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Impact of refrigerated storage on the bioactive compounds and antioxidant capacity of two Algerian carrot varieties (*Daucus carota* L.)

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Abstract. Carrot (*Daucus carota* L.) is one of the main root vegetables rich in bioactive compounds with appreciable health-promoting properties, largely consumed in Algeria. In the current study, the storage effect (at 4 °C throughout 12 days) on bioactive compound stability and the antioxidant activity of two Algerian orange carrot varieties (Supermuscade and Touchon) were investigated. Total phenolic content of samples was determined by the Folin–Ciocâlteu method. Antioxidant capacity was determined spectrophotometrically, based on the evaluation of Free Radical Scavenging Activity (FRSA) using DPPH radical and Ferric Reducing Power (FRP). The results showed that the Touchon variety is richer in phenolics, flavonoids, and carotenoids and presents higher antioxidant activity in comparison with the Supermuscade variety. At the end of storage, the bioactive compound content and antiradical activity increased significantly ($p < 0.05$). Also, an extremely significant correlation ($p < 0.001$) was observed between the antioxidant contents and the antioxidant capacities of aqueous carrot extracts.

1 Introduction

Vegetables generally possess a good antioxidant activity, which is linked to their high contents of phenolic compounds. Numerous studies have suggested that the phytochemical content and the corresponding antioxidant activity of the vegetable contribute to their protective effect against chronic and degenerative diseases. The evaluation of the antioxidant capacity of foods commonly consumed in the diet, mainly after storage, is of great importance.

Carrot (*Daucus carota* L.) is a vegetable belonging to the family of *Umbelliferae*, also known as *Apiaceae*. *Daucus* is the largest genus in the family (*Rubatzky et al.*, 1999). Carrot is a root vegetable largely consumed on a global level, particularly in Algeria. It plays an important role in human nutrition and constitutes a rich source of health-promoting ingredients, such as carotenoids (*Kammerer et al.*, 2004; *Pace et al.*, 2020; *Shami & Naz*, 2019), in which antioxidant β-carotene acts as an anti-mutagenic and immunity booster (*Saleh et al.*, 2019; *Sharma et al.*, 2020; *Young & Lowe*, 2018). The phytonutrient content of carrots also includes phenolics, polyacetylenes, L-(+)-ascorbic acid (AA), and tocopherol, wherefore it is classified as a vitaminized food (*Encalada et al.*, 2016; *Numan*, 2019; *Vorobiev & Lebovka*, 2020).

Several epidemiological and clinical studies suggest that a high intake of carrot plays an important role in metabolism regulation, retaining a healthy skin and vision, and decreasing the risks associated to different types of cancer (*Chen et al.*, 2018; *Deding et al.*, 2020; *Jayaprakasha et al.*, 2019; *Luo et al.*,

2017; *Nkondjock & Ghadirian*, 2004; *Soares et al.*, 2018; *Su et al.*, 2002; *Surh*, 2003; *Tiwari*, 2016; *Tomita et al.*, 2020), cardiovascular diseases (*Alissa & Ferns*, 2017; *Castelletti*, 2019; *Louis et al.*, 2018; *Nicolle et al.*, 2003; *Nicolle et al.*, 2004; *Soleti et al.*, 2020), and cataract (*Braakhuis et al.*, 2017; *Chen & Chen*, 2017; *Haslam*, 2019; *Stahl & Sies*, 2020). Moreover, carrot is considered beneficial against urogenital diseases (*Aslam et al.*, 2014; *Chakraborty et al.*, 2018; *Chohra & Ferchichi*, 2019). The health-promoting effects of carrot have been attributed to the various antioxidant components present in this root vegetable (*Numan*, 2019; *Pace et al.*, 2020; *Shami & Naz*, 2019; *Soares et al.*, 2018).

Carrot is a source of various crucial macro- and micronutrients, including carbohydrates, proteins, fats, vitamins, antioxidants, minerals (potassium and sodium), folic acid, fibres, and carotenoids (*Ahimed et al.*, 2012; *Ludong et al.*, 2017; *Madu & Bello*, 2018; *Naseer et al.*, 2019; *Que et al.*, 2019; *Surbhi et al.*, 2018; *Vorobiev & Lebovka*, 2020). It contains significant quantities of thiamine, riboflavin and is also rich in sugars (*Naseer et al.*, 2019; *Surbhi et al.*, 2018). Carrot comprises several carotenoids (α- and β- carotenes) (*Ahmad et al.*, 2019; *Ludong et al.*, 2017; *Pace et al.*, 2020), which are the main pigments responsible for their colour, presenting nutritional importance due to their provitamin A and antioxidant activity (*Ellison et al.*, 2017; *Yoo et al.*, 2020). The β-carotene constituent is the major carotenoid, followed by α-carotene, lutein, and the other minor carotenoids such as cryptoxanthin, lycopene, or zeaxanthin (*Hà & Nguyễn*, 2015; *Ahmad et al.*, 2019; *Pace et al.*, 2020).

