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# Shelf-Life of Bunched Carrots as Affected by Nitrogen Fertilization and Leaf Presence

**Anita Ierna [1], Rosario Paolo Mauro [2,*], Cherubino Leonardi [2] and Francesco Giuffrida [2]**

[1] Institute of BioEconomy, National Research Council (CNR-IBE), Via P. Gaifami 18, 95126 Catania, Italy; anita.ierna@cnr.it

[2] Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy; cherubino.leonardi@unict.it (C.L.); francesco.giuffrida@unict.it (F.G.)

* Correspondence: rosario.mauro@unict.it; Tel.: +39-095-4783314

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**Abstract:** Nitrogen (N) fertilization is essential for adequate earliness and the commercial attractiveness of carrots, but its excess could generate fast decay during postharvest, mostly in bunched carrots exhibiting their highly perishable leaves. A field experiment was conducted over the 2016–2017 growing season to address the effects of two N fertilization rates (120 and 240 kg N $ha^{-1}$, hereafter $N_{120}$ and $N_{240}$, respectively) and leaf presence/absence (leaf+ and leaf−) on physicochemical and compositional traits of carrots cv. 'Dordogne', after storage at 4.0 ± 0.5 °C, 95–96% relative humidity (RH) for 0, 3, 6, 12, and 24 days (hereafter $S_0$–$S_{24}$). Before storage, carrots were arranged in bunches and packaged in common low-density polyethylene (LDPE) pouches (thickness 95 µm), 54 × 24 cm size, with 16 holes of 5 mm size. $N_{240}$ carrots compared to $N_{120}$ showed higher cumulative weight loss (CWL) and firmness reduction, with differences at $S_{24}$ equal to 108 vs. 41 g $kg^{-1}$ fresh weight (FW) and 13.3 vs. 14.5 N, respectively. $N_{240}$ compared to $N_{120}$ increased also the color deviation ($\Delta E^*_{ab}$, +126%) and nitrates content (+93%) of carrots and slowed down their temporal increase of total polyphenols and antioxidant activity. Leaf+ carrots compared to leaf boosted CWL and firmness reduction, with differences at $S_{24}$ equal to 90 vs. 58 g $kg^{-1}$ FW and 12 vs. 17 N, respectively. In addition, leaf presence increased reducing sugars (+17%) and decreased nitrates (−24%) contents. This research has shown the possibility of improving the desirable quality and shelf-life of carrots by halving the N dose commonly supplied by growers and marketing bunched carrots within 12 days from the start of storage.

**Keywords:** *Daucus carota* L.; nitrogen rate; leaf presence; storage; shelf life

## 1. Introduction

Carrot (*Daucus carota* L.) is a biennial herbaceous crop belonging to the Apiaceae family, playing a role of primary importance among the root vegetables cultivated worldwide [1]. From a nutraceutical viewpoint, the species represents a valuable source of bioactive constituents, such as carotenoids, flavonoids, and hydroxycinnamic acid derivates, so being able to exert multiple beneficial effects on human health [2]. In Italy, the global production of carrot accounts for almost 547 ktons of roots obtained from about 14 kha of land [3], with a product intended both to fresh consumption or industrial processing. Under Mediterranean growth conditions, similarly to other horticultural crops [4], its cultivation usually takes place in sandy soils, which are characterized by low organic matter and, consequently, by low N reserves and mineralization potential. Consequently, N fertilization is perceived as essential to reach adequate earliness and yield levels, due to the pivotal role of N in ensuring an adequate crop growth rate, so allowing growers to adjust the harvest dates to meet factory and marketing schedules [5]. This leads often to unjustifiable N overdressing in the attempt to enhance as much as possible the

product's earliness and commercial attractiveness. Excessive N applications generate environmental problems such as groundwater contamination, greenhouse gases emission, and the eutrophication of aquatic ecosystems, thus playing a significant role in ecosystems deterioration [6]. When food safety is concerned, the impact of N overdressing in favoring the accumulation of harmful nitrates for human health, as well as in increasing the incidence of storage disorders and potential for faster postharvest decay, has been demonstrated in a multitude of vegetables [7]. When consumed as an unprocessed vegetable, carrot is present on the market all year round in form of fresh or cold-stored product, a condition deriving from both the wide environmental adaptability of the crop and the good storability of the roots [8]. Indeed, when deprived from leaves (topped carrots), they represent a suitable product for long-term storage under appropriated conditions (≈0 °C at 95–98% RH), being able to be kept up to 8 months before packaging and retail [9]. In Southern areas of Italy, characterized by mild winters (Mediterranean climate), carrot is grown during the autumn–spring period (plantings from September to November and harvesting from February to May), generating an off-season product, mainly destined to fresh consumption. These carrots are usually more appreciated by consumers than cold-stored ones, thanks to their higher freshness and superior organoleptic properties, and in consequence of this are marketed also in several EU countries, when long cold-stored stocks show both quantitative and qualitative issues. The product is often offered in bunches, with plants still exhibiting their leaves (bunched carrots), in order to emphasize their commercial attractiveness and freshness. As a result of their high metabolic activity, leaves are notoriously highly perishable organs during postharvest life [10]. Indeed, the leaves of carrot bunches restricts the options for commercial refrigerated stores, thus potentially accelerating the postharvest decay and product spoilage. Unfortunately, up to now, there is no information about the consequences of leaves presence on the postharvest physiology of this popular vegetable, just as there is no knowledge on the effects that N fertilization could have on the postharvest shelf-life in bunched carrots. While, on the one hand, N fertilization is an essential means to ensure a good development and a longer duration of carrot leaves in the field, on the other, N concentration in leaf tissues is tightly linked to their level of metabolic activity [11], so potentially altering the physiological balance among interrelated organs during postharvest life. Considering the lack of specific literature regarding carrot, the aim of the present research was to evaluate the effects N fertilization rate and leaf presence on postharvest shelf-life of fresh, off-season carrot roots.

