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Volatile, quality and olfactory profiles of fresh-cut polignano carrots stored in air or in passive modified atmospheres

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ABSTRACT

Volatile organic compounds (VOCs), quality attributes (respiration activity, total phenols and antioxidant activity) and olfactory profile by electronic nose of fresh-cut orange and purple Polignano carrots stored in Air or passive modified atmosphere (pMAP) were examined until 14 days of storage. The pMAP storage reduced the respiration rate, preserving the antioxidant activity and total phenols of the carrots. Moreover, it enhanced the peculiar aromatic notes of these carrots, as showed by Principal Component Analysis (PCA), which allowed also to select the VOCs associated to fresh carrots and to samples stored in Air or pMAP. Predictive models of selected volatiles by electronic nose data were build running Partial least squares regression (PLSR). In orange carrots, six significant models were obtained for β -pinene, δ -elemene, γ -curcumene, *cis* α -bisabolene, cariophyllene oxide and α -cedrol. In the case of purple carrots, E-nose signals were able to predict only two terpenes, *trans* β -cariophyllene and myristicin.

1. Introduction

Carrot (*Daucus carota* L.) is one of the most cultivated vegetables in the world with well-known antioxidant properties (Cefola et al., 2012). In the Apulia Region (Southern Italy), a multicolored landrace of *Daucus carota* L. has been cultivated for many decades by local growers (Cefola et al., 2012). This landrace, called yellow-purple Polignano carrot, is characterized by a cortex pigmentation that goes from yellow to dark purple, while the inner core tone can range from pale yellow to light green. The yellow-purple Polignano carrots are characterized by higher amounts of antioxidants, phenols and carotenoids, especially β -carotene, compared to the commercial orange one (Cefola et al., 2012). In particular, the purple carrot has an antioxidant activity of about four times greater than the commercial orange type and almost ten times higher than the yellow or orange roots (Cefola et al., 2012). Additionally, in contrast to the almost uniform aroma of the marketable cultivars, the aromatic notes of the yellow-purple Polignano carrots, characterized by a salty and freshness sensation, tend to be extremely peculiar and

make them particularly suitable to be consumed as fresh-cut product (Cefola et al., 2012). Moreover, it has been reported that yellow-purple Polignano carrot are very perishable (Renna et al., 2013). In this context, according to Alasalvar, Al-Farsi, Quantick, Shahidi, and Wiktorowicz (2005), a MAP of 5% O₂ and 5% CO₂ in N₂ can give better sensory quality and can extend the shelf-life of purple carrots, while no differences were revealed for orange colour. In another study, Esturk, Ayhan, and Gokkurt (2015) have reported that minimally processed carrot disks can keep their quality up to 14 days when stored at 4 °C in passive MAP (pMAP), using low density polyethylene material bags. Furthermore, a recent study carried out on fresh-cut purple Polignano carrots has shown that a MAP of 15% O₂ and 5% CO₂ is able to preserve the sensory visual quality, the nutritional value and the respiration rate during storage at 4 °C until 14 days (Pace et al., 2020).

Consumer preference of fresh and fresh-cut fruit and vegetables is primarily affected by flavour and aroma sensations, which are straight determined by volatile organic compounds (VOCs) profiles (Cozzolino, Martignetti, et al., 2016). Consequently, in last years the monitoring of

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the VOCs profiles of fresh-cut fruit and vegetable products during their storage in different packaging and/or temperature conditions has revealed to be very useful, in order to evaluate some metabolites, as quality markers of the most suitable preservation condition (Cozzolino et al., 2016a, 2016b). In particular, alterations of food typical aroma could also be accompanied by alterations in nutritional value, decreasing the shelf life of the product (Kader, 2013). Relative VOCs content is largely influenced by factors as cultivar, location and methods of cultivation, harvesting and storage conditions. Kiraci et al. (2016) have reported that colored carrots, respect to light colored ones, shown higher terpenes content, which have been described to play a primary role in determining carrot aroma.