Interest in the role of antioxidants in human health has promoted research in the field of food sciences to assess fruit and vegetable antioxidants and determine how their content and activity can be maintained or improved, as the content of phytochemical substances is influenced by numerous factors such as ripening, genotype, cultivation technique, or climatic conditions during the pre-harvest period, but operations carried out during the post-harvest storage are also very important. In order to extend shelf life and maintain the quality of fresh carrot, refrigerated storage is largely used. However, storage at low temperatures may affect the composition and the activity of carrot phytonutrients. Therefore, the main objective of the present study was to evaluate the impact of refrigerated storage at 4 °C throughout 12 days on the antioxidant compounds (total phenolics, total flavonoids, and total carotenoids) and antioxidant activity of two Algerian orange carrot varieties.

2 Materials and methods

Chemicals

Folin–Ciocâlteu reagent (FCR) was purchased from Biochem, Chemopharma (Montreal, Quebec); sodium carbonate from Sigma-Aldrich (Switzerland); aluminium chloride and potassium ferricyanide from Biochem, Chemopharma (Georgia, USA); gallic acid and β-carotene from Prolabo (Montreuil, France); 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma-Aldrich (Germany).

Samples and preparation of extracts

Two varieties of orange carrot (Supermuscade and Touchon) were purchased from the local market. The varieties are fresh and without infection or damage. The carrots were washed with distilled water and then stored in refrigerator for 12 days at a temperature of 4 °C. The choice of this period is related to the average carrot's shelf life after purchase, stored in consumers' refrigerators and then divided into four sampling periods. The tested parameters were determined before storage of the samples and after storage, therefore sampling every three days: after 3, 6, 9, and 12 days.

The edible portions were separated from the inedible portions with manual peeler. An amount of 10 g of edible portion of fresh carrots were grated into small pieces (2.4 cm length and 1 mm width) using a manual grater and mixed with 50 mL of distilled water. After 30 min of agitation, the homogenate was centrifuged at 4,500 g for 15 min at 5 °C (Sigma 2-16 K; Germany). The supernatant was collected and the residue re-extracted with 50 mL of distilled water. The collected supernatants were combined and then concentrated under vacuum at 35 °C using a BÜCHI rotavapour (R-200, Germany) until the volume of 10 mL was reached, and the extracts were stored at -10 °C until analysis.

Sample analysis

To evaluate the effects of storage at 4 °C, the fresh-stored carrots were analysed every three days regarding their antioxidant constituents and antioxidant activity as follows:

Determination of Total Phenolic Contents (TPC)

The TPC of carrot extract was estimated following colorimetric assay of *Naithani et al.* (2006). Briefly, to 100 μL of the diluted extract (1:1, V:V), 2.2

mL of sodium carbonate water solution (2%) was added and mixed thoroughly. After 3 min, 100 μL of FCR (50%) was added under mixing. The absorbance of the mixture was measured spectrophotometrically at the wavelength of $\lambda_{abs} =$ 750 nm by using a spectrophotometer (UV-mini 1240 Shimadzu, China). The results were expressed as milligram Gallic Acid Equivalent per one hundred gram of the fresh weight (mg GAE/100 g FW) using a standard curve ($y = 1.8986\,x, R^2 = 0.9973$).

Determination of Total Flavonoid Content (TFC)

The TFC of carrot extract was evaluated following the colorimetric assay of *Djeridane et al.* (2006). To 1.5 mL of extract, an amount of 1.5 mL of 2% aluminium chloride solution (w/v) was added. After 10 min, the absorbance was measured at the wavelength of $\lambda_{abs} = 410$ nm. The total flavonoid was reported as milligram quercetin equivalent per one hundred gram of the fresh weight (mg QE/100 g FW) using standard curve ($y = 0.0095\,x, R^2 = 0.991$).