## 2. Materials and Methods

### *2.1. Experimental Field, Plant Material, and Crop Management*

A field trial was carried out during the 2016–2017 growing season at a commercial farm located at Ispica plain, (Southeastern Sicily: 36°47′ N 14°54′ E, 42 m a.s.l.), which is one of the most typical areas for early carrot cultivation in Southern Italy. The climate is semi-arid Mediterranean, with mild winters, often rainless springs, and hot and dry summers. Frost occurrence is virtually unknown (only two events in 30 years), and this permits growing carrot in a winter–spring cycle (from November–December to May). The mean 30-year 1977–2006 monthly maximum and minimum temperatures ranges between 15.3 and 7.6 °C in February and 32.9 and 23.0 °C in August, respectively. Yearly rainfall is 480 mm, more than 50% of which concentrated from November to January [12]. During the experiment, meteorological data were obtained daily from SIAS (Servizio Informativo Agrometeorologico Siciliano). The soil is a moderately deep, calcic brown on the basis of the USDA Soil Taxonomy Classification [13], with sandy loam texture, which, at the beginning of the experiment, comprised low N content (0.8 g $kg^{-1}$) and low organic matter (12.2 g $kg^{-1}$), $P_2O_5$ available (57 mg $kg^{-1}$), $K_2O$ exchangeable (302 mg $kg^{-1}$), pH 7.7. Soil analyses were carried out according to the procedures approved by the Italian Society of Soil Science [14]. The experiment was arranged in a randomized complete block design with four replications, including two nitrogen rates (120 and 240 kg $ha^{-1}$ of N, hereafter referred as $N_{120}$ and $N_{240}$, respectively). $N_{120}$ was formulated on the basis of the N uptake by carrot crop with marketable yields of 40 t $ha^{-1}$ [15] and the available soil mineral N for the crop cycle at planting

equal to about 60 kg $ha^{-1}$ determined according to the methodology reported in a previous paper [16]. $N_{240}$ represents the conventional N fertilization rate commonly adopted by Mediterranean producers for enhancing yields. The cultivar 'Dordogne' was utilized, a hybrid of the Nantes-type, which is well-adapted to Mediterranean growing conditions, and it is usually adopted for the production of "early", bunched carrots. 'Dordogne' is characterized by a homogeneous and intense orange color and smooth roots, bright green leaves with an elegant and tidy posture, and excellent root/leaf balance. On November 5, 2016, seeds were sown at a ≈1 cm depth, through a precision seeder operating in twin rows (0.20 × 0.30 m) on an 0.80 m wide ridges; soon after seeding, the ridges were rolled uniformly. Actual density was 170 plants $m^{-2}$. Plot size was 3.6 m × 3.6 m, and consisted of 3 ridges, 3.6 m long. Tillage consisted in a preparatory work deep ploughing (~40 cm) and ridges setting with a bed-maker for the formation of raised ridges, ≈2 weeks before sowing. One week before sowing, 70 kg $ha^{-1}$ of $P_2O_5$ (as mineral superphosphate) and 300 kg $ha^{-1}$ of $K_2O$ (as K sulfate) were applied. At sowing was also supplied 25% of N (as ammonium nitrate), 25% at the stage of 5–6 leaves (71 days after sowing, DAS), 25% at the beginning of roots enlargement (114 DAS) and the remaining 25% at the stage of advanced root enlargement (156 DAS). Crop water requirements were completely satisfied by drip irrigation, supplying 100% of crop maximum evapotranspiration, when the accumulated daily evaporation, estimated through the Penman–Monteith equation, reached 30 mm. Over the crop cycle, 170 mm of irrigation water were applied. Weeds and pests' control were performed by applying metribuzin and pirimicarb when needed.