Despite several studies were accomplished on fresh-cut carrots stored in MAP, most of them were focused on phytochemicals or subjective sensory traits, such as off-odour and off-flavour. Nevertheless, no data are available on the VOCs profile of fresh-cut Polignano carrots during the MAP storage. Therefore, the aims of the present paper were to: study the VOCs profile of orange and purple Polignano carrots cold stored in Air or pMAP; identify putative volatile markers of freshness for purple and orange carrots; and predict VOCs by E-nose data using multivariate models.

2. Materials and methods

2.1. Plant material and experimental setup

Polignano carrots (*Daucus carota* L. var. *sativus*) were purchased from a local market in Polignano a Mare (Southern Italy) and transported in refrigerated conditions to the Postharvest Laboratory of CNR-ISPA to be processed. Based on the external colour, carrots were divided in orange and purple samples that were processed separately. In details, for each colour, groups of about 4.5 kg carrots were brushed, washed in tap water, dried and sliced with a thickness of approximately 0.5 cm, using a sharp knife. Then, carrots slices were immediately dipped in tap water for 5 min and dried using tissue paper. Three replicates of about 100 g each were kept for the initial determination (Fresh); the same amount of carrot slices were sealed in pMAP using micro-perforated polypropylene bags (25 × 25 cm, 30 µm, Carton Pack, Rutigliano, Italy), or placed in open polypropylene bags as control (Air). Overall, 36 bags (2 colours, orange and purple, × 3 replicates × 2 treatments, pMAP and Air, × 3 storage time, 6, 9 and 14 days) were prepared and stored at 4 (±1) °C. In pMAP samples, O₂ and CO₂ changes were monitored (gas analyzer CheckPoint, PBI Dansensor, Ringsted, Denmark) during storage.

2.2. Chemicals and reagents

Methanol, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, sodium chloride (NaCl), ethanol and 2-octanone were from Sigma-Aldrich (St. Louis, Mo., USA). Folin-Ciocalteu's phenol reagent was purchased from Merck (Germany). Ultra-pure water from a Milli-Q system (Millipore, MA, USA) with a resistivity at 25 °C of 18 MΩ·cm was used during all the experiments. Helium at a purity of 99.999% (Rivoira, Milan, Italy) was used as GC carrier gas. Solid Phase Micro-Extraction (SPME) fibres and glass vials were from Supelco (Bellefonte, PA, USA); the capillary GC-MS column HP-Innowax (30 m × 0.25 mm × 0.5 µm) was purchased from Agilent (Agilent Technologies, CA, USA).

2.3. Respiration rate

Respiration rate was measured initially and during storage (just after opening the bags), using a closed system, as reported by Kader (2002). About 100 g of sliced carrots, for each colour, storage time, treatment and replicate (n = 3), were put into 6 L sealed plastic jars (one jar for each replicate), allowing the accumulation of CO₂ up to 0.1% (standard

concentration of CO₂). The analysis of CO₂ was carried out using a gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA, USA) equipped with dual columns and a thermal conductivity detector, as reported by Renna et al. (2013). Respiration rate was expressed as µmol kg⁻¹ s⁻¹ CO₂. The same carrot samples were, then, used for the analysis reported below.

2.4. Antioxidant activity and total phenols

The same extraction was carried out for the analysis of antioxidant activity and total phenols. In details, for each replicate, 5 g of chopped sample were homogenized in 20 mL methanol/water solution (80:20 v/v) for 1 min, using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA), and then centrifuged (Labnet C2500-R Prism R™, Edison, NJ, USA) at 6440×g for 5 min at 5 °C. Antioxidant activity and total phenols were determined, using the DPPH and the Folin-Ciocalteu method, respectively, as reported by Cefola et al. (2012).

Antioxidant activity and total phenols are expressed on the basis of the fresh weight and reported as g kg⁻¹ Trolox using a Trolox calibration curve (82–625 µM; R² = 0.99) and as gallic acid (GA) equivalents (g kg⁻¹ GA), using gallic acid as standard (0.05–0.5 g L⁻¹, R² = 0.99).

