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Phytochemical Analysis and Antioxidant Properties in Colored Tiggiano Carrots

Aurelia Scarano [1], Carmela Gerardi [1], Leone D'Amico [1], Rita Accogli [2] and Angelo Santino [1,*]

1 C.N.R. Unit of Lecce, ISPA-CNR, Institute of Science of Food Production, 73100 Lecce, Italy; aurelia.scarano@ispa.cnr.it (A.S.); carmela.gerardi@ispa.cnr.it (C.G.); leone.damico@ispa.cnr.it (L.D.)

2 Orto Botanico Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, 73100 Lecce, Italy; rita.accogli@unisalento.it

* Correspondence: angelo.santino@ispa.cnr.it; Tel.: +39-0832422606

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Abstract: The carrot (*Daucus carota* L.) is an important vegetable source of bioactive compounds in the human diet. In the Apulia region (Southern Italy), local farmers have domesticated colored landraces of carrots over the years, strictly related to local cults and traditions. Amongst these, an important landrace is the carrot of Saint Ippazio or the Tiggiano carrot. In the present study, we evaluated the content of carotenoids, anthocyanins, phenolic acids, sugars, organic acids, and antioxidant activity in Tiggiano carrots. Our results indicated that yellow-purple carrots have the highest levels of bioactive compounds, together with the highest antioxidant capacity compared to the yellow and cultivated orange varieties. These data point out the nutritional value of purple Tiggiano carrots and may contribute to the valorization of this typical landrace.

Keywords: apulian landraces; bioactive compounds; polyphenols; Tiggiano carrot

1. Introduction

The carrot is a root vegetable widely consumed in human diet, either as fresh or processed in meals and beverages. The carrot have been ranked 10th among 39 fruits and vegetables for its multiple nutritional benefits [1]. Italy accounts for over 500,000 tons of production [2], with about 300,000 quintals coming from the Apulia region in 2017 [3]. Carrots are an important dietary source of carotenoids [4], mostly α-carotene and β-carotene, also known as provitamin A, since it can be converted to vitamin A once in the body. Despite western countries consuming commonly orange carrots, yellow or purple colored carrots are also cultivated in some parts of the U.S. [5], Europe, Turkey, and India [6]. The domestication of yellow and purple carrots arises from the oriental countries spreading to Italy starting from the 13–14th century [7,8]. In the Apulia region, a plan of preserving crop biodiversity has been launched from the Regional Administration (2007–2013 Rural Development Program, actions 214/3 and 214/4), with the aim of rescuing and valorizing the local landraces of crops traditionally cultivated in the rural areas. Several Apulian varieties are included in the list of vegetables at risk of genetic erosion [9], which constitutes an issue for the loss of the genetic traits important for the biodiversity and nutritional quality of the varieties [8–10]. In the Apulia region, three main ecotypes of colored carrots have been reported, whose names are strictly related to the production areas: Polignano (province of Bari, [11]), Zapponeta, (province of Foggia), and Tiggiano (which also includes Tricase and Specchia villages, in the province of Lecce [12]). Tiggiano carrot cultivation is strongly related to the popular cult of the Saint Ippazio, protector of male virility. In celebration of the saint, the colored carrots are usually sold as a pagan ritual concerning fertility, and to receive protection from the saint against hernias or male impotency. As in the case of Polignano carrot [8], one of the phenotypes of the Tiggiano carrot has a dark purple epidermis and a yellow-orange inner

core. In many varieties of purple carrots, the dark color has been attributed to the accumulation of anthocyanins in the taproots [5,13–16].

Anthocyanins are natural pigments widely occurring in plants, which contribute to the nutritional value of vegetable and fruits, due to their molecular antioxidant properties and their involvement in anti-aging and anti-inflammatory processes [17–19]. In plants, anthocyanins are synthetized by the flavonoid biosynthetic pathway, which leads also to the production of phenolic acids and other classes of polyphenols with healthy benefits [20–22]. In this study, we carried out the chemical profiling of antioxidant compounds and characterized the main classes of polyphenols, carotenoids, sugars, and organic acids, which can contribute to the quality and nutritional value of Tiggiano carrots and to the valorization of this local landrace.