### *2.2. Plant Harvest, Post-Harvest Handling and Sampling*

Plants harvest was carried out by hand on 17 May (193 DAS), on 13 $m^2$ wide plots (3.6 m × 3.6 m) per each fertilization level and replicate, taking care to avoid any damage to leaves. Within each experimental unit, harvested carrots were selected for uniform size and absence of defects, then arranged by hand in 20 bunches each containing 10 roots. Within 3 h from the harvest, all bunched carrots were brought to the laboratory, where they were washed through demineralized water to remove soil particles, and then, they were dried with paper towels. The storage trial was realized on 30 bunches per each N level, 15 of which were topped (by removing leaves with a knife at the collar level, hereafter leaf−) and leaving the remaining carrots with the leaves (leaf+). Then, all bunches (either topped or not) were packaged in low-density polyethylene (LDPE) pouches (thickness 95 µm) of 54 × 24 cm size, each with 16 holes of 5 mm sizes. This film was selected since it is commonly used by local commercial carrot packagers. All pouches were closed through a heat sealer MVS41X (Minipack-Torre, Dalmine, Italy), stored at 4.0 ± 0.5 °C, 95–96% RH, and sampled for analyses at the harvest date (unpackaged carrots, hereafter $S_0$) and at 3, 6, 12, and 24 (hereafter $S_3$, $S_6$, $S_{12}$, and $S_{24}$) days after storage. Overall, 60 bags were used, deriving from the factorial combination of 2 N fertilization rates, 2 carrot typologies (leaf+ vs. leaf−), and 5 sampling dates (0, 3, 6, 12, and 24 days after storage).

### *2.3. Determinations and Calculations*

#### 2.3.1. Physicochemical Traits

At each storage time, physicochemical traits were determined considering three packages as a single experimental unit. Carrot fresh weight was determined through a digital precision balance (±0.01 g) (Gibertini Europe, Novate Milanese, Italy); then, the cumulative weight loss (CWL) within the $S_3$–$S_{24}$ interval was calculated and expressed as g $kg^{-1}$ fresh weight (FW). Soon after, external chromatic coordinates were determined on five carrots within each package, according to McGuire [17], through a tristimulus Chroma Meter mod. CR-200 (Konica Minolta, Tokyo, Japan), on 2 points per root ≈1 cm below the plant collar. The obtained CIELab* coordinates, namely lightness (L*), green–red axis (a*), and blue–yellow axis (b*), were used to calculate the total color difference [$\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$], this last describing the color deviation recorded during postharvest storage ($S_3$–$S_{24}$ interval). Firmness was evaluated in the middle part of five roots per package, through a

Digital Texture Analyser mod. TA-XT2 (Stable Micro Systems, Godalming, UK) by using a cylindrical tip (Ø 2.0 mm), and defined as the force (N) needed to impress a 2 mm fruit deformation along the equatorial axis, at a speed of 10 mm $s^{-1}$. Dry matter (DM) content was determined through the gravimetric method on three taproots cut into slices (5 mm thick), after drying them at 105 °C in a thermo-ventilated oven (Binder, Tuttlingen, Germany) until constant weight was reached. In the same way, leaf DM was determined in leaf+ samples. Total solids content was also determined on three taproots sliced into slices of 5 mm, after drying at 65 °C in a thermo-ventilated oven (Binder, Tuttlingen, Germany) until constant weight to obtain dry material for determining the content of reducing sugars and nitrates.

#### 2.3.2. Compositional Traits

At each storage time, 5 roots per package were cut into discs with a thickness of 5 mm, frozen with liquid N, and placed at −80 °C until subsequent determination of the antioxidant activity and the total polyphenol content (TPC). Antioxidant activity was estimated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [18], measuring the absorbance at 517 nm (spectrophotometer Lamba 11, Perkin Elmer, San Jose, CA, USA), according to the method reported by Singh et al. [19]. Results were expressed as mg Trolox $kg^{-1}$ FW. TPC was quantified through the Folin–Ciocalteau assay [20] measuring the absorbance of the carrot extracts at 710 nm. Determinations were made on the basis of a standard calibration curve generated with known amounts of gallic acid and expressed as mg gallic acid equivalents (GAE) $kg^{-1}$ FW. Reducing sugars were quantified on the dry material and pressed through a 1 mm stainless steel sieve using the 3,5-dinitrosalicylic acid (DNS) method [21] and expressed as mg $kg^{-1}$ FW. Nitric N concentration in carrots was determined through ionic chromatography. To each dry and ground 0.5 g sample, 50 mL of ultrapure water was added, and the mixture was stirred for 30 min at 180 rpm. After filtering the extract and carrying out the appropriate dilution, reading was conducted with an ion-chromatograph (Dionex IC 25, 40 EG Eluent Generator, IonPacAS11-HC; Dionex Corp., Sunnyvale, CA, USA). Results were expressed as mg $kg^{-1}$ FW.

### *2.4. Statistical Procedure*

Collected and calculated data were firstly subjected to Shapiro–Wilk's and Levene's test, in order to check for normal distribution and homoscedasticity, respectively. Then, data were subjected to a three-way analysis of variance (ANOVA), based on a factorial combination 'nitrogen rate × leaf presence × storage time', according to the layout adopted during the experiment (Table 1). The DM content of leaves was subjected to a two-way ANOVA ('nitrogen rate × storage time') (data not shown).