2.5. Sample preparation and SPME procedure

The optimization of SPME extraction and desorption parameters were performed using commercial samples of carrots obtained from a local supermarket. Volatiles profiling was executed following the headspace SPME GC-MS method described by Cozzolino, Martignetti, et al. (2016), using the extraction temperature of 40 °C and the extraction time of 20 min. For sample preparation, 0.5 g of carrots sample were put in a 20 mL headspace vial with screw cap (Supelco, Bellefonte, PA, USA) containing 3 g of NaCl. Carrot samples were prepared by cutting several slices from the same package and taking 0.5 g from the whole sample, to get a representative sample from each bag. In each sample 3 µL of a stock solution of 100 mg L⁻¹ of 2-octanone, used as internal standard (IS), to guarantee the analytical reproducibility, were supplemented. Vials, sealed with a Teflon septum and an aluminium cap (Chromacol, Hertfordshire, UK), were shaken. The extraction and injection phases were automatically achieved by using an autosampler MPS 2 (Gerstel, Mülheim, Germany). The fibre was automatically put into the vial's septum for 20 min, to permit the volatiles adsorption onto the SPME fibre surface.

2.6. Gas chromatography–quadrupole mass spectrometry analysis (GC–qMS)

The SPME fibre was inserted into the injector port of the gas chromatograph apparatus, model GC 7890A, (Agilent Technologies, Santa Clara, USA) hyphenated with a mass spectrometer 5975 C (Agilent), wherein the volatiles were thermally desorbed and transported directly to a capillary column HP-Innowax (30 m × 0.25 mm × 0.5 µm, Agilent J&W) for the analysis. The oven temperature program was initially set at 40 °C for 2 min, ramped to 180 °C at 4 °C min⁻¹, then, after 1 min, the temperature was increased at 10 °C min⁻¹ at 240 °C and held for 5 min. Volatiles were analyzed consistent with the instrumental parameters already described (Cozzolino, Martignetti, et al., 2016; Cozzolino, Pace, et al., 2016). Each replicate was performed in triplicate by using a randomized sequence in which blanks were also run. The identification of volatiles was carried on using three different methods according to previous studies (Cozzolino et al., 2016a, 2016b).

The semi-quantitative data (Relative Peak Area, RPA %) of each metabolite were calculated in relation to the peak area of the IS. Areas were defined from the total ion current (TIC).