2. Materials and Methods

2.1. Plant Material

The carrots were grown and harvested in a local farm in Tiggiano (Lecce), South Italy. Samples were divided in three groups, based on the color of the epidermis of the carrots (yellow, orange, or purple). Five carrots for each group were used for the analyses. Carrots were cut, frozen in liquid nitrogen, grinded in a mortar, and stored at −80 °C for anthocyanin analyses. Alternatively, for the determination of phenolic acids, carotenoids, organic acids, sugars, and antioxidant activity, samples were lyophilized, finely powdered, and stored at 4 °C until their use. All analyses were performed in triplicate.

2.2. Extraction and Detection of Phenolic Acids

Two-hundred mg of freeze-dried powder of samples were extracted three times in methanol: water 80:20 (*v*/*v*), the extracts centrifuged, and the supernatants combined. Phenolic acids were detected at 320 nm by RP-HPLC DAD (Agilent 1100 HPLC system, Agilent Technologies Inc., Santa Clara, CA, USA). Separation was performed on a C18 column (5 UltraSphere, 80 A pore, 25 mm), with a linear gradient from 20% to 60% acetonitrile, in 55 min, with a flow of 1 mL/min at 25 °C. Concentrations were obtained by referring to calibration curves and results were expressed in μg/g dried weight.

2.3. Total Content of Anthocyanins

The total content of anthocyanins was determined on methanol extracts from fresh Tiggiano carrots using the pH differential method [23]. Fresh samples were frozen in liquid nitrogen and ground in a mortar. Two-hundred mg powdered samples were extracted three times in methanol: water 80:20 (*v*/*v*). The methanol extracts were mixed using the appropriate dilution factor, with two different solutions to obtain different pH values, prepared as previously described [23]: pH 1.0 potassium chloride buffer (0.025 M KCl) and pH 4.5 sodium acetate buffer (0.4 M $CH_3CO_2Na{\cdot}3H_2O$). After 15 min incubation at room temperature, the absorbance of the samples was measured at 520 nm and 700 nm (Shimadzu UV-1800, spectrophotometer, Kyoto, Japan). The total content of anthocyanins, expressed as cyanidin-3-glucosyde equivalents, was calculated according to the formula described in Lee et al. (2005) [23].

2.4. Sugar and Organic Acid Extraction and Quantification

Sugars and organic acids were extracted two times by mixing 200 mg of freeze-dried powder with 200 mg of PVPP in 10 mL of Milli-Q-water for 1 hour at room temperature. After centrifugation of the slurry (10 min at 4000× *g*), the supernatants were collected and 1 mL of extract was further centrifuged (10 min at 15,000× *g* and injected into the HPLC system (Agilent 1100 series)). The identification and quantification were performed by the HPLC system equipped with a pump system, a refractive index detector (RID) for sugar analysis, and a UV/Vis detector monitoring organic acids at 210 nm

onto an Aminex HPX-87H column (300 × 7.8, 9 µm) (Bio-Rad, Hercules, CA, USA), kept at 55 °C. The analytical method was the same as reported in Gerardi et al. (2015) [24].

2.5. Carotenoid Content

Samples were frozen in liquid nitrogen, grinded in a mortar until finely powdered, and stored at −80 °C until the analysis. Extractions were performed according to the method described in Koch and Holdman (2004) [25]. The supernatants were combined and dried with a rotary evaporator. Samples were resuspended in 1 ml of ethyl acetate and analyzed by RP-HPLC DAD as previously described [22].

2.6. Determination of Antioxidant Activity

The TEAC assay was performed as previously reported [26]. Briefly, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma-Aldrich, St. Louis, MO, USA) radical cations were prepared by mixing an aqueous solution of 2.45 mM potassium persulfate (final concentration) and an aqueous solution of 7 mM ABTS (final concentration) and were allowed to stand in the dark at room temperature for 12–16 h, before use. The ABTS radical cation solution was diluted in PBS (pH 7.4) to an absorbance of 0.40 at 734 nm. Trolox was used as antioxidant standard and to prepare a standard calibration curve (0–16 µM). After the addition of 200 µL of diluted ABTS to 10 µL of Trolox standard or extracts diluted in PBS, in each well of a 96 well-plate (Costar), the absorbance reading at 734 nm was taken 6 min after initial mixing using an Infinite200Pro plate reader (Tecan, Männedorf, Swizerland). Appropriate solvent blanks were run in each plate. All extracts were assayed at least at three separate dilutions and in triplicate. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of concentration of Trolox and the TEAC value expressed as Trolox equivalent (in µmolar) using Magellan v7.2 software.