### *2.5. Meteorological Conditions during the Experiment*

During the experiment, the meteorological data were in line with the long-term average, as total rainfall was 290 mm, mostly concentrated from November to March (overall 259 mm), with December being the rainiest month (71 mm). The mean maximum temperature gradually decreased from November (19.8 °C) to January (14.7 °C) and then increased up to 22.1 °C in May. Similarly, the mean minimum temperature passed from 14.1 °C in November to 8.8 °C in January and then reached the highest value (15.2 °C) during May.

Percentage data were Bliss' transformed before the ANOVA (untransformed data are reported and discussed), whereas multiple mean comparisons were performed through Fisher's protected least significant difference (LSD) test (at least for $p \leq 0.05$). All calculations were performed using Microsoft Excel® version 2016 and Minitab version 16.1.1 (Minitab Inc., State College, PA, USA).

**Table 1.** *F* values resulting from analysis of variance for all studied variables.

| | Df | CWL (g kg$^{-1}$ FW) | Root DM Content (%) | Firmness (N) | $\Delta E^*_{ab}$ | Nitrate Content (mg kg$^{-1}$ FW) | RSc Content (g kg$^{-1}$ FW) | TPC (mg GAE kg$^{-1}$ FW) | TEAC (mg Trolox kg$^{-1}$ FW) |
|---|---|---|---|---|---|---|---|---|---|
| Nitrogen rate (N) | 1 | 162.2 *** | 8.8 *** | 5.0 * | 323.8 *** | 103.3 *** | NS | 51.4 *** | 5.3 * |
| Leaf presence (L) | 1 | 50.4 *** | NS | 62.2 *** | NS | 20.0 *** | 13.8 *** | NS | NS |
| Storage time (S) | 4 | 65.6 *** | NS | 66.9 *** | 52.7 *** | 3.5 * | 26.1 *** | 49.1 *** | 105.6 *** |
| N × L | 1 | 28.1 *** | NS | NS | NS | NS | NS | NS | NS |
| N × S | 4 | 25.8 *** | NS | 6.7 *** | NS | NS | NS | 3.1 * | 19.6 *** |
| L × S | 4 | 11.3 *** | 4.9 *** | 5.1 ** | NS | NS | 4.4 ** | NS | NS |
| N × L × S | 4 | NS | NS | NS | NS | NS | NS | NS | NS |

Df: degrees of freedom; CWL: cumulative weight loss; DM: dry matter; RSc: reducing sugars; TPC: total polyphenols content; TEAC: Trolox equivalent antioxidant capacity; NS: not significant; *, **, ***: significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

## 3. Results and Discussion

### *3.1. Physicochemical Traits*

The nitrogen rate significantly influenced almost all the studied variables (Table 1). It is known that the increase in N fertilization may affect both nutritional and health-related traits of fresh carrots, either in a positive [22] or negative way [23,24]. Regardless of leaf presence and storage time, at $N_{240}$, we found a greater CWL of roots than at $N_{120}$ (66.5 vs. 23.0 g $kg^{-1}$ FW, +89%) (Table 2).

**Table 2.** Physicochemical traits of carrots as affected by the main factors.

| | | CWL (g $kg^{-1}$ FW) | Leaf DM Content (%) | Root DM Content (%) | Firmness (N) | $\Delta E^*_{ab}$ |
|---|---|---|---|---|---|---|
| N fertilization | $N_{120}$ | 23.0 b | 15.6 b | 11.7 b | 19.3 b | 0.38 b |
| | $N_{240}$ | 66.5 a | 16.4 a | 12.2 a | 20.2 a | 0.86 a |
| Leaf presence | leaf− | 32.6 b | - | 11.8 a | 21.3 a | 0.63 a |
| | leaf+ | 56.9 a | - | 12.4 a | 18.2 b | 0.62 a |
| Storage time | $S_0$ | - | 14.9 d | 11.7 a | 21.7 b | - |
| | $S_3$ | 15.1 d | 15.1 cd | 11.9 a | 23.4 a | 0.38 d |
| | $S_6$ | 28.5 c | 15.9 bc | 11.8 a | 20.7 b | 0.55 c |
| | $S_{12}$ | 60.7 b | 16.1 b | 12.1 a | 19.0 c | 0.74 b |
| | $S_{24}$ | 74.7 a | 18.2 a | 12.7 a | 13.9 d | 0.81 a |

CWL: cumulative weight loss; DM: dry matter. Different letters among factor means indicate significance at Fisher's protected LSD test ($p \leq 0.05$).

Excessive soil N can cause increased weight loss during storage in some vegetables [25], which is a common feature, whose extent depends on pre-harvest practices (including N fertilization) and varietal traits, as well as on the length and conditions of storage [26,27]. The significance of the "nitrogen rate × leaf presence" (Table 1) interaction indicates that the presence of leaves accentuated the effects of the N fertilization. Indeed, passing from $N_{120}$ to $N_{240}$, there was a higher CWL increase in leaf+ (from 26.1 to 87.6 g $kg^{-1}$ FW, +236%) than in leaf− carrots (from 20.0 to 45.3 g $kg^{-1}$ FW, +126%) (Figure 1). It is likely to presume that leaves promoted the CWL of roots through the transpiration-driven water flow, functionally connecting roots and stomata. Moreover, during postharvest storage, the CWL in $N_{120}$ roots was quite constant up to $S_{12}$; then, it significantly increased between $S_{12}$ and $S_{24}$ (from 22.2 to 41.1 g $kg^{-1}$ FW, +85%), whereas the $N_{240}$ supply boosted the CWL, most of all between $S_6$ and $S_{12}$ (from 41.8 to 99.2 g $kg^{-1}$ FW, +137%), so that within the $S_3$–$S_{24}$ period, the CWL increase was equal to 552% and 200%, in $N_{240}$ and $N_{120}$, respectively (Figure 2A).

