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# Morpho-Biometrical, Nutritional and Phytochemical Characterization of Carrot Landraces from Puglia Region (Southern Italy)

**Anna Bonasia [1], Giulia Conversa [1,*], Corrado Lazzizera [1], Giuseppe Gambacorta [2] and Antonio Elia [1]**

[1] Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, 71100 Foggia, Italy; anna.bonasia@unifg.it (A.B.); corrado.lazzizera@unifg.it (C.L.); antonio.elia@unifg.it (A.E.)

[2] Department of Soil, Plant and Food Sciences, University of Bari, 70126 Bari, Italy; giuseppe.gambacorta@uniba.it

* Correspondence: giulia.conversa@unifg.it

**Abstract:** The explorations as a part of the regional BiodiverSO Programme of vegetable genetic resource rescue revealed that in the *arenili* (sandy shores) of "Salterns of Margherita di Savoia" (SMS), a coastal landscape area of Puglia region (southern Italy), along the commercial genotypes of small rooting species, landraces are still cultivated. The morpho-biometrical, nutritional and phytochemical properties of two carrot landraces ("Carota a punta lunga" and "Carota a punta tonda") and a commercial carrot hybrid ("Presto") collected from the SMS area are examined. The study highlighted that the pedological conditions of the *arenili* of the SMS area are the main driving force in controlling the nutritional and nutraceutical characteristics of carrot, conferring to genotypes grown in this area a high profile in comparison with literature data. In the site of cultivation of *arenili*, a large variability in the morpho-qualitative traits emerged among carrot genotypes. "Carota a punta tonda" stands for a promising genotype being very rich in phenols (209.8 mg $kg^{-1}$ fw) (mainly di-caffeic acid derivative and chlorogenic acid), ß-carotene (21,512 µg 100 $g^{-1}$ fw), and high antioxidative proprieties. "Carota a punta tonda" could be considered as a healthy product for consumers and also amenable to selection for breeding purpose. Increasing the knowledge about nutritional and nutraceutical properties of local landraces may push the preference of consumers beyond the local community and, at the same time, farmers can be stimulated to continue their cultivation. Thus, the promotion of their on-farm/in situ conservation (cultivation) could represent an efficient strategy for agro-biodiversity preservation.

**Keywords:** *Daucus carota* L.; phenols; ß-carotene; *arenili*



**Citation:** Bonasia, A.; Conversa, G.; Lazzizera, C.; Gambacorta, G.; Elia, A. Morpho-Biometrical, Nutritional and Phytochemical Characterization of Carrot Landraces from Puglia Region (Southern Italy). *Sustainability* **2021**, *13*, 3940. https://doi.org/10.3390/su13073940

Academic Editor: Richard A. Niesenbaum

Received: 24 February 2021
Accepted: 25 March 2021
Published: 2 April 2021



## 1. Introduction

The agricultural intensification with modern plant breeding focused on few high-yielding crops and varieties. Thus, a wide range of plant species and varieties, representing an important component of agro-biodiversity, have been highly threatened or endangered [1].

The landrace, namely "locally adapted variety", a genotype well-adapted to an "environment" where the landrace itself has been selected by farmers ("holders" or "keepers"), thus evolved in a certain ecogeographical area and adapted to the edaphic and climatic conditions and to its traditional management and uses [2,3]. According to a more recent and inclusive definition, the landraces consist of cultivated varieties that have evolved and may continue evolving, using conventional or modern breeding techniques, in traditional or new agricultural environments within a defined ecogeographical area and under the influence of the local human culture [4].

It is worth mentioning that a higher resistance to pests, diseases, and abiotic stresses of landraces could embrace a sustainable agriculture system facing global climate changes [5].

Additionally, their use is a potential way to achieve social-ecological resilience, i.e., the capacity of human-environment systems to absorb shocks induced by changes, so that the system continues to support human well-being [6].

Due to a recent increasing demand for functional foods, many recent studies have highlighted the potential of the exploitation of landraces, mostly associated with the high content of phytochemicals, secondary metabolites with bioactive and antioxidant properties—phenols and carotenoids in carrot landraces [7–10], glucosinulates and vitamic in turnip landraces [11], phenols in garlic landraces [12].

It is significantly assessed that various parameters such as genetic background, soil conditions, cultivation technique and climatic conditions could affect the composition and content of bioactive compounds of landraces as well as biometrical, nutritional and organoleptic properties [13,14].

Many landraces have already disappeared from cultivation and many others are considered highly susceptible to erosion [3], mainly due to the introduction of the modern cultivars. A consequence of modern breeding practices determined the replacement of landraces [15].

In Italy, genetic agro-diversity has been eroded [16] and the Puglia region (southern Italy) is suffering from continuous genetic erosion, especially for vegetable genetic resources [17]. The recent project "Biodiversity of the Puglia" (BiodiverSO, https://biodiversitapuglia.it//, accessed on 3 March 2021), launched by the Puglia Region Administration, under the Rural Development Program, aimed at seeking, identifying and collecting plant resources at risk of genetic erosion, and enacting their protection and recovery [18].

The BiodiverSO program highlights how the Puglia region is an example of how local vegetable varieties are still interacting with modern horticulture: it is very rich in local varieties of vegetables and some of them are still used by regional communities [18,19].

The explorations of the above-cited vegetable genetic resource rescue programme (BiodiverSO) revealed that in the *arenili* located in the "Salterns of Margherita di Savoia" (SMS), a coastal landscape in the North of Puglia region, which falls in the Barletta-Andria-Trani (BT) and Foggia (FG) provinces, [20], several vegetable landraces are still cultivated along with commercial vegetable genotypes [19]. The *arenili* are sandy shores, laid out in a comb system of long narrow field strips, located between the widest salterns in Europe (the salterns of Margherita di Savoia) and the Adriatic sea. Nowadays, they are extended for 30 km alongside the sea and widened to about 2 km in the countryside near the villages of Zapponeta (FG) and Margherita di Savoia (BT) and the city of Barletta (BT). The *arenili* are the result of a long work of reclamation of an ancient coastal lake-lagoon in Puglia ("Salpi" lake), a swampy area covered with vegetation immersed in 10 cm of saltwater [21]. This work provided for the removal of sand from the retro-dunal cordon and its positioning in the marshy area to a height of at least 100 cm above an impermeable layer of clay ("clay table"), on which underground aquifers flow. According to their origin, the *arenili* are shallow sandy soils (1–2 m deep), light grey, without stones and easy to work, very permeable, with a low water holding capacity, sub-alkaline reaction, low content of active limestone, averagely endowed in exchangeable potassium and poor in microelements. The existing "clay table" under the sandbank creates the conditions for a water reserve of rainwater during the autumn–winter period. The water reserve is available for plant growth thanks to capillary rise. However, when high-intensity rainfall occurs, flooding may be experienced by the crops. Despite seawater infiltration into the aquifers occurring during the dry season, the saline layer is far deeper than the crop root apparatus. These peculiarities of *arenili* make them suitable for the cultivation of root vegetables at autumn–winter cycle. The possibility to extend the period of cultivation is strictly linked with the availability of fresh irrigation water in the September–November and April–June periods, provided by the regional consortium network for collective irrigation.