Determination of Total Carotenoid Content (TCC)

Carotenoids were extracted from the samples using the method of *Sass-Kiss et al.* (2005). In brief, 20 mL mixture of hexane-acetone-ethanol (2:1:1, V: V: V) was added to 0.5 g of homogenized fresh carrot samples. After 30 min of agitation, the supernatant was collected, and the residue was added with 10 mL hexane for a second extraction. The absorbance of the combined hexane layers was measured at the wavelength of $\lambda_{abs} = 450$ nm. The TCC in carrot samples was determined from the standard curve using β-carotene ($y = 0.1282\,x, R^2 = 0.996$), and the results were expressed as milligram β-carotene equivalent per one hundred gram of the fresh weight (mg βCE/100 g FW).

Antioxidant activities

DPPH Free Radical Scavenging Activity (DPPH-FRSA)

The antiradical activity of carrot extracts against DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical was evaluated according to the method described by *Peschel et al.* (2006). In brief, 500 μL of the extract was mixed with 2 mL methanolic solution of DPPH; the mixture was left in the dark for 90 min before measuring the absorbance at the wavelength of $\lambda_{abs} = 517$ nm. The reduction of DPPH was calculated using the following formula:

$$\text{DPPH radical scavenging activity } (\%) = [(A_c - A_e)/A_c] \cdot 100, \qquad (1)$$

where: A_c was the absorbance of the control, and A_e was the absorbance in the presence of the sample extracts.

Ferric Reducing Power (FRP)

The reducing power of carrot extracts was measured according to the method described by *Bhandari & Kawabata* (2004). Briefly, 1 mL of carrot extract, 0.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide solution (1% w/v) were mixed in a test tube and reacted for 20 min at 50 °C. The tubes were cooled immediately, and 0.5 mL of trichloroacetic acid (10%) was added in. After centrifugation at 3,000 g during 10 min (Sigma 2–16 K; Germany), 1 mL of supernatant was mixed with 1 mL of distilled water and 100 μL of ferric chloride (0.1% w/v) and reacted for 10 min. Then, the absorbance at the wavelength of $\lambda_{abs} = 700$ nm was measured. The FRP of carrot extracts was determined from the standard curve using Trolox standard ($y = 0.002\,x, R^2 = 0.997$), and the results were expressed as milligram Trolox equivalent per one hundred gram of the fresh weight (mg TE/100 g FW).

Statistical analysis

All data are reported as mean ± standard error of mean of three replicates. The analysis of variance (ANOVA) at $p < 0.05$ was calculated using STATISTICA 5.5 (StatSoft, Inc., USA) in order to determine the significant differences between the results. Correlations were performed using the correlation matrix at three different significance levels ($p = 0.05$, 0.01, and 0.001).

3 Results and discussion

Total Phenol Content (TPC)

Phenolic compounds are the most abundant antioxidants in the human diet and are widespread constituents of fruits and vegetables. These compounds are of considerable interest due to their antioxidant properties. Phenolic compounds in carrots are primarily found with a single aromatic ring known as phenolic acids. Major phenols found in carrots are chlorogenic, caffeic, and p-hydroxybenzoic acids along with numerous cinnamic acid derivatives. Chlorogenic acids are hydroxycinnamic acid derivatives formed by the esterification of cinnamic acids, such as caffeic, ferulic, or p-coumaric acids, with L-quinic acid (*Hà & Nguyễn*, 2015). The TPC of the aqueous carrot extracts of the

Supermuscade variety was significantly different (12.70 ± 0.65 mg/100 g FW) from that of the Touchon variety (38.81 ± 0.44 mg/100 g FW) (*Figure 1*). The two varieties present a significant difference ($p < 0.05$); the Touchon variety contains 2.5-fold more phenolics than Supermuscade. These results are in agreement with those reported by *Alasalvar et al.* (2001), who noted differences on the phenolic content of carrot varieties. In orange, yellow, and white varieties, the phenolic content varies from 7.74 to 16.2 mg/100 g; for purple carrot, it was 74 mg/100 g of fresh weight. Furthermore, the phenolic content of carrots has varied from 12.59 to 290.18 mg GAE/100 g FW (*Koley & Singh*, 2019). *Alasalvar et al.* (2005) have reported that orange and purple carrots contain 34.8 and 102 mg/100 g respectively. *Yu et al.* (2005) and *Cieslik et al.* (2006) found that the phenolic content of carrot was 198 and 15.6 mg/100 g FW respectively. Moreover, in orange carrot varieties, the phenolic content varied from 18.7 to 58.6 mg/100g FW (*Leja et al.*, 2013). These differences on the TPC may be caused by varietal differences, the geographic origin, and solvent and/or extraction method or measurement.