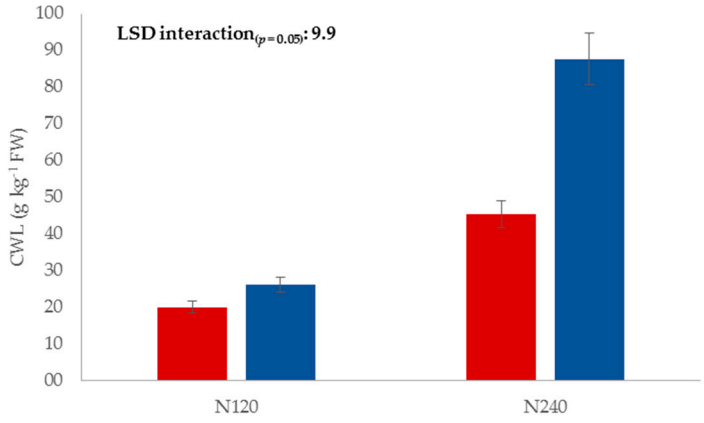


**Figure 1.** Cumulative weight loss (CWL) in carrots as affected by "nitrogen fertilization × leaf presence". Red histograms: leaf−; blue histograms: leaf+. Vertical bars indicate the standard error ($n$ = 12).

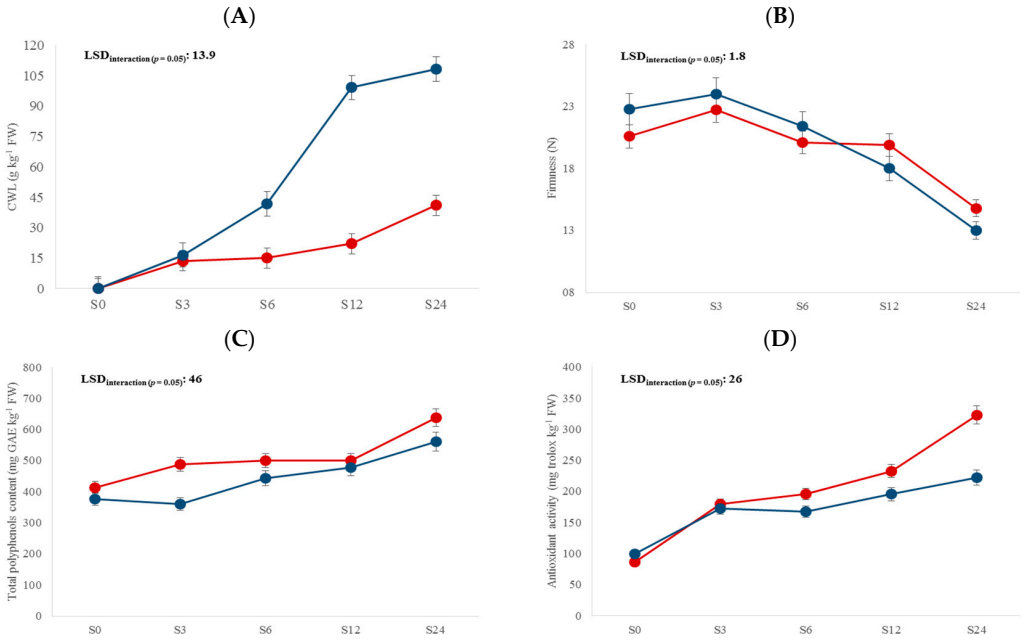


**Figure 2.** Cumulative weight loss (**A**), firmness (**B**), total polyphenols content (**C**), and antioxidant activity (**D**) in carrots as affected by "nitrogen fertilization × storage time" interaction. Each time point represents means ± standard error ($n$ = 6). Red line: $N_{120}$; blue line: $N_{240}$.

In both leaf+ and leaf− carrots, average CWL values equal to 15.1 g $kg^{-1}$ FW were found at $S_3$, which are reputedly within the norm of storage carrots. It was found that short-term storage (2 days) at very high air humidity did not excessively stress the bunched carrots [28]. After this storage time, leaf+ carrots displayed a more abrupt increase in weight loss, most of all between $S_6$ and $S_{12}$ (from 30.0 to 87.9 g $kg^{-1}$ FW, 193%), as compared to the leaf− ones (from 26.9 to 33.5 g $kg^{-1}$ FW, 24%), so that at the end of postharvest storage, it was recorded a 48% higher CWL in leaf+ than in leaf− carrots (89.2 vs. 60.1 g $kg^{-1}$ FW, respectively) (Figure 3A).