Nowadays, the cultivations in the *arenili* of the SMS area are mainly based on commercial genotypes of carrot, potato and onion, but also on a typical landrace of onion ("Cipolla

Bianca di Margherita"), recently recognized with the protected geographical indication (PGI) European label by the Italian Ministry of Agricultural, Food, and Forestry Policies (MiPAAF-PGI, 2015), all destined to national market.

Thanks to the above-cited BiodiverSO Project, it has been revealed that in the *arenili* of the SMS area located around Margherita di Savoia (BT) village, along with the hybrid cultivars of carrots, the local growers still cultivate landraces of orange carrots, mostly for family consumption. The Puglia region also counts the cultivation of other carrot landraces, recognized by regional mark as Traditional Italian Agri-food Products (TIAP), such as the "yellow-purple Polignano carrot" (YP-PC), a multi-coloured-root landrace grown in Bari province (MiPAAF-TIAP, 2015), and "Tiggiano Carrots" (TC), a landrace with a dark purple epidermis and a yellow-orange inner core, cultivated in Lecce Province (MiPAAF-TIAP, 2004). Their morpho-chemical properties along with the traditional gastronomic uses have been highlighted—for YP-PC [7,8,10,22] and for TC [9]—contributing to expand their consumption beyond the local community and to increase their cultivation by holders.

Thus, the identification of the morphological, nutritional and nutraceutical properties of the resources at high risk of erosion could represent an efficient strategy for agro-biodiversity preservation.

Aiming to promote the consumption and the conservation of two carrot landraces grown in the *arenili* around Margherita di Savoia (BT), the present work describes the morpho-biometrical, nutritional and phytochemical properties, with a special interest in the composition of minerals, organic acids, sugars, phenols, ß-carotene, and in the antioxidant capacity of roots, in comparison with a commercial carrot genotype grown in the same site of cultivation.

## 2. Materials and Methods

### *2.1. Plant Materials, Collecting Site and Sampling*

Three carrot genotypes were directly collected from fields of local growers during the spring period in 2017. Two out of three carrot genotypes were landraces, which in relation to the shape of the tip of the taproots, are named by the growers as "Carota a punta tonda" (CPT, meaning: rounded tip carrot) and "Carota a punta lunga" (CPL, meaning: elongated tip carrot). These landraces can be considered "creole" as they derive from cultivars introduced to the area over 50 years ago, and since then have been selected and auto-propagated by the local farmers [19]. CPL probably derives from a variety called "99" ("Fiumicino"), belonging to the "Nantes" type (Figure S1), and CPT from a variety called "66", belonging to the "Imperator" type (Figure S1).

Along with the landraces, the hybrid F1 "Presto", an orange type carrot (Vilmorin-Mikado, Fano, PU, Italy), henceforth referred to as the commercial genotype (CG), was also collected.

The site of cultivation was the *arenili* in the "Salterns of Margherita di Savoia" area, specifically located around Margherita di Savoia (BT) village. In general, the carrot growers adopted low-input techniques, particularly no or little fertilizer for landraces. The climate conditions of the *arenili* are those of the main municipality in the SMS area: Margherita di Savoia (41°22′31″ N; 16°9′13″ E) (0–10 m a.s.l.), characterized by a mild climate (annual mean temperature-Tmean 16.2 °C) with hot summers (August, Tmean 25.4 °C, Tmax 31.2 °C) and mild winters (Tmean 7.3 °C), and by quite scarce rain (489 mm), mostly concentrated during late autumn and winter (data reported by https://www.ilmeteo.it/portale/medie-climatiche/Margherita+di+Savoia, for the last 30 years; accessed on 4 May 2020).

For each genotype, samples of 20 ± 0.5 kg of roots were well mixed to obtain three independent replicates, each consisting of 50 roots. Morphological (equatorial and longitudinal diameter) and physicochemical measurements (fresh and dry weight, dry matter concentration, colour, Total Soluble Solids, pH, total titratable acidity) were carried out in duplicate on all fresh materials of each replicate. Chemical analyses were performed in triplicate on a representative sample of the fresh material, for each replicate. The representative sample was obtained by a 2 cm-cut and blended root bulk. For chemical analyses,

the samples were previously freeze-dried (CoolSafe ScanVac; LaboGene, Allerød, Denmark), powdered, packed in hermetic jars and then stored in the dark at $-18 \pm 1$ °C until the analyses were carried out.

### *2.2. Morphological Measurements and Physicochemical Analysis*

The morphological measurements (equatorial and longitudinal diameter) and the colour of roots were performed by images from an image acquisition station (Immagini & Computer, Bareggio, Italy), equipped with 4 Tornado Esaver white lamps (23 W) (Philips Lighting Italy s.p.a., Milan, MI, Italy), a Nikon D5200 camera and Image Pro Plus 7.0 software (Media Cybernetics Inc., Rockville, MD, USA). Colour indices were based on the C.I.E.L.a.b. scale 1976: L*, indicating lightness/darkness, ranging from 0 (black) to 100 (white) value in a greyscale; a* reflecting greenish (if negative) to reddish (if positive) tonality; b* indicating bluish (if negative) to yellowish (if positive) tonality. In addition, the derived parameters, hue angle (h°) and chroma index (C*) were evaluated, indicating, respectively, the hue and the vividness/dullness.

The dry matter concentration was calculated as dry weight (DW)/fresh weight (FW)*100. In order to determine the DW, fresh material was freeze-dried (CoolSafe ScanVac; LaboGene, Allerød, Denmark).

Total Soluble Solids (TSS) of samples was assayed using the refractometric method (digital refractometer DBR35, Giorgio Bormac s.r.l., Carpi, Italy), according to the method given in Association of Official Agricultural Chemists-AOAC (2000).

To measure pH, a fresh sample (10 g) was blended in 100 mL of distilled water. The pH was measured using a pH meter (Hanna Instruments Italia s.r.l., Villafranca, Italy).

Total titratable acidity was determined according to the method given in AOAC (2000). Fresh samples were homogenized into a blender and then passed through filter paper. The filtrate was diluted with distilled water and the pH was measured (Hanna Instruments Italia s.r.l., Villafranca, Italy). The same filtrate was then titrated with 0.1 N NaOH solution up to an end-point of 8.2. The result is expressed as grams of citric acid per 100 g of fresh weight.

### *2.3. Chemical Analysis*

#### 2.3.1. Minerals

Ashes were determined by muffle furnace according to the AOAC method 923.03. Inorganic ions were analysed by ion chromatography (Dionex ICS 3000; Dionex-ThermoFisher Scientific, Waltham, MA, USA). Inorganic cations were extracted from lyophilised samples (1 g), previously ashed (in a muffle furnace at 550 °C for 6 h) and acid digested (20 mL of 1 mol $L^{-1}$ HCl in boiling water for 30 min), before injection into the ion chromatography system. For inorganic anions, the lyophilized samples (0.5 g) were extracted with 50 mL of eluent solution (3.5 mM sodium carbonate and 1.0 mM sodium bicarbonate) in a shaking water bath at room temperature for 30 min. The mixture was filtered through Whatman n. 2 paper. The filtrates were filtered again through 0.22 μm Millipore filter, before injection into the ion chromatography system. The ion chromatography system was equipped with: an isocratic pump; conductivity detector; a model AS-DV auto-sampler; a self-generating ERS-500 suppressor (4 mm); an Ion-Pac AS23 analytical column (4 mm × 250 mm, particle size 6 μm); and an eluent solution (3.5 mM sodium carbonate and 1.0 mM sodium bicarbonate) at a flow rate of 1 mL $min^{-1}$ (Dionex-ThermoFisher Scientific, Waltham, MA, USA) (specifically, for anion analysis); and a self-generating DRS-600 suppressor (4 mm); an IonPack CS12A analytical column (4 × 250 mm, 5 μm); and an eluent solution (20 mM methanesulfonic acid) at a flow rate of 1 mL $min^{-1}$ (Dionex-ThermoFisher Scientific, Waltham, MA, USA) (specifically, for cation analysis).