2.7. Statistical Analysis

Values were expressed as mean ± SD of three independent experiments. One-way analysis of variance (ANOVA) was performed, followed by separation of means with Tukey's multiple comparison test, using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA).

3. Results

Tiggiano carrots are characterized by different colors of epidermis. Figure 1a shows representative yellow, orange and yellow-purple carrots analyzed in this study. It is worth mentioning that sections of the purple carrots showed that this color was mainly limited to the epidermis, whereas the cortex and the vascular cylinder showed a typical purple-orange and yellow-pigmented color, respectively (Figure 1b).

Due to their colors, we firstly investigated the presence of anthocyanins (Figure 2a). The anthocyanins content was over 100 mg cyanidin-3-glucoside equivalents/100 g of fresh weight in Tiggiano yellow-purple carrots. As expected, anthocyanins were undetected in the yellow and orange carrots. We also analyzed the content of phenolic acid, another phytochemical belonging to the group of polyphenols. As shown in Figure 2b, the phenolic acid content in the Tiggiano carrots was higher compared to the orange commercial cultivar used as control. Among the Tiggiano carrots, the yellow-purple ones showed the highest amount of these polyphenols, with chlorogenic acid as the main representative (−2.6 mg/g dry weight, compared to −1 mg/g dry weight detected in the commercial carrot cultivar), followed by caffeic acid (−0.26 mg/g DW compared to −0.18 mg/g DW in the commercial carrot cultivar). Other phenolic acids, mainly *p*-coumaric acid and ferulic acid, were detected in traces.

Figure 1. (**a**) Multicolored phenotypes of Tiggiano carrots. (**b**) Sections of a purple Tiggiano taproot in detail. The epidermis shows the typical purple color followed by the cortex (purple-orange pigmented) and the inner core, represented by the vascular cylinder (yellow pigmented).

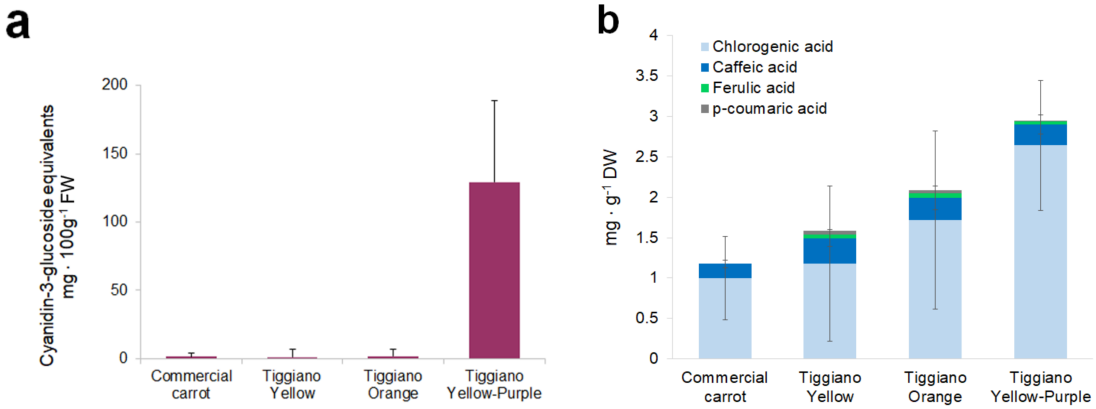

Figure 2. (**a**) Anthocyanins and (**b**) phenolic acids content in Tiggiano carrots compared to a commercial cultivar. Data are expressed as means (n = 3) ± SD.

We investigated the content of organic acids and sugars, which are an important quality parameter and are involved in the taste and flavor of vegetables, influencing the overall level of acidity of the fruits. Three main organic acids were identified in Tiggiano carrots: malic, tartaric, and citric acids. As shown in Figure 3a, malic acid was the most abundant, both in commercial and Tiggiano carrots. Malic acid content in the Tiggiano carrots was higher than in the commercial cultivar but this result was not statistically significant. On the other hand, the level of citric acid was significantly lower in Tiggiano carrots than in the commercial orange cultivar.