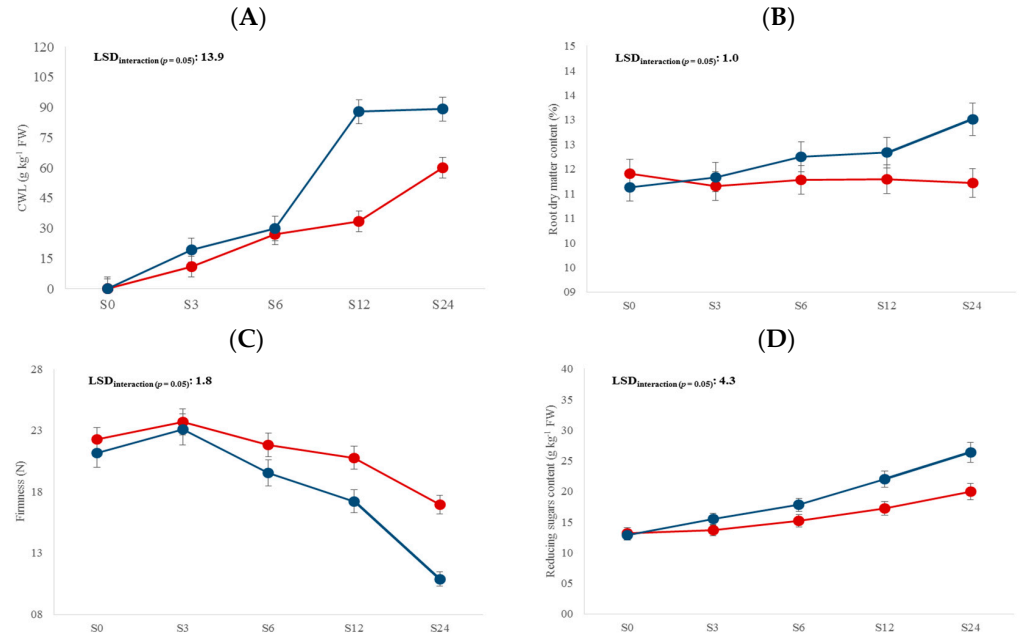


**Figure 3.** Cumulative weight loss (**A**), root dry matter content (**B**), firmness (**C**), and reducing sugars content (**D**) in carrots as affected by "leaf presence × storage time" interaction. Each time point represent means ± standard error ($n$ = 6). Red line: leaf−; blue line: leaf+.

The weight loss values of almost 9% we found at $S_{12}$ in leaf+ carrots is considered as a threshold for acceptable marketable quality [29], while leaf− carrots had, at the same storage time, a CWL of about 3%, which is in line with weight loss values usually found in carrots stored in bags for a short time [30]. The loss of water in leaf+ carrots from leaves was certainly favored by the use of macro-perforated film pouches, which by their nature provide very high mass exchange rates and usefully allow oxygen ingress and carbon dioxide exit. Similar weight loss values are reported for macro-perforated films in "cime di rapa" (*Brassica rapa* L. subsp. *sylvestris* L.) [31] and minimally processed carrots [32]. Water losses also led to a reduction in leaves hydration degree throughout the storage period. A similar DM content of leaves in $N_{120}$ and $N_{240}$ up to $S_{12}$, but at $S_{24}$, the $N_{240}$ carrots proved a greater increase of their leaf DM content (up to 19.8%) than the $N_{120}$ ones (16.5%) (Figure 4).

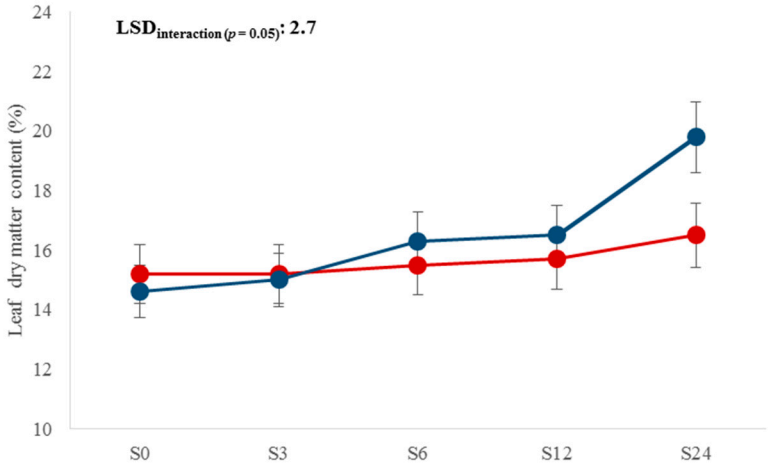


**Figure 4.** Leaf dry matter of carrots as affected by "nitrogen fertilization × storage time" interaction. Each time point represents means ± standard error ($n$ = 6). Red line: $N_{120}$; blue line: $N_{240}$.