#### 2.3.2. The Simple Carbohydrates and the Sweetness Index

Samples were analysed according to Rohrer [23] using ICS 3000 System (Dionex-ThermoFisher Scientific, Waltham, MA, USA) high-performance anion-exchange chro-

matography with pulsed amperometric detection (ED50; Dionex-ThermoFisher Scientific, Waltham, MA, USA), equipped with a Carbopac PA-1 column (CarboPac PA1 Analytical, 4 × 250 mm; particle size 10 µm) (Dionex-ThermoFisher Scientific, Waltham, MA, USA) maintained at 30 °C. Glucose was eluted with NaOH (150 mM) at a flow rate of 1.0 mL $min^{-1}$ for 15 min. Glucose was identified by a comparison of the retention times with standard. Peak areas were analysed using Dionex Chromeleon software (version 6.80, Dionex-ThermoFisher Scientific, Waltham, MA, USA). Total simple carbohydrates were extracted from 15–30 mg of lyophilized sample by adding 15 mL of ultrapure water and using shaking water baths (Foss, Padova, Italy) for 45 min at room temperature. Then, the mixture was centrifuged at 19,000× *g* (10 min); the supernatant was collected, filtered through a 0.45 µm membrane filter and analysed as previously described. Carbohydrates were identified by a comparison of the retention times with those of sugar standards. Peak areas were analysed using Dionex Chromeleon software (version 6.80, Dionex-ThermoFisher Scientific, Waltham, MA, USA).

The sweetness index (SI = (g glucose 100 $g^{-1}$ FW) × 1.00 + (g fructose 100 $g^{-1}$ FW) × 2.30 + (g sucrose 100 $g^{-1}$ FW) × 1.35)) was also assessed.

#### 2.3.3. Organic Acids

A lyophilized sample (0.3 g) was placed in a 50 mL tube and 20 mL of metaphosphoric acid (0.1%) was added. The sample was mixed in a shaking water bath at room temperature for 15 min, then centrifuged (16,000× *g*, 4 °C for 15 min) (SR16L, ThermoFisher Scientific, Waltham, MA, USA). The supernatant was collected, filtered and stored at 4 °C until analysed according to González-Castro et al. [24] with some modifications.

Organic acids were separated by ICS 3000 HPLC System (Dionex-ThermoFisher Scientific, Waltham, MA, USA) equipped with: an isocratic pump, a 10 µL injection loop, an AS-DV auto-sampler, a Hydro-RP 80A column (250 × 4.60 mm) (Phenomenex Inc., Castel Maggiore, BO, Italy), maintained at 30 °C combined with a UV-visible detector (RLSC Diode Array Detector) (Dionex-ThermoFisher Scientific, Waltham, MA, USA), set to a wavelength of 210 nm, and Chromeleon version 6.50 software.

The eluent consisted of 100 mM $Na_2SO_4$ at pH 2.6 adjusted with methanesulfonic acid at a flow rate of 1 mL $min^{-1}$. Individual organic acids were identified by comparing retention times with those of available standards.

#### 2.3.4. Phenolic Compounds

A lyophilized sample (0.05 g) was placed in a 2 mL Eppendorf tube with the addition of 1 mL of 80% methanol in water. The sample was mixed for 1 min, sonicated for 5 min and then centrifuged (4000× *g* 4 °C for 20 min). The clear supernatant was diluted 1:1 with acetonitrile:water (10:90, *v*/*v*) solvent mixture containing 0.1% formic acid, and filtered using re-generated cellulose filters of 0.22 µm pore diameter. The analysis was performed according to Pasqualone et al. [25] with some modifications using the Ultra High Performance Liquid Chromatography (UHPLC) Dionex Ultimate 3000 RS system (quaternary pump, auto-sampler, column oven and diode array detector (DAD), coupled with the HESI-II probe with the LTQ Velos Pro ion trap mass spectrometer (Dionex-ThermoFisher Scientific, Waltham, MA, USA). The separation of compounds was performed on Hypersil GOLD aQ C18 column, 100 mm length, 2.1 mm ID and 1.9 µm particle size (Waters, Milford, MA, USA) maintained at 30 °C. A binary mobile phase was used: (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, at a constant flow of 0.2 mL/min. The gradient program of solvent B was as follows: 0–30 min from 10% to 70%, 30–33 min isocratic 70%, 33–33.1 min from 70% to 10%. The mass spectrometry (MS) conditions were: capillary temperature 320 °C; source heater temperature 280 °C; nebulizer gas $N_2$; sheath gas flow 30 arbitrary units; auxiliary gas flow 15 arbitrary units; capillary voltage—2.8 kV, S-Lens RF Level 60%. Data were acquired in negative ionization mode.

Phenolic compounds were identified by comparing elution times, molecular ions, and MS/MS fragmentation patterns of the experimental spectra with those obtained by the

available pure standard compounds or by tentative methods using reported data from the literature. Calibration curves were created to obtain quantification results and were based on the UV signal of each available standard. When no commercial standard was available, a similar compound from the same phenolic group was used as a standard.

#### 2.3.5. Antioxidant Capacity

The antioxidant capacity (AC) was determined by ABTS assay based on the formation of the radical $ABTS^+$ by the reaction of ABTS (2,2-Azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid) (7 mM $L^{-1}$) with 140 mM $L^{-1}$ of potassium persulphate. In brief, 20 µL of the extract was reacted with 1 mL of $ABTS^+$ radical. The hydrophilic fraction was extracted twice from lyophilized samples using 80% (*v*/*v*) methanol in a shaking water bath (22 °C) for 15 min and by centrifugation (19,000× *g*; 10 min). The supernatants were combined. The lipophilic components were extracted twice with 1 mL of hexane, using the above conditions. The antioxidant activity was assessed as TEAC (trolox equivalent antioxidant capacity) according to Re et al. [26].

#### 2.3.6. ß-Carotene

ß-carotene was extracted and quantified as reported by Taungbodhitham et al. [27] with some modifications. Lyophilized samples (0.1 g) plus 0.05 g of $MgCO_3$ (to neutralize cytosolic acids) and 0.01 g of celite (for better tissue disruption) were extracted with 10 mL of ethanol:hexane (4:3 by volume); 1 mL of pyrogallol solution (5%) was added as an antioxidant. The mixture was placed in a mechanical shaker for 15 min and then centrifuged at 5000× *g* for 10 min, and the supernatant was collected. The residue was re-extracted; the two extracts were combined and decanted into a 50 mL tube. The supernatant hexane phase was then transferred into another tube, and the lower aqueous phase was discarded.