Using the method described in Materials and Methods, we were able to obtain a simultaneous identification and quantification of organic acid and monosaccharides. Concerning the monosaccharides, the most abundant was glucose followed by fructose, where either yellow or orange Tiggiano carrots showed a significant higher glucose content (0.5 g/g of dry weight) compared to the commercial carrot and purple/orange Tiggiano carrot.

Next, we analyzed the carotenoid content in the different colored Tiggiano carrots. As illustrated in Figure 4, β-carotene was the main carotenoid found in our analyses, reaching the highest levels in the commercial and yellow-purple carrots. Significantly low levels were recorded in yellow/orange Tiggiano carrots. Concerning lutein, a significantly higher level was observed in yellow-purple Tiggiano carrots (−90 µg/g DW) compared to the commercial or yellow/orange Tiggiano carrots.

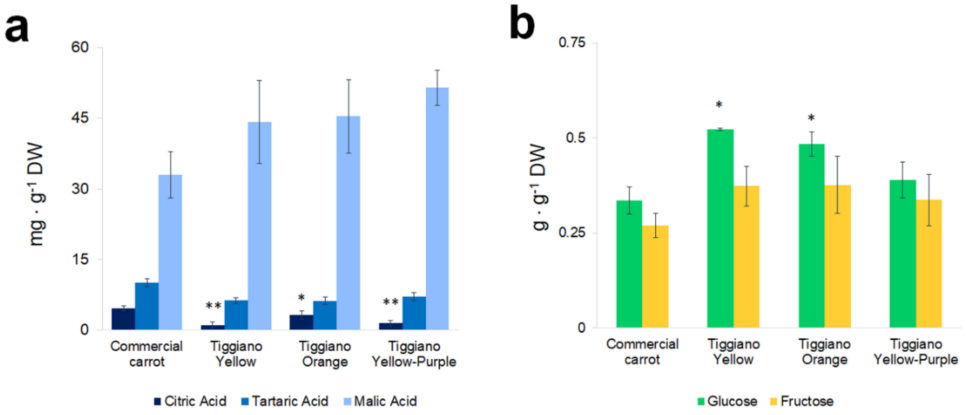

Figure 3. (**a**) Main organic acids and (**b**) sugars detected in Tiggiano carrots compared to the commercial cultivar. Data are expressed as mean ± SD. Significance assumed at * $p < 0.05$, ** $p < 0.01$.

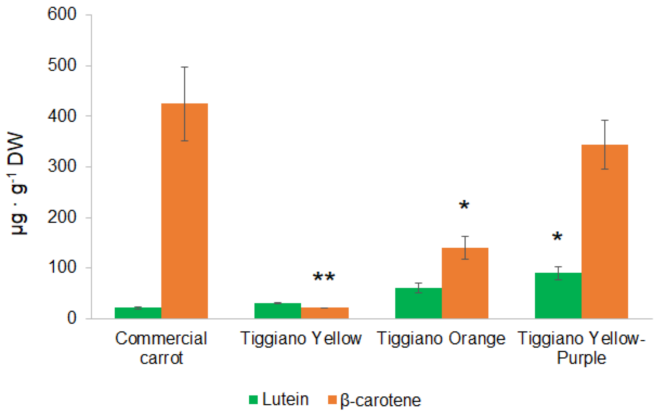

Figure 4. Carotenoid content in Tiggiano carrots compared to a commercial cultivar. Significance assumed at * $p < 0.05$, ** $p < 0.01$.

Finally, we measured the antioxidant activity in Tiggiano carrots by the Trolox Equivalent Antioxidant Capacity (Figure 5). Our results pointed out a similar anti-oxidant capability in the extract of yellow or orange Tiggiano carrots and the commercial carrot. Notably, the extracts from purple carrots showed a significant higher antioxidant capacity in the hydrophilic fraction, which doubled that of the commercial carrot and those from the yellow/orange Tiggiano carrots.