This seems to suggest that the $N_{240}$ leaves had a higher transpiration rate, which is a feature that is consistent with the known effects of N in accelerating leaf metabolism and transpiration rate [33]. A higher average root DM content was recorded in $N_{240}$ (12.2%) than in $N_{120}$ carrots (11.7%) (Table 2), and, with the increasing storage time from $S_3$ to $S_{24}$, a significant root DM rise in leaf+ (from 11.8 to 13.2%) unlike the leaf− roots (in which DM content remained constant between the 11.6–11.7% range) (Figure 3B). Dry matter content values of about 12% are in accordance with the values for conventional carrots found in the literature [34]. The greater DM content in $N_{240}$ carrots, compared to the $N_{120}$ ones, was at the base of their greater firmness (20.2 vs. 19.3 N, respectively) (Table 2). However, their faster CWL resulted in their more marked loss of firmness, since the $N_{240}$ carrots showed the highest firmness reduction along the postharvest period, especially between $S_6$ and $S_{24}$ (from 21.4 to 13.3 N, −38%), compared to the $N_{120}$ roots (from 20.0 to 14.5 N, −27%) (Figure 2B). Moreover, our results showed that leaf+ carrots had higher firmness reduction within the $S_3$-$S_{24}$ period (from 23.1 to 10.9 N, −53%) than the leaf− ones (from 23.7 to 17.0 N, −22%) (Figure 3C), so confirming that CWL was essentially a transpiration-driven process, resulting in a lower hydration status of the leaf+ roots. However, it should be noted that in leaf+ carrots the firmness reduction at $S_{12}$ was quite low (−22%), showing how bunched carrots for at least half of the postharvest period can keep a good firm texture thanks to the storage conditions used (cold and high humidity). This does not happen realistically during retail sale when they are normally exposed to nearly free convection in ambient air of rather low humidity. However, 12 days is a long enough time that can also allow a long journey to export the product, as long as the storage conditions are maintained until the time of retail sale. Moreover, at $S_{12}$, no differences in the visual appearance of both taproots and leaves were found nor symptoms of infection resulting from bacteria and molds (data not reported). Certainly, it would have been important to test the satisfaction of the product, also verifying the taste, aroma, flavor, and microbial counts

that we intend to do in a subsequent research. Among the main factors, $\Delta E^*_{ab}$ of carrot proved to be responsive to N fertilization, as it was significantly higher in $N_{240}$ (0.86) than in $N_{120}$ fertilized roots (0.38) (Table 2), meaning that $N_{240}$ carrots showed a more vivid and shiny color. This seems in accordance with the finding that the biosynthesis of carotene is an N-demanding process [35]. $\Delta E^*_{ab}$ progressively increased during storage with a similar trend among the two N rates (Table 2). However, $\Delta E^*_{ab}$ values greater than 1, which is the threshold above which two colors can be optically distinguished [36], were not recorded throughout the entire storage period, which is consistent with high qualitative status in terms of roots fresh weight and turgor.

### 3.2. Compositional Traits

Regardless of the other factors, the highest N rate promoted the nitrate content of carrots, almost by doubling it (105.9 mg $kg^{-1}$ FW) in comparison to the $N_{120}$ roots (54.9 mg $kg^{-1}$ FW) (Table 3).

**Table 3.** Compositional traits of carrot roots as affected by the main factors.

| | | Nitrate Content (mg $kg^{-1}$ FW) | RSc Content (g $kg^{-1}$ FW) | TPC (mg GAE $kg^{-1}$ FW) | TEAC (mg Trolox $kg^{-1}$ FW) |
|---|---|---|---|---|---|
| N fertilization | $N_{120}$ | 54.9 b | 17.4 a | 512 a | 194 a |
| | $N_{240}$ | 105.9 a | 17.3 a | 440 b | 181 b |
| Leaf presence | leaf− | 91.6 a | 16.0 b | 485 a | 199 a |
| | leaf+ | 69.2 b | 18.7 a | 467 a | 176 a |
| Storage time | $S_0$ | 91.8 a | 13.0 e | 395 e | 93 d |
| | $S_3$ | 89.7 a | 14.5 d | 424 d | 175 c |
| | $S_6$ | 78.1 b | 16.5 c | 472 c | 181 c |
| | $S_{12}$ | 76.5 b | 19.6 b | 490 b | 214 b |
| | $S_{24}$ | 65.9 c | 23.2 a | 600 a | 272 a |

RSc: reducing sugars; TPC: total polyphenols content; TEAC: trolox equivalent antioxidant capacity. Different letters among factor means indicate significance at Fisher's protected LSD test ($p \leq 0.05$).