To overcome the problem of carotenoid overestimation by the presence of chlorophyll, a saponification step was included during extraction. In brief, an equal volume of 10% methanolic KOH was added to the recuperated hexane phase, the mixture was shaken vigorously for 1 min and placed on ice for 15 min. After centrifugation at 5000× *g* for 10 min, the supernatant (hexane phase) was collected and washed 2 times with 15 mL of NaCl 10% solution and 2 times with 15 mL water. The aqueous phase was discarded. All samples were stored at −25 °C until analysis.

The ß-carotene was separated using a gradient HPLC method with an ICS 3000 System (Dionex-ThermoFisher Scientific, Waltham, MA, USA) which included: a gradient pump, a 10 µL injection loop, C18 5 µm reverse-phase ion-exchange columns (Kinetex Core-Shell, Phenomenex Inc., Castel Maggiore, BO, Italy), combined with a UV-visible detector (RLSC Diode Array Detector) (Dionex-ThermoFisher Scientific, Waltham, MA, USA), set to a wavelength of 445 nm, and Chromeleon version 6.50 software (Dionex-ThermoFisher Scientific, Waltham, MA, USA). The oven temperature was set at 35 °C. Mobile phase A consisted of acetonitrile:methanol:tris buffer (0.1M, pH 8) (84:2:14); mobile phase B had a methanol:ethyl acetate ratio of 68:32. Compounds were separated using the following program, with a flow of 1 mL $min^{-1}$: mobile phase A for the first 5 min gradually followed by eluent B from the 5th to the 17th minute; isocratically B from the 17th to 30th minute; 1 min to returning to 100% A and finally 5 min at 100% A. The ß-carotene was identified by comparing retention times and spectra with its standard.

### *2.4. Statistical Analysis*

One-way analysis of variance was performed using the Statistical Analysis Software (SAS, Cary, NC, USA). The least significant difference (LSD) test ($p = 0.05$) was used to establish differences between means.

## 3. Results and Discussion

### *3.1. Morphological and Physicochemical Features of Carrot Genotypes*

The morphological and physicochemical traits of the examined genotypes—"Carota a punta tonda" landrace (CPT, meaning: rounded tip carrot); "Carota a punta lunga" landrace (CPL, meaning: elongated tip carrot), and the commercial genotype (CG) (hybrid F1 "Presto")—are reported in Table 1.

**Table 1.** Morpho-biometrical and physicochemical features of carrot roots.

| | Fresh Weight | Equatorial Diameter | Length | Dry Matter | L* | C* | h° | pH | TSS [3] | TA [4] |
|---|---|---|---|---|---|---|---|---|---|---|
| | (g) | (mm) | (mm) | (g $kg^{-1}$ fw) | | | | | (°Brix) | (g 100 $g^{-1}$ fw) [5] |
| CPL [1] | 72.4 ± 6.7 b | 23.0 ± 1.1 b | 177 ± 6 b | 97.8 ± 0.9 c | 72.0 ± 0.4 a | 27.6 ± 0.6 b | 51.5 ± 0.3 b | 6.4 ± 0.03 b | 4.5 ± 0.1 c | 0.13 ± 0.0 ab |
| CPT [1] | 52.2 ± 6.7 b | 24.6 ± 1.3 b | 158 ± 7 c | 165.5 ± 3.2 a | 64.8 ± 1.0 b | 32.8 ± 1.2 a | 56.4 ± 0.6 a | 6.4 ± 0.02 b | 7.3 ± 0.5 a | 0.14 ± 0.01 a |
| CG [1] | 151.3 ± 10.2 a | 31.0 ± 1.2 a | 226 ± 6 a | 114.8 ± 1.5 b | 65.8 ± 0.6 b | 33.0 ± 0.9 a | 50.5 ± 0.5 b | 6.6 ± 0.02 a | 5.5 ± 0.2 b | 0.12 ± 0.0 b |
| Significance [2] | *** | *** | *** | *** | *** | *** | *** | *** | *** | ** |

[1] CPL, "Carota punta lunga"; CPT, "Carota punta tonda"; CG, commercial genotype. [2] ** and *** significant at $p \leq 0.01$ and $p \leq 0.001$, respectively. Means (n = 6) (±standard error) in columns not sharing the same letters are significantly different according to least significant difference (LSD) test ($p = 0.05$). [3] TSS, total soluble solids. [4] TA, titratable acidity. [5] Citric acid monohydrate.

The average root weight (62 g) and equatorial diameter (23.8 mm) of the landraces were much lower in comparison with the CG, with CPT showing the shortest roots (157.6 mm) and CG the longest (226.2 mm). The low standard biometrical features of the landraces, compared with the tested CG, could be strictly related both to their genetic characteristics and to the environmental factors, including the technical practices. Specifically, very limited agronomic input is provided by the local growers.

Colour is one of the most important physical properties of raw and processed food since it can firstly affect consumer buying decisions [28]. The examined carrot roots were characterized by a positive value of b* (24.9) and a* (18.6) indices—indicating the yellowish and reddish colour components, respectively—by a resulting hue value (52.8°) allowing colour identification as orange. A slight colour variability emerged among genotypes and between the examined landraces, with CPL showing less colourfulness and vividness of roots (highest L*, lowest C*).

Concerning the dry matter (DM) content, CPT and CPL showed the highest and the lowest values, respectively; although both landraces are cultivated with low inputs, their response lets us suppose that the genetic effect is predominant, confirming that genotype is one of the main determinants in the variability of the morpho-biometrical traits in carrots, as widely reported in the literature [29,30].

The DM of our landraces, in particular, that of CPT, was higher than that of a multi-coloured-root landrace from the Puglia region, named "yellow–purple Polignano carrot" (YP-PC) (82 g $kg^{-1}$ fw), grown very close to the Adriatic Sea coast border in the south of Bari province (Polignano a Mare, 40°59′31″ N; 17°13′17″ E) [7]. The lower moisture concentration, related to a higher resistance to quality deterioration [31], could be indicative of a better shelf-life performance.

The value of both total soluble solids (TSS) and titratable acidity (TA), respectively, indicative of the content of all soluble substances (mainly free sugars, but also organic acids, soluble pectins, amino-acids) and the "weak" organic acids [32], was the highest in CPT, consistent with the DM content. Since extensive studies on several vegetables correlate TSS with the perceived sweetness [33], a sweeter flavour can be inferred in CPT. CG showed the highest pH of juice, in an expected reverse relation with TA, indicating a slightly lower tartness than the landraces.

A higher firmness and a higher dry matter content (i.e., lower moisture) presuppose a higher mechanical resistance [34] and a higher resistance to quality deterioration [31] during the conservation of a product. According to the literature evidence [35,36], TSS values are strictly correlated to the firmness of roots (usually measured by a texture meter). Thus,

the landrace CPT, endowed with the highest firmness (TSS) and the lowest moisture, should be suitable for long storage, but all that has to be confirmed only in a further investigation.

### *3.2. Nutritional and Nutraceutical Features of Carrot Genotypes*

#### 3.2.1. Minerals

The anion and cation concentrations of the carrots studied are reported in Table 2.