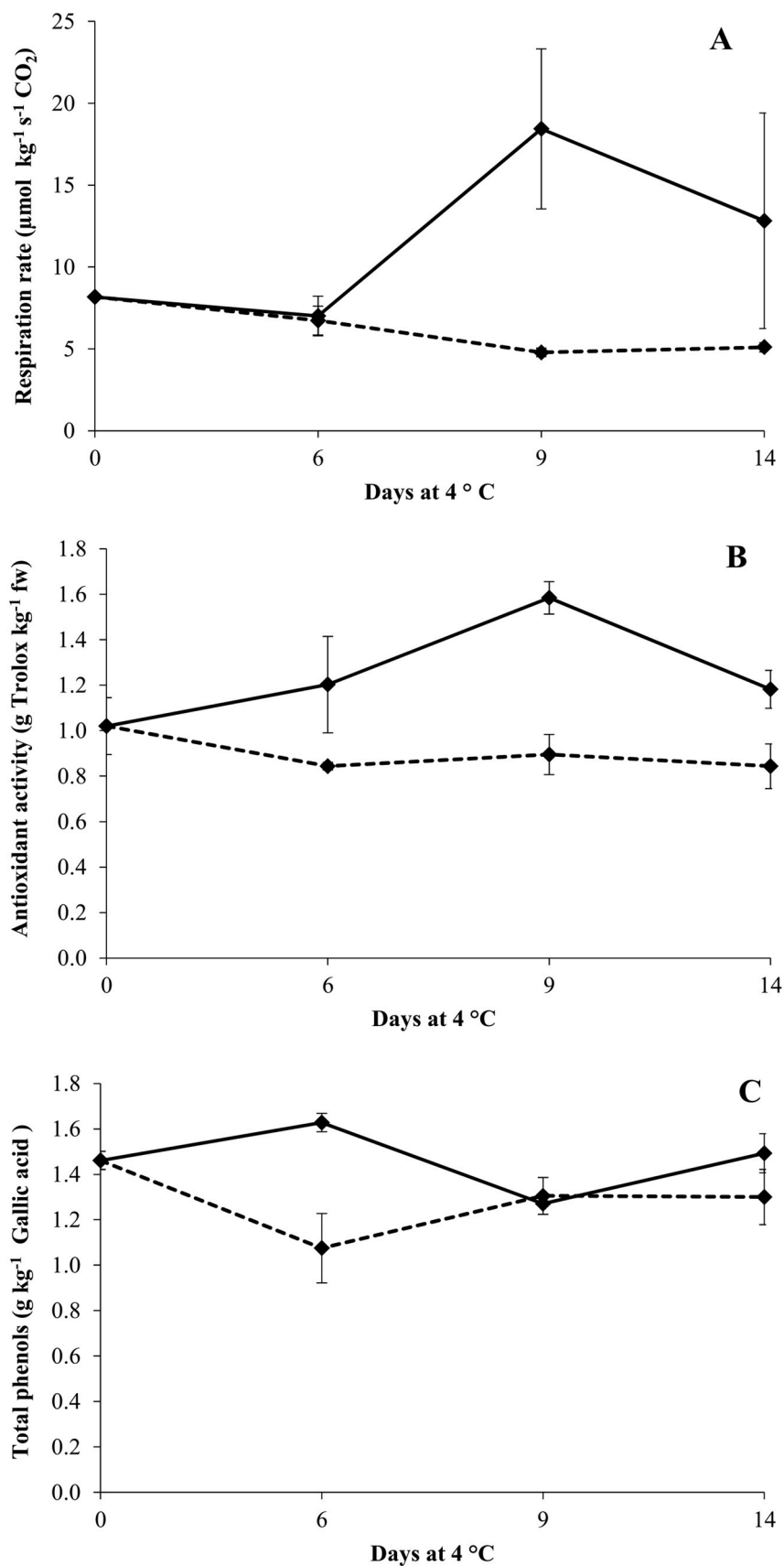


Fig. 1. Changes in respiration rate (A), antioxidant activity (B) and total phenols (C) in fresh-cut purple Polignano carrots stored at 4 °C in passive modified atmosphere (pMAP) or in Air for 14 days. Antioxidant activity and total phenols are expressed on the basis of the fresh weight. - -♦- pMAP —♦— Air. Mean \pm standard deviation.

2.7. Evaluation of carrot samples by electronic nose

A commercial portable Electronic nose (E-nose, PEN 3, Airsense Analytics Inc., Schwerin, Germany, including the Win Muster software) was used to analyze the olfactory profiles of Polignano carrots. The instrument is equipped with an array of 10 metal oxide semiconductors (MOS) chemical sensors which differ in thickness and chemical composition, to provide selectivity towards volatile classes (Laurienzo et al., 2013). Due to the high operating temperatures (200–500 °C), VOCs transferred to the surface of the sensors are totally combusted to carbon dioxide and water, leading to a change in the resistance. The MOS sensors response, expressed as resistivity (O), is based on changes in conductivity caused by the adsorption of gas molecules and on consequent surface reactions. Orange and purple Polignano carrots were analyzed separately by E-nose, evaluating the fresh roots and the cold stored samples at 6, 9 and 14 days. Specifically, 4 g of each sample were placed in 45 mL air tight glass vials, closed with a screw cap with Poly (1, 1,2,2-tetrafluoroethylene) (PTFE)/silicone septum, equilibrated at 30 °C for 30 min and analyzed at 22 ± 2 °C and at $50 \pm 5\%$ relative humidity (RH). In the course of the measurement period, the E-nose sucked the gaseous metabolites from the headspace of the sample through the sensory array at 400 mL/min for 80 s, during which changes in sensor resistance were recorded by computer. Each sample was analyzed in 5 replicates and data were collected every 1 s. When the volatile compounds reach the measurement chamber the sensor conductivity changes, increasing in the first seconds and then stabilizing by reaching a steady state. A second pump transported the filtered air to the sensor array at 600 mL/min for 400 s to rinse the system between two consecutive samples. The acquired signals in the range 4–8 s of the sensors 2, 6 and 8 were used in the correlation analysis with VOCs obtained by SPME GC-MS.