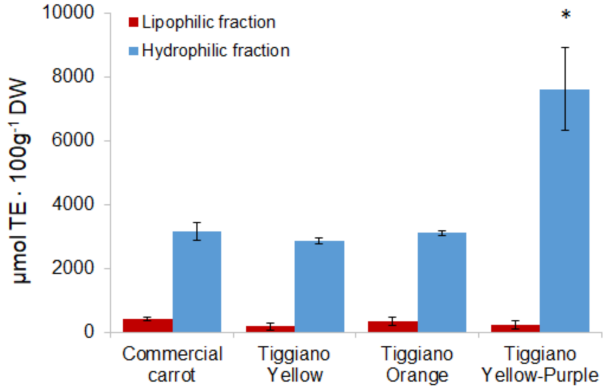

Figure 5. Antioxidant profile of Tiggiano carrots compared to a commercial cultivar. Significance assumed at * $p < 0.05$.

4. Discussion

In this study, we carried out a phytochemical and antioxidant profiling of the main components associated with the quality and nutritional value of the Apulian colored Tiggiano carrots. Yellow and orange Tiggiano carrots showed different features in respect to the yellow-purple carrots. Compared to the yellow-purple ones, the yellow and orange Tiggiano carrots showed higher glucose and lower citric acid levels. Both these parameters influence the organoleptic properties of food products [27], since sugars reduce the perception of acidity, affecting the taste and the sweetness of fruits [7,28]. On the other hand, the reduced levels of citric acid in all Tiggiano carrots may influence the astringency sensation [29].

Notably, the yellow-purple Tiggiano carrots also showed a similar β-carotene content, but higher lutein levels compared to the commercial cultivar or yellow/orange Tiggiano carrots, a result that can further contribute to the nutritional quality of these colored fruits.

Moving to other phytochemicals related to the nutritional value of fruit crops, our results clearly indicated that high levels of phenolic acids (such as caffeic acid and chlorogenic acid) and anthocyanins were specifically associated with yellow-purple carrots. All these compounds have been associated to the health-promoting properties of fruits and vegetables [19,22]. Our results are in agreement with other studies, which reported a higher content of these phytochemicals in the cortex of different purple carrot varieties [5].

Tiggiano carrots show similarities with Polignano carrots, another Apulian landrace previously characterized [8]. Similarly to the Tiggiano yellow-purple carrots, Polignano carrots are also characterized by a high content of phenolic acids and polyphenols assayed by Folin-Ciocalteau assay [8]. Furthermore, Polignano carrots showed a higher glucose and fructose content than the commercial carrots [8] confirming our results on carbohydrate content of Tiggiano carrots. Taken together, these results revealed interesting similarities in these two landraces. Further studies are now in progress to verify the genetic relationships between Tiggiano and Polignano carrots.

Anthocyanins are natural pigments with a protective function against abiotic (UV irradiation, cold) or biotic stresses. However, the reasons why the anthocyanin biosynthetic pathway is activated in yellow-purple and not in orange or yellow carrots are still unknown. In Tiggiano yellow-purple carrots, we also found a higher antioxidant capacity compared to the other carrots, which likely contributes to increasing the nutritional quality of the fresh product. On these bases, their consumption might have positive effects on human health. Other studies indicate that diets rich in anthocyanins and phenolic acids have protective effects against oxidative stress, involved in the onset of aging processes and several human pathologies [21,22].

5. Conclusions

Crop landrace conservation is an emerging challenge to be addressed, due to progressive susceptibility to the genetic erosion of local crops, as in the case of the Apulian carrot varieties. In general, the cultivation of landraces is decreasing because of the modern cropping systems that use hybrids with features mainly satisfying the requests of the global market. Nevertheless, some local varieties still maintain genetic traits adapted to be more efficient in stress defense and nutritive uptake and, in some cases, they are able to accumulate phytonutrients, thus showing interesting features for the human diet. In the local carrot landraces analyzed in this study, besides their role in nutritional quality, the presence of bioactive compounds highlights on the possible activation of the anthocyanin biosynthetic pathway in the taproots. Therefore, further studies will be useful to elucidate the genetic features of the different Apulian carrots landraces. Despite the fact that it is still unclear if the Apulian colored carrots derive from the same genetic background, they represent important local genetic resources, to be preserved and valorized.

Author Contributions: A.S. (Aurelia Scarano), L.D., C.G. performed the experiments; A.S. (Aurelia Scarano), L.D., C.G., A.S. (Angelo Santino) analyzed the data; A.S. (Aurelia Scarano), R.A., A.S. (Angelo Santino) wrote the paper with the approval of all the authors.

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Conflicts of Interest: The authors declare no conflict of interest.

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