**Table 2.** Mineral concentration (mg 100 $g^{-1}$ fw) of carrot roots.

| Genotype [1] | Ashes | Cations | | | | | Anions | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | K | Ca | Na | Mg | Total | Cl | $PO_4$ | $SO_4$ | $NO_3$ |
| CPL | 637 ± 26 b | 261 ± 11 b | 183 ± 11 b | 50 ± 1 c | 26.1 ± 1.7 b | 2.0 ± 0.1 b | 92.9 ± 5.1 a | 36.7 ± 2.3 b | 16.2 ± 1.2 c | 3.8 ± 0.4 c | 36.2 ± 3.3 a |
| CPT | 1041 ± 29 a | 449 ± 9 a | 277 ± 11 a | 109 ± 6 a | 60.1 ± 6.5 a | 1.9 ± 0.3 b | 88.1 ± 2.6 a | 50.5 ± 3.1 a | 22.0 ± 1.9 b | 15.1 ± 0.5 a | 0.5 ± 0.1. b |
| CG | 604 ± 16 b | 249 ± 8 b | 144 ± 6 c | 75 ± 4 b | 27.2 ± 1.4 b | 2.7 ± 0.2 a | 88.4 ± 4.3 a | 49.7 ± 1.2 a | 28.4 ± 1.4 a | 7.9 ± 0.8 b | 2.5 ± 0.2 b |
| Significance [2] | *** | *** | *** | *** | *** | * | ns | *** | *** | *** | *** |

[1] CPL, "Carota punta lunga"; CPT, "Carota punta tonda"; CG, commercial genotype. [2] ns, * and *** not significant or significant at $p \leq 0.05$ and $p \leq 0.001$, respectively. Means (n = 9) (±standard error) in columns not sharing the same letters are significantly different according to LSD test ($p = 0.05$).

The CPT landrace stands out for the higher ash content (+66%), which can be related to the total cation concentration (+76%). CPT showed, indeed, the highest K, Ca and Na concentrations, while Mg was the highest in CG. The total anion concentration was not affected by the genotype, but significant differences emerged between them by considering the individual anions. CPL had the lowest Cl, $PO_4$ and $SO_4$, but the highest $NO_3$ concentration. CPT had the greatest $SO_4$ concentration and, along with CG, it showed the highest and the lowest Cl and $NO_3$ concentrations, respectively. In this study, the genotype seems to be the dominant factor in controlling the variability in anion/cation concentration/composition, although different agronomic practices were applied to the landraces and the CG.

From a human nutrition point of view, K is an essential nutrient for the human body since its intake is associated with lower blood pressure and stroke incidence [37]. In particular, a low "Na/K" ratio in vegetables is advisable for lowering the risk of hypertension and blood pressure [38]. According to this ratio, CPT and CG showed the highest value (0.21), while CPL showed the lowest (0.15) ($p \leq 0.001$).

The level of K in the genotypes collected from the *arenili* of SMS was in agreement with that reported in the reference food composition databases, such as that of the Italian National Centre of Agriculture (INRAN-CREA) [39] and the European Food Safety Authority (EFSA) [40], and in line with that found in the Polignano area [7] (Table S1). The genotypes, grown in the *arenili* area, accumulated much lower Na than the INRAN-CREA [39] and the United States Department of Agriculture (USDA) [41] standard values, and also than the genotypes grown in fields along the coastal border in Polignano a Mare [7] (Table S1).

All the genotypes grown in the *arenili* of SMS had a lower "Na/K" ratio (on average, 0.18) than that grown in the other Apulian landrace (YP-PC, 0.24) or another commercial genotype (e.g., hybrid F1 "Presto", 0.31), grown in heavier soils [7], thus highlighting the better health value of the carrots from *arenili*, in particular for the CPL landrace.

In general, the Ca level in all genotypes grown in the *arenili* of the SMS area was much higher than the standards reported by INRAN-CREA [39], EFSA [40] and USDA [41] or observed for other genotypes grown in other parts of the Puglia region [7] (Table S1). In contrast, the Mg level of the genotypes grown in *arenili* was very poor in comparison with the standard values reported in Italian [39] and international [40,41] databases or the genotypes grown in other parts of the same region [7] (Table S1). These differences underline that, apart from the genotype, the environment has a large weight on affecting the cation concentration and profile in carrots [42,43].

The genotypes with the higher nitrate concentration had a lower Cl accumulation, following the well-known antagonistic uptake pattern between Cl and $NO_3$ [44]. A decrease in the osmotic values of vacuoles is prevented mainly by increasing Cl when plants accumulate less nitrate [45].

As a whole, genotypes grown in the *arenili* of the SMS area accumulated much lower nitrate levels than the genotypes grown in other areas of the same region (such as from the Polignano a Mare area [7]) and the genotypes from the North and East of Europe [46,47] (Table S1). This evidence confirms that, apart from the genotype, other environmental factors (climate, soil properties, agronomic practices) [48] may greatly affect the nitrate content accumulation. The very low level of nitrate in all examined genotypes could be strictly linked to the physical-chemical properties of *arenili*, sandy soils unable to hold nitrate ions and in which nitrate leaching is favoured. Additionally, the frequent flooding in *arenili* could cause a significant decline in the uptake of N, as observed by several authors [49,50]. A decrease in N concentration has been reported in the roots of *Distylium chinense* shrub under 60 days of flooding [51].

According to the classification of vegetables in terms of their capacity to accumulate nitrate in edible products [52,53], carrot genotypes grown in the *arenili* of the SMS area can be considered "very low" nitrate-accumulating vegetables (130.7 mg $kg^{-1}$ fw, on average). Even if no limits for nitrate content are set for carrots by European Union (EU) Regulations, the problem of nitrate concentration is crucial even for this vegetable, considering that EU Regulation 1258/2011 imposes a nitrate concentration limit for baby food (200 mg $kg^{-1}$ fw) and carrots constitute one of the most frequently used raw materials for these kinds of products. Thus, considering that the vegetable nitrate assumption is associated with the increased risk of gastrointestinal cancer [54], the low level of nitrate in the examined genotypes implies a high health quality profile for these roots.

#### 3.2.2. Simple Carbohydrates

The genotypes were distinctively differentiated in their amount of total carbohydrates and in their individual soluble sugars, as reported in Table 3.

**Table 3.** Concentration of simple carbohydrates (g 100 $g^{-1}$ fw) of carrot roots.

| Genotype [1] | Sweetness Index | Simple Carbohydrates | | | |
|---|---|---|---|---|---|
| | | Total | Sucrose | Glucose | Fructose |
| CPL | 3.4±0.5 b [3] | 2.5 ± 0.4 b | 2.2 ± 0.4 b | 0.19 ± 0.020 a | 0.14 ± 0.01 b |
| CPT | 5.4 ± 0.5 a | 3.8 ± 0.3 a | 3.3 ± 0.3 a | 0.13 ± 0.020 b | 0.34 ± 0.05 a |
| CG | 0.8 ± 0.2 c | 0.6 ± 0.1 c | 0.5 ± 0.1 c | 0.02 ± 0.004 c | 0.05 ± 0.02 c |
| Significance [2] | *** | *** | *** | *** | *** |

[1] CPL, "Carota punta lunga"; CPT, "Carota punta tonda"; CG, commercial genotype. [2] *** significant at $p \leq 0.001$. [3] Means in columns (n = 9) (±standard error) not sharing the same letters are significantly different according to LSD test ($p = 0.05$).