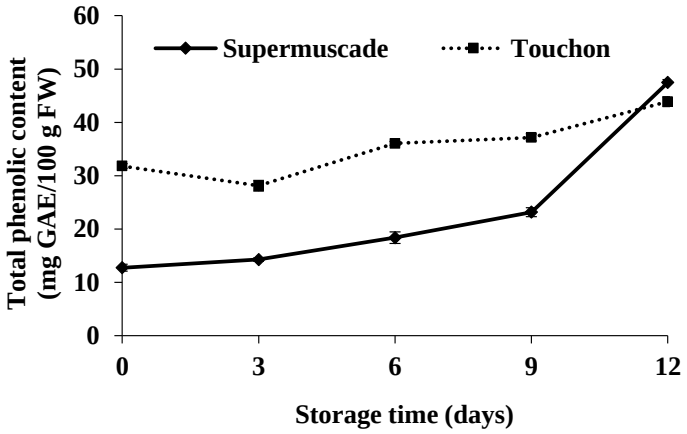

Figure 1. Effects of refrigerated storage on the phenolic content of carrot

Figure 1 shows that TPC increased significantly from the third day of storage until the 12th day. After 12 days of storage, TPC increased with 73.18% and 25.52% for Supermuscade and Touchon respectively. These results are in accordance with those obtained by *Zhang et al.* (2005), where the phenolic content of the Kend, Ricardo, and Stefano carrot varieties increased after 10 days of storage at 4 °C. Increase of TPC due to storage may be a result of the increased transcription of genes encoding the corresponding biosynthetic

enzymes (*del Rosario Cuéllar-Villarreal et al.*, 2016; *Dixon & Paiva*, 1995), i.e. changes in phenolic compound metabolism (*Alasalvar et al.*, 2005) and the synthesis of these compounds during storage (*Klimczak et al.*, 2007). According to *Tavarini et al.* (2008), the increase of total phenolic content during storage could be attributed to changes occurring in phenol metabolism as well as to the increase of phenylalanine ammonia lyase (PAL). PAL has been found to be associated with post-harvest disorders induced after prolonged storage at low temperature (*Martinez-Tellez & Lafuente*, 1997; *Zhao et al.*, 2019b).

Total Flavonoid Content (TFC)

The presence of phenolic compounds in carrots influences the organoleptic properties of fresh and processed carrots, including colour, bitterness, and aroma. Therefore, they could be used as a good quality indicator during processing and storage (*Hà & Nguyễn*, 2015). *Ahmad et al.* (2019) reported that carrots are rich in phenolic acids as well as in anthocyanins, a class of flavonoids. Flavonoids are among the most studied phytochemicals in foods of plant origin and include a large number of different molecules with various biological activities. Similarly to phenolic compounds, the TFC of Supermuscade and Touchon aqueous extracts significantly differed with rates of 3.20 ± 0.04 and 7.93 ± 0.21 mg/100 g FW respectively (*Figure 2*). These results are in accordance with those reported by *Miean & Mohamed* (2001) and *Marinova et al.* (2005), who registered carrot flavonoid contents of 3.7 and 26.7 mg/100 g respectively.

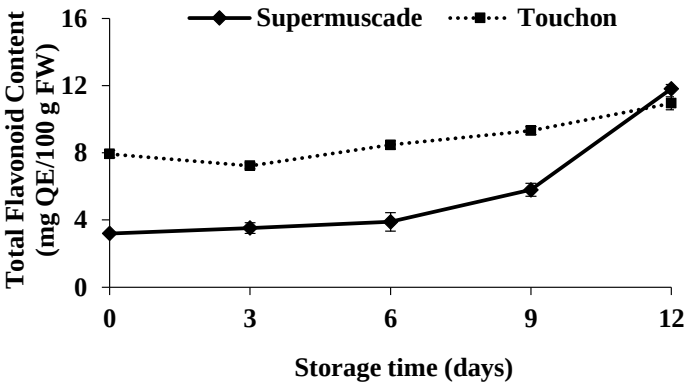

Figure 2. Effects of refrigerated storage on the flavonoid content of carrot

Furthermore, in orange carrots, a flavonoid content of 5.33 mg CE/100 g FW was indicated by *Singh et al.* (2018a). Similarly, *Leja et al.* (2013) and *Koley & Singh* (2019) estimated the highest amount of flavonoids in carrots. On the contrary, *Al-Dabbas et al.* (2015) recorded low flavonoid content in carrots with a value of 0.029 μg/g. The published data may vary according to the extraction methods, sample preparations, and other factors such as cultivars, post-harvest handling, and processing conditions (*Hà & Nguyễn*, 2015). In addition, *Dixon & Paiva* (1995) stated that the flavonoid composition of plants depends on the temperature, the solar exposition, the cellular damage, and the available quantities of phosphorus and nitrogen. Furthermore, *Ahmad et al.* (2019) reported that phenolic compounds are affected by multiple factors such as the cultivar, storage conditions and temperature, fertilizer application, processing procedures, and various biotic and abiotic stress factors.