2.8. Statistical analysis

The effect of treatment (pMAP or Air), storage time (6, 9 and 14 days at 4 °C) and their interaction on respiration rate, antioxidant activity and total phenols were tested for each carrot colour by a two-way Anova using StatGraphics Centurion XVI.I (StatPoint Technologies, Inc., USA). When the interaction (treatment x storage time) was significant, data were presented as graphs with mean values \pm standard deviation. When interaction between factors resulted not significant and data were affected only by main factors, mean values were separated applying Fisher's Least Significant Difference (LSD) with significant difference at $p \leq 0.05$. One-way Anova was carried out to evaluate significant difference for each volatile during storage in Air or pMAP, using StatGraphics Centurion XVI.I (StatPoint Technologies, Inc., USA).

For each carrot colour, a principal component analysis (PCA) was run, using VOCs as variables and the following cases: Fresh, Air samples cold stored for 6, 9 and 14 days (Air-6d, Air-9d, Air-14d) and pMAP carrots cold stored for 6, 9 e 14 days (MAP-6d, MAP-9d, MAP-14d). PCA analysis was carried out using STATISTICA software. For each carrot colour, VOCs data and sensor response values carried out by E-nose were mediated. A matrix composed by 7 rows (Fresh, MAP-6d, MAP-9d, MAP-14d, Air-6d, Air-9d, Air-14d) and 63 columns, for the purple carrots, and 64, for the orange one, (60 VOCs for purple and 61 VOCs for orange, and 3 E-nose signals, S2, S6 and S8) was obtained. Data were subjected to partial least squares regression (PLSR) analysis by using the software The Unscrambler X. For both type of carrot, firstly three PLSR models were run with the aim to select the main VOCs predictors for each E-nose signal. Successively, the VOCs associated to the sensors were considered to build PLSR models with the purpose to predict them using the three E-nose signals.

3. Results and discussions

3.1. Headspace gas composition, respiration rate, antioxidant activity and total phenols

Inside pMAP bags, the steady state was reached after 2 days in purple carrots ($15.8 \pm 1.1\%$ O₂ and $5.2 \pm 0.7\%$ CO₂) and after 3 days in orange ones ($14.0 \pm 1.0\%$ O₂ and $6.7 \pm 0.6\%$ CO₂).

For both colored Polignano carrots the effect of treatment (pMAP or Air), time of storage (6, 9 and 14 days at 4 °C) and their interaction on respiration rate, antioxidant activity and total phenols were investigated by performing a two-way Anova. Results showed that treatment affected all the parameters analyzed with the exception of antioxidant activity and total phenols in orange carrots, while storage time affected only antioxidant activity in purple roots (Table S1). In purple carrots, the interaction treatment x time was significantly observed for all the quality parameters considered (Table S1).

Fig. 1A, which displays changes in respiration rate during storage of purple Polignano carrots, shows that this parameter at harvest was $8.2 (\pm 0.1) \mu\text{mol kg}^{-1} \text{s}^{-1} \text{CO}_2$ (Fig. 1A). The use of pMAP allowed to slightly reduce the rate of respiration during storage, which reached the value of $5.1 (\pm 0.3) \mu\text{mol kg}^{-1} \text{s}^{-1} \text{CO}_2$ after 14 days at 4 °C (Fig. 1A). In Air samples respiration rate was averagely higher than pMAP, though a great variability among replicates was revealed. For orange carrots the effect of the main factors on the respiration rate is reported in Table S2. Starting from the value of $9.6 (\pm 0.01) \mu\text{mol kg}^{-1} \text{s}^{-1} \text{CO}_2$ at harvest (data not shown), respiration rate in orange carrot did not change significantly during storage. On the contrary, the use of pMAP allowed to reduce significantly the respiration rate compared to sample stored in Air (Table S2). Consequently, both colored Polignano carrots showed a similar behavior: a reduction of respiration rate in pMAP samples respect to Air ones. These findings are comparable to some previous results about the effect of atmosphere modification on reducing respiration rate in fresh-cut carrots (Iqbal, Rodrigues, Mahajan, & Kerry, 2009; Pace et al., 2020; Simões, Allende, Tudela, Puschmann, & Gil, 2011). As showed in Fig. 1B, the antioxidant activity of purple Polignano carrots at harvest was $1.0 (\pm 0.1) \text{g kg}^{-1}$ Trolox, keeping almost constant during storage in pMAP. In Air samples, however, antioxidant activity increased significantly until $1.6 (\pm 0.1) \text{g kg}^{-1}$ Trolox at day 9, even though the final value at the end of the storage was $1.2 (\pm 0.1) \text{g kg}^{-1}$ Trolox, not different from the initial one (Fig. 1B). In orange Polignano carrots antioxidant activity, at harvest, was $0.2 (\pm 0.01) \text{g kg}^{-1}$ Trolox (data not shown), however no significant changes of this parameter was observed during storage and comparing treatments (Table S1). As regards total phenols, the content in purple Polignano carrots at harvest was $1.5 (\pm 0.04) \text{g kg}^{-1}$ GA and remained almost constant in both treatments until the end of the storage, with a mean value of $1.4 (\pm 0.1) \text{g kg}^{-1}$ GA (Fig. 1C). In orange Polignano carrots the initial content in total phenols was $0.4 (\pm 0.02) \text{g kg}^{-1}$ GA; during storage it remained almost constant in both treatments (data not shown). Similar data have been reported by Cefola et al. (2012), who have founded an antioxidant activity in purple Polignano carrots 10-fold higher than in orange one. These authors specified that the elevated antioxidant activity recorded in the purple carrots can be only ascribed to the cortex tissues. Concerning total phenols, Cefola et al. (2012) reported that orange Polignano carrot showed a mean phenol content about 24% lower respect to the purple one. In the present study the differences in both antioxidant activity and total phenol contents between purple and orange Polignano carrots were less evident, probably because the analysis was performed on the whole carrot slices, regardless the cortex and the inner core.