CG was the poorest in total and in any of the single simple carbohydrates, while CPT stands out for the highest total level of sugars (in particular, sucrose and fructose), consistent with DM accumulation (Table 1). In terms of the sweetness index (SI), CPT was confirmed the highest in sweetness, while the CG, despite having a high DM (Table 1), had the lowest sugar concentration and SI, thus resulting in a lower sweetness. In the *arenili* cultivation area, the genotype appears as the main determinant of sugar variability, as also reported by Simon et al. [55].

In our experience, the total simple sugars in the studied genotypes were, on average, lower than those reported in the INRAN-CREA [39] and USDA [41] databases and for several other commercial genotypes grown in Sweden [56], Slovenia [57], Denmark [58], Poland [59] and Ukraine [60] (Table S1). The value of free sugars in our genotypes was also lower than that reported for landraces grown in Tunisia [61] and for landraces grown in the Puglia region, such as YP-PC [7] and another multi-coloured local landrace named "Tiggiano Carrots" (TC) [9] (grown close to the Adriatic Sea coast in the villages of Tiggiano −39°54′10″ N 18°21′54″ E, Tricase −39°55′48″ N 18°21′15″ E, and Specchia −39°56′20″ N 18°17′52″ E, in the south of Lecce province) (Table S1). Considering these pieces of evidence, it is clear that, beyond the genotype, some other factors significantly determine the sugar content [55,59,62,63]. It is quite likely that the particular pedological properties

of *arenili* could cause a stressed growing condition: the frequent flooding experienced by the plants in this site could have determined a large use of the carbohydrate reserves (sugars) of the plant as carbon skeleton donors in the biosynthesis of secondary metabolites (phenols, carotenoids), useful to counteract the oxidative stress damage. Thus, the lowest sugar concentration could be an index of the high-stress conditions, particularly affecting the CG.

It has been observed in mung bean [64,65] and cucumber plants [66] that a plant's ability to reduce sugars for proper metabolic function (glycolytic activity) helps plants to tolerate waterlogged conditions.

In our work, no sugars were detected besides the two monosaccharides (hexose, fructose and glucose) and a disaccharide (sucrose), but several experimental works show a wider composition including xylose (pentose) [67], maltose (disaccharide) [68] (performing the same HPLC assay as in our experiment), sedoheptulose (heptose) or other minor hexoses such as scylloinositol, myoinositol and mannitol in several genotypes grown in Spain (thanks to a different analysis implemented) [69]. In our genotypes, despite the more limited composition, sucrose was the main carbohydrate (85% of total sugars), which is in line with the literature evidence [62]. It is considered the major transport and storage carbohydrate in the underground organs of plants such as carrots [70]. In rare cases, glucose has been reported as the main sugar in carrot, as in an open-pollinated carrot of the Chantenay type "Kämpe" [56,71] and as in local landrace TC [9].

The incidence of reducing monosaccharides in other local landraces (fructose, 28% and 45% in YP-PC; glucose, 27% and 55% in TC) [7,9] was higher than that found in the genotypes grown in *arenili* (fructose, 12%; glucose, 3%). Considering that in the last part of the cycle the concentration of the two hexose monosaccharides (fructose and glucose) decreases in favour of an increase in disaccharide sucrose [62], it can be speculated that in our case, the high incidence of sucrose at harvest could be considered as an index of reaching the maturity stage faster.

#### 3.2.3. Organic Acids

We investigated the concentration and the composition of the organic acids in carrot, as reported in Table 4.

**Table 4.** Concentration of organic acids (mg 100 $g^{-1}$ fw) of carrot roots.

| Genotype [1] | Organic Acids | | | | |
|---|---|---|---|---|---|
| | **Total** | **Quinic Acid** | **Malic Acid** | **Ascorbic Acid** | **Oxalic Acid** |
| CPL | 333.5 ± 18 a | 64.7 ± 5.0 a | 266.2 ± 17.2 a | 2.6 ± 0.1 b | 0.0 ± 0.0 b |
| CPT | 142.9 ± 5.7 b | 16.9 ± 0.5 b | 109.5 ± 5.9 b | 11.1 ± 0.4 a | 5.4 ± 0.6 a |
| CG | 48.5 ± 10.6 c | 13.5 ± 3.0 b | 26.0 ± 5.0 c | 3.8 ± 0.8 b | 5.2 ± 1.1 a |
| Significance [2] | *** | *** | *** | *** | *** |

[1] CPL, "Carota punta lunga"; CPT, "Carota punta tonda"; CG, commercial genotype. [2] *** significant at $p \leq 0.001$. Means in columns (n = 9) (±standard error) not sharing the same letters are significantly different according to LSD test ($p = 0.05$).

CG showed the lowest total organic acid concentration and CPL (333.5 mg 100 $g^{-1}$ fw; 31.7 g $kg^{-1}$ dw) the highest, consistent with the highest amount of malic and quinic acids. The genetic factors along with the growing practices appear to be by far the dominant sources of variation in the amount and composition of organic acids.

The total organic acid content in our genotypes was slightly lower than that found in the local landrace TC [9], but also in several commercial varieties grown elsewhere (Slovenia, Denmark, Latvia) [57,58,72] (Table S1). In the light of these data, the pedological conditions of *arenili* could be considered so stressing as to induce a large consumption of organic acids; in the same manner, like sugars, these compounds could probably be destined to biosynthetic pathways of secondary metabolites, useful to disable the oxidative damage.

The organic acid composition of the examined genotypes showed little variation compared with other literature data. A wider composition of organic acids, including

eight components, was found in carrot samples from the Latvia market, also reporting the presence of citric (42%), tartaric (33%) fumaric (4%), succinic (0.6%) and benzoic (0.07%) acids, beside malic (6.6%), ascorbic (1.0%) and oxalic (12%) acids, detected by implementing the same assay (HPLC) we used [72]. In our study, although a more limited composition of organic acids was recorded (only four), malic acid was the predominant acid (79%), in agreement with the literature which reports malate along with citrate as the most abundant acids in carrot [73]. The prevalence of malate has also been detected in the TC landrace (90%) [9], in the commercial variety "Bolero F1" grown in Denmark (93%) [58], and in commercial varieties grown in Slovenia (46%) [57].

In our work, malate was followed by quinate (17%), ascorbate (7%) and oxalate (1%).

Quinic acid, which is scarcely present in carrots [72,74,75], was detected in appreciable amounts in genotypes grown in the *arenili* of the SMS area since the plants often experienced flooding due to the native pedological origin of *areneli*. In Vandoorne et al. [76], an increase in quinic acid has been reported in the roots of chicory after long-term intermittent flooding stress. We suppose that the synthesis of this compound was elicited, especially in the CPL landrace, since protracted flooding due to downpours occurred during the last 15 days before harvesting in fields where CPL carrots were grown (personal communication from local growers). As highlighted by Zhang et al. [77], the beneficial properties of quinic acid isolated from Cat's Claw (*Uncaria tomentosa* DC.) are noteworthy. It is considered a natural in vivo antioxidant, and a safe and reasonable anti-ageing candidate with great potential.