Similarly to TPC, TFC increases significantly ($p < 0.05$) from the third day of storage at 4 °C to the 12th day. In fact, TFC increases with 72.92% (Supermuscade) and 27.58% (Touchon) after 12 days of storage. These results are in agreement with those reported by *Lafuente et al.* (2011), *Gorrepati & Bhagat* (2018), and *Youryon & Supapvanich* (2019). In addition, *Ahmad et al.* (2019) reported that in a recent study conducted by *Kamiloglu et al.* (2015) with black carrots it was found that after 20 weeks of storage the preserved amount of main flavonoids (anthocyanins) in samples stored at 4 °C (53.4%–81.0%) was higher than in samples stored at 25 °C (7.8%–69.3%). Moreover, *Del Caro et al.* (2004) noted an increase in the flavonoid content of lemon after 12 days of storage at 4 °C, explained by the stimulation of phenylalanine ammonia lyase (PAL) activity and consequently a synthesis of these compounds. According to *Gorrepati & Bhagat* (2018), the increase of flavonoid content after refrigerated storage may be attributed to stress due to low temperature. On the other hand, further studies have shown a decrease in flavonoid content in carrot (*Al-Dabbas et al.*, 2015), lettuce (*DuPont et al.*, 2000), and fresh-cut onion during storage (*Berno et al.*, 2014). These differences may be explained by the differences in the time and/or temperature of the storage.

Total Carotenoid Content (TCC)

Carotenoids are compounds very sensitive to light, heat, air, and other variables; consequently, their determination, involving the steps of extracting, can be accompanied by degradations and/or loss. For this reason, it is important to make a careful evaluation of the analytical procedure to avoid causes of variation and inaccuracies (*Chiosa et al.*, 2005). *Figure 3* shows the effects

of storage at 4 °C throughout 12 days on the TCC of the two orange carrot varieties. The varieties analysed present significant differences ($p < 0.05$); the TCC of Touchon variety (19.09 ± 0.06 mg/100 g FW) was initially 2.2-fold higher than that of the Supermuscade variety (8.9 ± 0.1 mg/100 g FW). This is in agreement with values reported by *Alasalvar et al.* (2005), who found that the total carotenoid of purple carrots (19.5 ± 0.05 mg/100 g) was 2.3-fold higher than that of orange carrots (8.6 ± 0.2 mg/100 g). *Edwards et al.* (2002) reported that the total carotenoid content of the Apache variety was 11.45 mg/100 g, while *Sun & Temelli* (2006) recorded carrot carotenoids content of 15 mg/100 g. In addition, *Scarano et al.* (2018), *Singh et al.* (2018a), and *Hasan et al.* (2019) estimated the highest amount of carotenoids in carrot compared to the results found in the present study. *Koley & Singh* (2019) noted that the β-carotene content in various coloured carrot genotypes ranged between 0 and 4.62 mg/100 g and high β-carotene content was observed in the orange-coloured genotype. These differences in TCC are probably due to the extraction method and/or the sensibility of the measurement method, the varietal differences and geographic origin of the sample analysed. According to *Sun & Temelli* (2006), total carotenoid content can be influenced by carrot genotype, development stage, and growing conditions such as temperature and use of fertilizers.

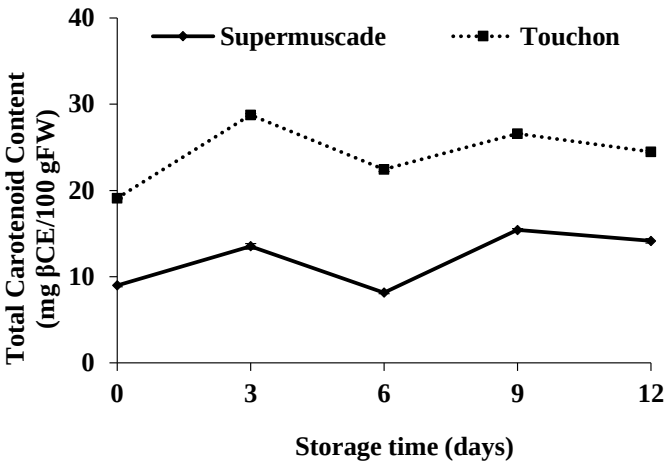

Figure 3. Effects of refrigerated storage on the carotenoid content of carrot

Figure 3 shows that the evolution of TCC during storage varies significantly. An increase of TCC has been noted after 3 and 9 days of storage, which is not