3.2. Determination of VOCs profile of fresh-cut orange and purple polignano carrots during cold storage in air or pMAP

SPME GC-MS analysis of Polignano carrots cold stored in Air or

Table 1

Volatile metabolites detected in purple and orange “Polignano” carrots and their identification codes.

Metabolite	Code	RI _t /RI _{sp}	ID	Metabolite	Code	RI _t /RI _{sp}	ID
Terpenes				Ketones			
α-Pinene	T1	1014/1014	RI/MS/S	5-Nonanone	K1	1324/1325	RI/MS
α-Thuiene	T2	1018/1018	RI/MS/S	6-Methyl-5-epten-2-one	K2	1336/1336	RI/MS/S
Camfene	T3	1057/1057	RI/MS/S	2-Nonanone	K3	1386/1387	RI/MS/S
β-Pinene	T4	1082/1085	RI/MS/S	Alcohols			
Sabinene	T5	1100/1098	RI/MS/S	1-Esanolo	A11	1353/1353	RI/MS/S
δ-3-Carene	T6	1126/1127	RI/MS/S	1-Esanol-2-etil	A12	1493/1492	RI/MS/S
Phellandrene	T7	1147/1148	RI/MS/S	1-Octanolo	A13	1558/1558	RI/MS/S
β-Mircene	T8	1154/1154	RI/MS/S	Esters			
α-Terpinene	T9	1169/1170	RI/MS/S	2-Ethyl-1-hexyl propionate	E1	1447/1450	RI/MS/S
DL-Limonene	T10	1190/1190	RI/MS/S	Ethyl nonanoate	E2	1539/1541	RI/MS/S
β-Ocimene	T11	1234/1233	RI/MS/S	Lactones			
γ-Terpinene	T12	1240/1240	RI/MS/S	γ-Octalactone	L1	1916/1914	RI/MS/S
Cimene	T13	1267/1268	RI/MS/S	γ-Nonalactone	L2	2035/2036	RI/MS/S
α-Terpinolene	T14	1279/1279	RI/MS/S	γ-Decanolactone	L3	2194/2183	RI/MS/S
1,3,8 para Mentatriene	T15	1389/1391	RI/MS	Others			
α-Cubebene	T16	1454/1454	RI/MS/S	Tridecane	O1	1297/1300	RI/MS
δ-Elemene	T17	1467/1470	RI/MS	Acido butanoico	O2	1626/1625	RI/MS/S
α-Copaene	T18	1490/1491	RI/MS/S				
Bornil acetato	T19	1578/1577	RI/MS/S				
α-Bergamotene	T20	1581/1583	RI/MS				
trans β-Cariofillene	T21	1591/1592	RI/MS/S				
Isosativene	T22	1630/1639	RI/MS/S				
β-Farnesene	T23	1658/1659	RI/MS/S				
α-Cariofillene	T24	1669/1670	RI/MS				
α-Amorfene	T25	1683/1679	RI/MS				
γ-Curcumene	T26	1691/1688	RI/MS				
Germacrene D	T27	1705/1703	RI/MS				
Zingiberene	T28	1719/1718	RI/MS				
β-Bisabolene	T29	1726/1726	RI/MS				
trans α-Farnesene	T30	1730/1732	RI/MS				
trans γ-Bisabolene	T31	1751/1745	RI/MS				
β-Sesquiphellandrene	T32	1769/1767	RI/MS/S				
cis α-Bisabolene	T33	1773/1761	RI/MS				
Cuparene	T34	1810/1819	RI/MS/S				
p Cimen-8-ol	T35	1817/1818	RI/MS				
Geranil acetone	T36	1844/1846	RI/MS/S				
Cariofillene ossido	T37	1860/1859	RI/MS/S				
Carotolo	T38	1976/1977	RI/MS				
α-Cedrolo	T39	2018/2019	RI/MS/S				
Miristicina	T40	2152/2149	RI/MS/S				
Aldehydes							
Esanale	Ald 1	1072/1072	RI/MS/S				
Eptanale	Ald 2	1183/1184	RI/MS/S				
2-Esenael	Ald 3	1222/1226	RI/MS/S				
Octanal	Ald 4	1286/1287	RI/MS/S				
Nonanale	Ald 5	1392/1392	RI/MS/S				
Decanale	Ald 6	1503/1503	RI/MS/S				
2-Nonenael	Ald 7	1536/1535	RI/MS/S				
2-Decenale	Ald 8	1648/1655	RI/MS/S				