Oxalic acid is considered anti-nutritional due to its effect in reducing the dietary Ca availability, and additionally, it induces the formation of kidney stones [78]. Not to neglect its toxicity in humans, the minimum lethal dose of oxalic acid for an adult is set at 5 g [79].

In the examined genotypes, this organic acid (CPT vs. CPL and CG, 0.0 vs. 5.3 mg 100 $g^{-1}$ fw) was found, on average, in low concentrations (0.38 g $kg^{-1}$ dw) in comparison with other carrot genotypes, as grown in Latvia [72] or in market samples from Pakistan [80], which showed appreciable amounts of oxalic acid (Table S1). Based on the minimum lethal dose for an adult, only a huge consumption of roots from the genotypes of the SMS area could cause severe health effects.

As well as oxalic acid concentration, Ca content must be considered to make safe conclusions about the toxicity limits of this acid in vegetables. Given the recommended safe ratio of "oxalic acid/Ca", established as lower than 2.5 [81], or of "Ca/oxalic acid", established as higher than 0.3 [82], the daily consumption of the examined genotypes, in particular, CPL, should not impair dietary calcium absorption ("oxalic acid/Ca" ratio, 0.00 vs. 0.06, CPL vs. CPT and CG, $p \leq 0.01$).

Ascorbic acid, the main biologically active form of vitamin C, was distinctively higher in the CPT landrace (11.1 mg 100 $g^{-1}$ fw; 0.67g $kg^{-1}$ dw) than in the CPL and CG (3.2 mg 100 $g^{-1}$ fw; 0.29 g $kg^{-1}$ dw, on average). The average values of all the collected genotypes were higher than those reported in the literature for other commercial varieties and than those reported in the Italian and international standard food composition databases (Table S1). The above-mentioned stressing conditions also seem to act in eliciting the production of this organic acid, with a large variability that can be attributed to the genotype effect. In this regard, the CPT landrace must be emphasized as being very interesting.

#### 3.2.4. Phenols

Phenols, along with carotenoids, are important bioactive compounds in carrots [13]. The concentration of total and individual phenolic compounds in our genotypes is reported in Table 5.

**Table 5.** Phenolic compounds (mg $kg^{-1}$ fw) in the roots of carrot genotypes.

| Phenols | RT (min) | UV Max (nm) | [M-H]$^-$ | *m*/*z* Ions | Genotype | | | Significance [1] |
|---|---|---|---|---|---|---|---|---|
| | | | | | "Carota a Punta Lunga" | "Carota a Punta Tonda" | Commercial Genotype | |
| chlorogenic acid | 3.54 | 324 | 353 | 191-179 | 15.2 ± 3.1 b | 75.6 ± 16.7 a | 13.3 ± 1.0 b | * |
| caffeic acid | 5.26 | 321 | 387 | 341 | 0.10 ± 0.06 a | 0.00 ± 0.00 a | 0.00 ± 0.00 a | ns |
| 5-p-coumaroyl-quinic acid | 7.01 | 312 | 337 | 191-163 | 4.70 ± 2.45 b | 9.38 ± 1.99 a | 1.00 ± 0.12 b | * |
| 5-feruloyl-quinic acid | 8.19 | 325 | 367 | 191-193 | 3.25 ± 0.50 b | 11.8 ± 5.0 a | 8.67 ± 0.89 b | * |
| caffeic acid derivate1 | 8.64 | 326 | 365 | 202-185-179 | 11.9 ± 2.4 b | 33.4 ± 10.6 a | 5.40 ± 0.39 b | * |
| caffeic acid hexoxide | 9.75 | 327 | 341 | 179-135 | 0.30 ± 0.04 a | 0.47 ± 0.13 a | 0.35 ± 0.05 a | ns |
| ferulic acid derivative | 10.95 | 327 | 379 | 185-141 | 3.70 ± 0.45 b | 9.77 ± 4.66 a | 3.82 ± 0.44 b | * |
| di-caffeoyl-quinic acid | 11.9 | 324 | 515 | 353-354-191 | 0.52 ± 0.23 a | 0.60 ± 0.09 a | 0.62 ± 0.21 a | ns |
| di-caffeic acid derivative | 12.26 | 327 | 527 | 365 | 42.9 ± 10.8 b | 60.7 ± 21.6 a | 31.6 ± 3.6 b | * |
| caffeic acid derivative2 | 12.7 | 328 | 515 | 353-185 | 2.87 ± 0.75 b | 1.53 ± 0.57 b | 8.90 ± 0.59 a | ** |
| caffeic/ferulic acid derivative | 14.4 | 326 | 541 | 379 | 4.77 ± 0.81 b | 6.50 ± 2.16 b | 9.22 ± 0.96 a | * |
| Total phenols | | | | | 90.3 ± 11.6 b | 209.8 ± 54.5 a | 82.9 ± 4.1 b | * |

[1] Significance: ns, * and ** not significant or significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. Means (n = 9) (±standard error) in rows not sharing the same letters are significantly different according to LSD test ($p = 0.05$).

The phenolic profile of the examined carrots was only represented by the phenolic acid component, in particular, by 11 hydroxycinnamic acids. The di-caffeic acid derivative followed by chlorogenic acid were the most abundant. The genotype distinctively affected the concentration and phenolic profile, except for 3 out of the 11 compounds, such as caffeic acid, caffeic acid hexoxide, and di-caffeoyl-quinic acid, all detected at a very low quantity (<0.6 unit).

The total level of phenolic compounds in the CPT landrace was about 2.5 times higher than in the CG and CPL due to the contribution of almost all individual compounds. CG highlighted a higher amount of only two compounds: caffeic acid derivative 2 and caffeic/ferulic acid derivative.

Although the low input of nutrients in which the landraces are grown could represent an abiotic stress factor pushing on the up-regulation of the biosynthesis of secondary metabolites as phenols [13,83], the differences in the accumulation of phenols seem mainly imputable to the genotype.

The phenolic amount in all genotypes from *arenili* was substantially greater than that reported for commercial genotypes grown elsewhere and also in the Apulian landrace TC (Table S1). Thus, this evidence confirms that environmental factors are great determinants of phenolic variability [13,84] as well as the stressed growing conditions related to the *arenili* origin (such as frequent flooding). The flooding stress experienced by plants might also be related to the high accumulation of phenols. A significative shift towards the synthesis of these secondary metabolites in plants could be useful to counteract oxidative stress, particularly in CPT. Similarly, in azuki beans under waterlogging conditions, a high amount of flavonoids has been reported, along with the enzymatic antioxidant mechanism (an increase in the activity of POD, PPO, GSH, SOD, CAT enzymes) [85].

In the current study, the qualitative profile of phenolic compounds is in agreement with that observed in several studies on carrot [57,86–89], where "chlorogenic acids", esters of quinic acid with caffeic, ferulic, or coumaric acids, are prevalent.

CPT landraces highlighted an appreciable amount of chlorogenic acid (36%), followed by the di-caffeic acid derivative (29%). On the contrary, for CPL and CG, the di-caffeic acid derivative (48%, CPL; 38%, CG) was the most abundant, followed by chlorogenic acid (17%, CPL; 16%, GC). The amount of chlorogenic acid in genotypes from *arenili*, in particular in CPT (76 mg $kg^{-1}$ fw; 0.458 g $kg^{-1}$ dw), was substantially higher than the values reported in the literature (Table S1).

