABACAK, 2019). Our experiment showed that the carotenoids profile was constituted mainly by β-carotene, α-carotene and lutein, whose contents varied distinctly among the carrot landraces, β-carotene and α-carotene being more highly abundant than lutein in orange coloured carrot .

These findings are in conformity with previous studies showing that α-carotene and β-carotene are the major carotenoids in orange carrots, unlike in yellow carrots (NICOLLE *et al.*, 2004; SURLES *et al.* 2004; BARANSKI *et al.*, 2010; JOURDAN *et al.*, 2015). Carrots with orange skin colour have been shown to possess higher amounts of total carotenoids than yellow carrots (ALASALVAR *et al.*, 2001; GRASSMANN, 2007; SUN *et al.*, 2009; SINGH *et al.*, 2018).

These new results indicate that Tunisian landraces are richer in carotenoids than ''Nante'' hybrids: namely, Nante/Berlikum (60.21 mg/100 g); Nante/Maestro (76.47 mg/100 g); Nante/Forto (72.45 mg/100 g); Nante/Bolero (72.93 mg/100 g) and Nante/Champion (79.47 mg/100 g) (RAKCEJEVA *et al.*, 2012).

Carotenoids biosynthesis depends on the cultivar (NICOLLE *et al.*, 2004) and is related to genetic factors (ROSENFELD *et al.*, 1997). Also, these compounds can be affected by the season (HORVITZ *et al.*, 2004), environmental conditions (SIMON, 2000), plant maturity (PHAN and HSU, 1973) and soil (HART and SCOTT, 1995). In fact, high temperatures and dry weather promote an increase in carotenoids contents (FIKSELOVÁ *et al.* 2010). Carotenoids have been found to be more abundant in carrots grown in clay soil than in carrots grown in sandy soil (MARTÍN-DIANA *et al.*, 2007; RICO *et al.*, 2007).

Cultivation practices can affect the content of biochemical compounds. Carotenoids accumulation was stimulated by fertilization based on NPK (SMOLEN and SADY, 2009), while it was inhibited by regular irrigation (FIKSELOVÁ *et al.*, 2010).

It has been noted that the post-harvest and storage conditions affect the carotenoids concentration (SAINI *et al.*, 2015). A temperature of 2-3 °C and 90% relative humidity reduced the carotenoids content by an average of 11% (MATĚJKOVÁ and PETŘÍKOVÁ, 2010). Hence, lyophilization is the most adequate method for better preservation of the nutritional quality of vegetables (SAINI *et al.*, 2014). The carotenoids and provitamin A

contents of carrots are important contributors to human health and the variability of carotenoids (determined using reliable HPLC methodology) could be considered as a parameter of selection in breeding programs (SIMPSON, 1983).
Sugars were also quantified due to their contribution to carrot taste and sweetness. Like carotenoids, the sugars content varies among the different parts of the carrot root. For example, total sugars are higher in the crown section (at the shoulders) than in the tip section (SEKOLI *et al.*, 2016).
Significant differences were observed in individual sugars as well as total sugars. Fructose and glucose were more abundant than sucrose and galactose, the latter being detected in Tunisian carrot landraces in lower amounts. Galactose has not been determined in similar research related to carrots and other crops. The results seem to be in direct contrast to the findings of DOLORES *et al.* (1999), ALASALVAR *et al.* (2001) and SIMON (1985), who showed sucrose to be the major soluble sugar, followed by glucose and fructose. The sugar content in plants, which contributes to sweetness, is controlled by the genotype and the environment (SIMON *et al.*, 1980.a, b; 1982; 1985). It is a polygenic control with a heritability estimated at 0.45 (BARANSKI *et al.*, 2012). The variation in sugars has been the subject of many studies, but sometimes the results are contradictory. SUOJALA *et al.* (2000) found low sugar levels in warm seasons, whereas NILSSON (1987), HOGSTAD *et al.* (1997) and ROSENFELD *et al.* (1998) noted the highest sugar levels at high temperatures. Fertilization can affect the variation in sugars. In this context, SEKOLI *et al.* (2016) found that the sucrose content increased proportionally with the increase in fertilization.
According to MELO *et al.* (2006), carrot belongs to the group of vegetables characterized by low phenolic contents since its mean content is <100 mg catechin equivalents/100 g of fresh weight. In our study, the phenolic compounds varied greatly among the carrot landraces. NICOLLE *et al.* (2004) found sizable differences in the content of total phenols among different carrot varieties (white, yellow, orange, dark-orange and purple), the values ranging between 4.3 and 4.4 mg GAE/g of dry weight for yellow carrot and between 3.3 and 6.0 mg of GAE/g of dry weight for orange carrots. However, in our study the carrots showed a lower content of total phenols, varying from 24.6 to 41.4 mg GAE/100 g of dry weight for yellow carrots and from 24.1 to 36.7 mg GAE/100 g of dry weight for orange carrots. Consequently, Tunisian carrot landraces appear to have lower contents of total phenols compared with other data of the scientific literature. This difference could be explained by the influence of the genetic factors, environmental conditions and variety (BRAVO, 1998). In addition, the phenolic compounds concentration depends on the fertilization method (SMOLEN and SADY, 2007). For example, the provision of nitrogen permits high concentrations of phenolic compounds (SMOLEN and SADY, 2009). Also, ROZEK *et al.* (2000) noted that the content of phenolic compounds are influenced more by the soil (light, medium or heavy texture) and climatic conditions than by fertilization.
The flavonoids are one of the most important groups of phenolic compounds since they exhibit important biochemical and pharmacological effects. Indeed, they contribute to the protection against reactive oxygen species (ROS) and the inhibition of platelet aggregation. They also have anti-inflammation, anti-atherosclerotic, antitumor, antimicrobial and antiallergic effects (KOLEY *et al.*, 2014). Quercetin, luteolin, kaempferol and myricetin are the main types of flavonoids found in carrot (BAHORUN *et al.*, 2004). However, the information related to the flavonoids content in carrot genotypes is limited. The low values of flavonoids in Tunisian carrot landraces are explained by the absence of anthocyanins, which are the most abundant type of flavonoids in the root and are responsible for purple and black colours (SINGH *et al.*, 2018). Different authors (LEJA *et*

al., 2013; KOLEY *et al.*, 2014; SINGH *et al.*, 2018) have reported lower amounts of flavonoids in orange and yellow carrots in comparison to purple, rainbow and black carrots.
Principal component analysis (PCA) and clustering (AHC) were performed to classify landraces on the basis of their similarities. Information obtained by the PCA can help breeders to distinguish between highly differentiated landraces to be used for plant breeding programs.

5. CONCLUSION

The contents of sugars and phenolic compounds in the roots varied significantly among 14 Tunisian carrot landraces due to agronomic and genetic factors. The colour, an important organoleptic characteristic in carrot, was significantly correlated with the content of carotenoids, oranges landraces having higher contents of α-carotene and β-carotene, whereas yellow landraces had more lutein. Considering the high variability of the biochemical parameters and taking into account their role in the nutritional value and health benefits of carrots, these results will be helpful in research and breeding programs to improve the overall quality of this plant food.

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