the case after 6 days of storage, when a slight decrease was recorded in TCC. This effect may be attributed to the cis-trans isomerization of carotenoids during storage. According to *Vieira et al.* (2018), this behaviour may be related to the matrix disruption and the polyene chain instability of carotenoids, promoting their isomerization or oxidation. Overall, the storage of carrot at 4 °C for 12 days causes a significant increase in TCC, with a percentage of 36.46% in Supermuscade and 21.98% in Touchon varieties. Similar results were obtained by *Murcia et al.* (2009), *Preethi et al.* (2018), and *Nunes et al.* (2019). *Berger et al.* (2008) stated that the storage of carrot for 14 days at 4 °C causes a significant increase in carotenoid content: 8% and 23% for Nevis and Kingston varieties respectively. These results were explained by the carotenoid biosynthesis during storage and the good extractability of these pigments after enzymatic decomposition, i.e. the fibrous structure of the carrot matrix could be disaggregated by cellulases and hemicellulases, and further quantities of carotene could be released during the extraction process. According to *Ahmad et al.* (2019), carotenoids are influenced by two main factors – namely, inherited characteristics and environmental conditions (during growth and packaging and/or storage conditions and temperature).

Many authors have reported a decrease in total carotenoid content during storage of fruits and vegetables. The Kintoki variety lost 30% of total carotenoid after 9 weeks of storage at 1 °C (*Mayer-Miebach & Spieß*, 2003). Similarly, a decrease of TCC was obtained by *Alasalvar et al.* (2005) in purple and orange carrots after 13 days of storage at 5 ± 2 °C and by *Macura et al.* (2019) in purple carrots. These differences registered in relation to TCC as effects of storage may be explained by the differences in storage temperature and duration and/or the varietal differences.

Antioxidant activity

In order to assess the antioxidant capacity of orange carrots during storage, two methods based on different reaction mechanisms were applied. The first is radical scavenging activity, which is based on the extract's ability to neutralize DPPH radical, and the second is ferric reducing power (FRP), which is based on the ability of the extract to reduce Fe^{3+}.

DPPH Free Radical Scavenging Activity (DPPH-FRSA)

The results obtained (*Figure 4*) indicate that the antiradical activity of aqueous carrot extracts presents significant differences at $p < 0.05$. The inhibition percentages of the DPPH radical were initially 10.90% and 20.60% for

Supermuscade and Touchon respectively. These results indicate that the antiradical activity of the Touchon variety was roughly 2-fold higher than that of the Supermuscade variety. These results are comparable with those reported by *Singh et al.* (2018b). *Singh et al.* (2018a) claimed that the antioxidant activity of coloured tropical carrots ranged from 1.22 to 43.98 μmol TE/g FW. *Koley & Singh* (2019) reported that the antioxidant activity varied from 0.58 to 29.72 μmol TE/g in the carrots' genotype. Also, *Leja et al.* (2013) noted a high antioxidant capacity in orange, white, and yellow roots, showing radical scavenging activity with a rate of 6%, and only the red roots had a high activity with a percentage of 9.3%. *Gajewski et al.* (2007) found higher antioxidant capacity in methanolic extracts from purple carrots as compared to extracts from orange and yellow carrots. *Yen et al.* (2008) observed very high, reaching even 80–98%, DPPH neutralization activity in red carrot roots; however, these authors used very high (2–20 mg DM/cm^3 of extract) tissue concentration. Furthermore, in their study on some selected fruits in Ekiti State, Nigeria, *Ogunlade et al.* (2019) noted that carrots presented a good antioxidant activity comparatively to other fruits as tangerine, lime, or watermelon. These differences may be related to differences in varieties and/or geographic origin. According to *Smeriglio et al.* (2018), antioxidant activity is not only related to the main constituents, but it may be modulated by several other compounds, wherefore the concepts of synergism and antagonism can be highly relevant.