This finding is coherent with previous studies claiming excess N fertilization responsible for high N accumulation in carrots [37,38]. Nitrates are naturally occurring compounds in vegetables, since their presence in plants is connected with the N transformations to amino acids and proteins [39]. Usually, the concentration of nitrate is relatively low in carrots [40]; however, supra optimal N supply has been shown to cause undesirable high nitrate levels in carrots, whether supplied as mineral fertilizer [41] or by the mineralization of residues of previous crops [19,37,42]. In this research, the nitrate content does not represented a safety problem, because even the maximum nitrate concentration found (about 106 mg $kg^{-1}$ FW in the $N_{240}$ carrots) was far below the safety threshold value for children food (250 mg $NO_3$ $kg^{-1}$ FW) [42]. Interestingly, the leaf presence proved a significant reduction of nitrates content (from 91.6 to 69.2 mg $kg^{-1}$ FW, −24%) (Table 3). This is probably due to the fact that leaves induce a more dynamic metabolism by consuming nitrates at a faster rate than leaf− carrots, making bunched carrots healthier than topped taproots. Furthermore, during postharvest storage from $S_0$ to $S_{24}$, nitrates content significantly decreased (from 91.8 to 65.9 mg $kg^{-1}$ FW, −28%), which is in agreement with what was previously found [43].

Regarding reducing sugars content, no differences were found in relation to the N supply, and this outcome was consistent with other authors, that found small [44] or no influence [37] of the N supply on glucose, fructose, or sucrose concentration. Differently, the leaf presence acted to significantly increase the reducing sugars concentration (Table 3). Such differences in response to leaf presence proved to be time-dependent, as they were more marked beyond $S_6$, reaching the maximum values at $S_{24}$ (26.3 and 20.0 g $kg^{-1}$ FW, in leaf+ and leaf−, respectively) (Figure 3D). This outcome can likely be attributed to the leaf–driven stimulation of the enzyme sucrose synthase, which catalyzes the decomposition of sucrose (the reserve carbohydrate in carrots) to uridine diphosphate glucose in the storage tissue [45]. On the other hand, we cannot exclude that the highest reducing sugars content in leaf+ carrots was

also a result of their higher concentration effect, following the greater root water loss in response to leaf presence. In this sense, there was a clear analogy among the temporal trends of root DM and reducing sugars contents, overall indicating that transpiration largely prevailed on respiration in influencing this compositional trait of carrots. In other words, there was a negligible consumption of simple sugars through respiration activity, which is likely due to the relatively short-term storage and optimal storage conditions, particularly low temperatures. This result is consistent with most experiments recording only minor changes in the glucose content in root vegetables during cold storage [46].

On the average of the other factors, the $N_{240}$ promoted a 20% decrease in TPC of carrots as compared to $N_{120}$ (512 vs. 440 mg GAE $kg^{-1}$ FW) (Table 3). Such a result directly supports the carbon nutrient balance (CNB) hypothesis that predicts a higher level of carbon-based secondary metabolites, such as polyphenols, under limited N availability [47]. Similarly, some studies on the pot cultivation of carrot plants fertilized with Ca $(NO_3)_2$ showed a decrease in the content of phenolic compounds in roots when compared to control without N fertilization [48]. The total content of phenolic compounds in carrot is a cultivar characteristic [49], but it is known that it is greatly modified by the rate of N and the method of N fertilization [27], foliar nutrition [50], and N form [48,51]. When considered over the $S_0$–$S_{24}$ storage period, the $N_{240}$ carrots proved a steeper TPC rise over time (from 336 to 572 mg GAE $kg^{-1}$ FW, +70%) than the $N_{120}$ ones (from 454 to 628 mg GAE $kg^{-1}$ FW, +38%) (Table 3). In globe artichoke [52], the TPC significantly increased during cold storage for both samples receiving 200 (N200) or 400 (N400) kg N $ha^{-1}$, while it decreased in N unfertilized samples. The authors stated that the behavior of N200 and N400 samples may be related to their higher proneness to degenerative phenomena following postharvest conditions of storage, which may have stimulated the phenylpropanoid metabolism and, therefore, the increase of TPC in their tissues [53]. The antioxidant activity during storage followed a trend similar to TPC, and this is attributable to antioxidant activity of total phenolic compounds [54].

## 4. Conclusions

The research highlighted that the N fertilization rate and leaf presence had an important effect on the postharvest shelf-life of fresh carrot roots. Supplying 240 kg $ha^{-1}$ of N, compared to 120 kg $ha^{-1}$, resulted in greater weight loss, color variations, and roots nitrates content as well as in a reduction of phenols and antioxidant activity. Consequently, the rate of 120 kg N $ha^{-1}$, which corresponds to the crop N uptake, has been shown to improve the quality and shelf-life of carrots, as well as making savings for the farmer and ensuring a more environmentally friendly crop management. The presence of the leaves led to dramatic changes in root appearance, mostly in terms of loss of weight and firmness, which are the most important parameters of quality and shelf-life in carrot. These differences were highly evident from the 12th day of storage, which therefore represents a deadline for their shelf-life when stored at 4 °C. However, bunched carrots compared to topped ones showed higher reducing sugars content and lower nitrates content, the former traits being appreciated by the consumer from a sensorial viewpoint, together with the attractive presence and freshness given by leaves. Overall, these preliminary results have shown the possibility of improving the desirable quality and shelf-life of bunched carrots halving the dose of nitrogen that is usually supplied, maintaining optimal storage conditions, and marketing carrots within 12 days from the start of storage.

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