Antioxidant capacity and other properties, such as anti-inflammatory, cardio-protective, anti-microbial, and neuroprotective effects of phenols (chlorogenic acids and caffeic acids), in vegetables such as carrot [90] are well known. Thus, the properties of the observed genotypes, in particular of the CPT landrace, in the preservation of human health could be emphasized due to their richness in these compounds.

#### 3.2.5. ß-Carotene

Carotenoids are a class of pigments that play essential functions in plants against abiotic or biotic stresses and have an important role in human health [91]. Numerous carotenoids have been isolated from natural sources, and about 20 can be detected in human plasma and tissues [92]. Among the latter, the most abundant are β-carotene, α-carotene, lycopene (carotenes) and lutein, β-cryptoxanthin, and zeaxanthin (xanthophylls). β-carotene mainly has pro-vitamin A activity [93], which is essential for normal organogenesis, immune functions, tissue differentiation, and eyesight [94]. Moreover, β-carotene has been found to have a positive effect on thyroid function [95] and an antioxidant and anti-inflammatory effect in cardiovascular care [96]. β-carotene was the most abundant of the carotenoids (carotenes) in carrot roots; therefore, it was analysed in the current work (Table 6).

**Table 6.** Concentration of ß-carotene and antioxidant capacity of carrot roots.

| Genotype [1] | ß-Carotene | Antioxidant Capacity (AC) | | |
|---|---|---|---|---|
| | | Hydrophilic (H-AC) | Lipophilic (L-AC) | Total (T-AC) |
| | (μg 100 $g^{-1}$ fw) | (μmol T.E. $kg^{-1}$ fw) [3] | | |
| CPL | 18,202 ± 2038 b | 577 ± 35 c | 288 ± 17 b | 865 ± 52 c |
| CPT | 21,512 ± 706 a | 1458 ± 114 a | 200 ± 31 c | 1659 ± 121 a |
| CG | 16,746 ± 1029 b | 806 ± 53 b | 403 ± 26 a | 1209 ± 80 b |
| Significance [2] | ** | *** | *** | *** |

[1] CPL, "Carota punta lunga"; CPT, "Carota punta tonda"; CG, commercial genotype. [2] ** and ***, significant at $p \leq 0.01$ and $p \leq 0.001$, respectively. Means in columns (n = 9) (±standard error) not sharing the same letters are significantly different according to LSD test ($p = 0.05$). [3] T.E., Trolox Equivalent.

The CPT landrace stands out for the highest concentration of ß-carotene, while the CG and the CPL landrace accumulated a lower and a similar amount. In agreement with other findings [13,63], even in the specific area of cultivation of SMS, the genotype can be assessed as the main factor affecting ß-carotene content in carrot.

In all the studied genotypes, the value of ß-carotene was much higher than that reported by the Italian and international food composition databases or that reported in several landraces or commercial varieties grown worldwide (Table S1). Over the genotype, the carotenoids and ß-carotene contents are also reported to be greatly elicited by environmental conditions [97]. In the same manner, like phenols, the pedological properties of *arenili* (such as frequent flooding) could highly elicit the concentration of this secondary metabolite, particularly in CPT. These findings are in line with the increase in carotenoid concentration that has been reported in cucumber plants subjected to waterlogging [66].

Thus, all these comparisons with literature data underline the high nutritional value of the collected genotypes, particularly the CPT. It should be noted that based on the provitamin A activity of β-carotene (12 μg corresponds to 1 μg of Retinol Activity Equivalent—RAE) [98], the intake of vitamin A per serving size (120 g) and the percentage of the Recommended Dietary Allowance (RDA) (900, 700, and 350 μg RAE $day^{-1}$ for men, women, and children) [99] have been calculated. The results (2100 μg RAE per serving size of CPT) highlight that one serving portion of CPT roots fulfils 233, 300, and 600% of RDA for men, women and children, respectively.

#### 3.2.6. Antioxidant Activity

The antioxidant capacity (AC) as total (T-AC), characterized by the hydrophilic (H-AC) and the lipophilic (L-AC) components, is reported in Table 6.

The H-AC (associated with phenols, vitamin C and others) and L-AC (associated with carotenoids, tocopherols, lipophilic phenols and other lipophilic compounds) accounted for 76% and 24% of T-AC, respectively. The CPT landrace showed the highest, while the CPL the lowest T-AC, consistent with H-AC. L-AC seems not to be related to the ß-carotene content, showing an inverse relationship both in CPT and CG.

In the latter, probably some other lipophilic compounds (such as terpenes) present in carrot roots [100] and not examined in the present work contribute to increasing this component of AC.

The highest T-AC in CPT could be related to its highest vitamin C (Table 4) and phenolic (Table 5) concentrations. The high correlation between the total phenol concentration and the T-AC, which has also been reported by several research works [86,87], underlines that the phenolic compounds have a strong influence on the overall scavenging activity in carrot.

## 4. Conclusions

This study highlights a morphological, nutritional and nutraceutical profile of carrot landraces ("Carota a punta tonda" and "Carota a punta lunga") still cultivated in *arenili* in the "Salterns of Margherita di Savoia" (SMS) area of the Puglia region (Italy) alongside a common commercial hybrid (CG) ("Presto").

The particular pedological conditions of the *arenili* in the SMS area emerged as the main driving force in determining the quantitative-qualitative profile of carrots, mainly in terms of nutritional (low "Na/K" ratio, nitrate and oxalic acid, but low sugars) and nutraceutical (high phenols and ß-carotene) properties when compared with carrots grown elsewhere.

In the *arenili* conditions, a large variability also emerged among genotypes as the "Carota a punta tonda" landrace exhibits high levels of minerals, phenols and ß-carotene, along with high antioxidative proprieties, resulting in a promising genotype as a potential candidate as a functional food and/or selection material for breeding purposes.

The knowledge of the health properties of these resources at high risk of erosion moves towards the diversification of the current food market and the valorisation of the local agro-biodiversity. The promotion of their consumption and their cultivation could contribute to protecting the agro-biodiversity by in situ/on-farm conservation.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/su13073940/s1, Figure S1: Main typologies of carrot in the world (source for graphic—www.vilmorin.com, accessed on 4 May 2020). Table S1: Averaged values of traits of genotypes—two landraces (LRs) and the hybrid F1 "Presto"—grown in *arenili* in "Salterns of Margherita di Savoia" (SMS) in Puglia region (Italy) in comparison to those reported in standard food composition databases and in the scientific literature.

**Author Contributions:** Conceptualization and methodology, A.B., G.C.; Data curation, A.B., G.G.; Formal analysis, C.L.; Funding acquisition, A.E., G.C.; Supervision, A.E., G.C.; Validation, A.E., G.C.; Visualization, A.E.; Writing—original draft, A.B.; Writing—review and editing, A.B., G.C., A.E. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Regione Puglia Administration under the "Rural Development Programme" (RDP) 2014–2020—Project "Biodiversity of vegetable crops in Puglia (BiodiverSO)". Measure 10, Sub measure 10.2, Operation 1 "Programme for the conservation and the valorisation of the genetic resources in agriculture".

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

**Acknowledgments:** The authors are grateful to all the growers who kindly donated their roots and to Paolo La Rotonda for valuable help in the laboratory analysis.

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

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