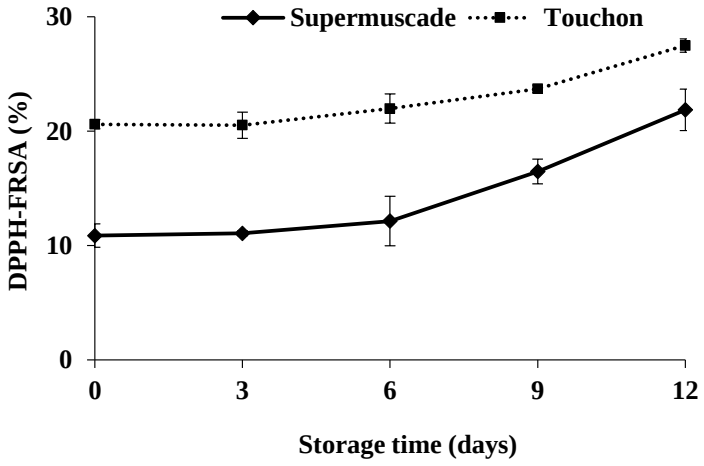

Figure 4. Effects of refrigerated storage on the antiradical activity of carrot

Figure 4 shows that the antiradical activity of carrot varieties increases progressively from the 6^{th} day until the 12^{th} day of storage at 4 °C. Indeed, the antiradical activity increased by 50.22% in Supermuscade and 25.06% in Touchon aqueous extracts. This effect is probably due to the increase of bioactive compounds (TPC, TFC, and TCC) during storage. Similar results were reported by *da Silva et al.* (2018), *Magalhães et al.* (2019), and *Zahoor & Khan* (2019). According to *Shivashankara et al.* (2004), an increase in antioxidant activity during the storage of vegetables was generally attributed to the phenolic content. *Lattanzio et al.* (1994) reported that during the storage of vegetables the cellular walls lost their integrity, which led to browning due to the enzymatic oxidation of phenolic compounds. *Zahoor & Khan* (2019) explained this increase in antioxidant activity by the occurrence of Maillard reactions during storage. Moreover, *Pinelo et al.* (2004) reported that these oxidations were coupled with the formation of highly polymerized phenolic compounds, possessing higher antioxidant activity in comparison with their natural precursors. However, many other authors have registered that storage decreases antioxidant activity in different foods (*Ang & Deocampo*, 2019; *Corleto et al.*, 2018; *Louaileche & Djaoudene*, 2016; *Murcia et al.*, 2009; *Nayik & Gull*, 2018; *Vieira et al.*, 2018). In fact, these differences in the effects of storage may be explained by the variety in duration and/or the different storage temperatures.

Ferric Reducing Power (FRP)

The presence of reductants causes the reduction of Fe^{3+} ferricyanide complex to the ferrous form; Fe^{2+} is monitored by measuring the formation of Perl's Prussian blue at the wavelength of $\lambda_{abs} = 700$ nm. Ferric reducing ability may serve as an indicator of the antioxidant potential. Prior to storage, the analysed carrots exhibited a ferric reducing ability of 25.46 mg TE/100 g FW for Supermuscade and 94.20 mg TE/100 g FW for Touchon (*Figure 5*). These results indicate that the FRP of Touchon variety was 3.7-fold higher than that of Supermuscade variety due to the highest content of bioactive phytochemicals (TPC, TFC, and TCC) in the Touchon variety. Our results approximate the value reported by *Allane & Benamara* (2019). Moreover, *Šeregelj et al.* (2017) noted a high reducing ability of carrot extracts obtained with ethanol-acetone solvent mixture with a rate of 7368.07 and 3167.91 μmol TE/100 g DW. However, a low reducing power was registered for the ethyl acetate extract with a level of 71.19 μmol TE/100 g DW. The observed differences may be explained by the differences in the polarity of solvents used for

carrot extraction, yielding different compositions of the extracts. The ethanol, acetone, and ethyl acetate extracts had the highest total bioactive compound content (i.e. carotenoids and polyphenols) resulting in superior reducing power (*Šeregelj et al.*, 2017). In addition, the modifications in the extraction procedures, in particular the homogenized sample weight: solvent volume ratio, extraction solvent type and technical extraction, and the varietal differences cannot be excluded in this case.

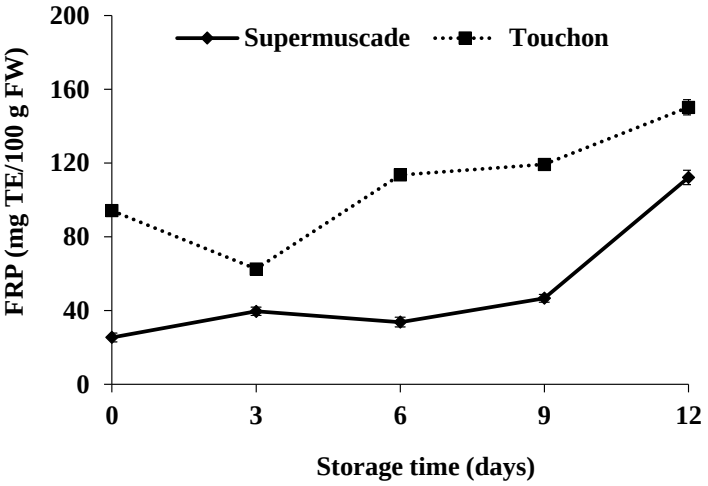

Figure 5. Effects of refrigerated storage on the reducing power of carrot

As shown in *Figure 5*, FRP increases gradually from the third day of the storage at 4 °C until the 12th day. The initial values of the ferric-reducing potential of orange carrot extracts are 25.46 mg TE/100 g FW (Supermuscade) and 94.20 mg TE/100 g FW (Touchon). It rises with a proportion of 77.30% for Supermuscade and 37.28% for Touchon. This effect could be attributed to the increase of the antioxidant compounds (TPC, TFC, and TCC) during storage, which is responsible for the antioxidant power. These results are in concordance with those reported by *Kallithraka et al.* (2009), *Zhao et al.* (2018), and *Zhao et al.* (2019a).

Nevertheless, several other authors have registered a decrease of reducing power during storage: *Saci et al.* (2015), *Louaileche & Djaoudene* (2016), *Panigrahi et al.* (2018), and *Ang & Deocampo* (2019). These differences observed regarding the impact of storage conditions on reducing power may be attributed to the differences in storage conditions (time and temperature) and/or the food matrices.

Correlations

Correlation analysis was used to explore the relationship between the different measured variables. The correlation matrix presented in *Table 1* revealed a correlation between bioactive compound content and antioxidant activity. A strong positive correlation was observed between total phenolic, total flavonoid, and total carotenoid content ($r = 0.99$ and $r = 0.85$). The antioxidant capacity of aqueous extracts was influenced by the content of bioactive compounds; FRSA and FRP activities were highly and significantly correlated ($p < 0.001$) with TPC ($r = 0.83$ and $r = 0.82$ respectively), TFC ($r = 0.85$ and $r = 0.83$ respectively), and TCC ($r = 0.75$ and $r = 0.63$ respectively). This indicates that these compounds are most responsible for the free radical scavenging ability and ferric-reducing power of the investigated carrots. A similar trend was observed in carrot extracts by other researchers, who observed a direct significant association between the total phenolics, total flavonoids, total carotenoids, and antioxidant ability (*Carrillo et al.*, 2017; *Koley & Singh*, 2019; *Koley et al.*, 2014; *Leja et al.*, 2013; *Singh et al.*, 2017) of root vegetables. Furthermore, a strong positive significant correlation was also observed between both antioxidant assays of FRSA and FRP ($r = 0.93$); this may be due to the presence of molecules displaying simultaneously antiradical and reducing properties. Similar results were reported by *Louaileche & Djaoudene* (2016) in orange jam and *Koley & Singh* (2019) in various carrot genotypes.

Table 1. Correlation matrix between the phytochemical content and antioxidant activity of orange carrots

	TPC	**TFC**	**TCC**	**FRSA**	**FRP**
TPC	1.00				
TFC	0.99***	1.00			
TCC	0.85***	0.85***	1.00		
FRSA	0.83***	0.85***	0.75***	1.00	
FRP	0.82***	0.83***	0.63***	0.93***	1.00

Notes: **TPC**: Total Phenolic Content; **TFC**: Total Flavonoid Content; **TCC**: Total Carotenoid Content; **FRSA**: Free Radical Scavenging Activity; **FRP**: Ferric-Reducing Power *** $p < 0.001$: extremely significant correlations)

4 Conclusions

In conclusion, the result of this investigation confirmed that Algerian orange carrots are a good source of bioactive molecules. The Touchon variety is richer in TPC (38.81 ± 0.44 mg/100 g FW), TFC (7.93 ± 0.21 mg/100 g FW), and TCC (19.09 ± 0.06 mg/100 g FW) and presents higher antioxidant activity (20.60% and 94.20 mg TE/100 g FW for FRSA-DPPH and FRP respectively) in comparison with the Supermuscade variety. Refrigerated (4 °C) storage for 12 days caused a significant increase of the bioactive compounds (phenolics, flavonoids, and carotenoids) and antioxidant activity of orange carrots as well as an increase of PAL, which led to an increase in antioxidant activity. Moreover, there was a strong linear correlation between the antioxidant compounds and antioxidant activity, which confirmed that these substances are the main compounds responsible for the carrots' antioxidant activity. Therefore, the refrigerated storage of carrots is a promising method that not only extends shelf life but also improves the bioactive compound content and antioxidant activity of carrots.

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