For each sample, data are mean values of 3 replicates.

RI_t: Relative retention indices on polar column reported in literature by www.pherobase.com; www.flavornet.org; www.ChemSpider.com; webbook.nist.gov; RI_{sp}: Relative retention indices calculated against n-alkanes (C₈–C₄₀) on HP-Innowax column.

ID: Identification method as indicated by the following: RI: Kovats retention index on a HP-Innowax column; MS: NIST and Wiley libraries spectra; S: co-injection with authentic standard compounds on the HP-Innowax column.

pMAP at different times of storage allowed to totally identify 60 volatiles in the purple and 61 VOCs in the orange root. Excluding aldehydes, 7 in fresh purple and 8 in the fresh orange samples, for both colored carrots the identified VOCs included the same chemical classes containing the same compounds: terpenes (40), ketones (3), alcohols (3), esters (2), lactones (3) and others (2). Particularly, Table 1 lists, for both Polignano carrots, the VOCs abbreviation code, the experimental and literature reported Kovats index and the identification methods. According to the literature, the volatile profiles of both varieties were characterized by a predominance of terpenes, in number and content, compared to the other VOCs (Guimarães et al., 2016).

In order to explore, during the preservation time, the effect of the packaging atmosphere (pMAP or Air) on the VOCs profile, for each carrot variety, the SPME GC-MS semi-quantitative data, calculated from the percentage ratio (% RPA) between the area of each metabolite and

that of 2-octanone (IS), were subject to a one-way Anova. Results, summarized in Tables S3 and S4 for purple and orange carrots, respectively, show that differences between the % RPA values are statistically significant for all the compounds, except for β-farnesene (T23) for purple carrots (Table S3) and for γ-nonalactone (L2) for orange samples (Table S4).

Table S3 shows that, 46 of the 60 VOCs identified in the purple carrot were common to all the treated samples and observed for the entire storage period. Comparing the volatile patterns of both fresh and preserved carrots, it was possible to infer that α-copaene (T18), isosativene (T22), γ-curcumene (T26), trans α-farnesene (T30), cuparene (T34), decanal (Ald5), 2-nonenal (Ald6), 5-nonanone (K1) and 1-hexanol (A11), absent in Fresh, appear both in pMAP and Air samples starting from the 6th day of preservation (Table S3). Alternatively, α-amorphene (T25), present in Fresh and pMAP for the entire storage, is always absent in Air.

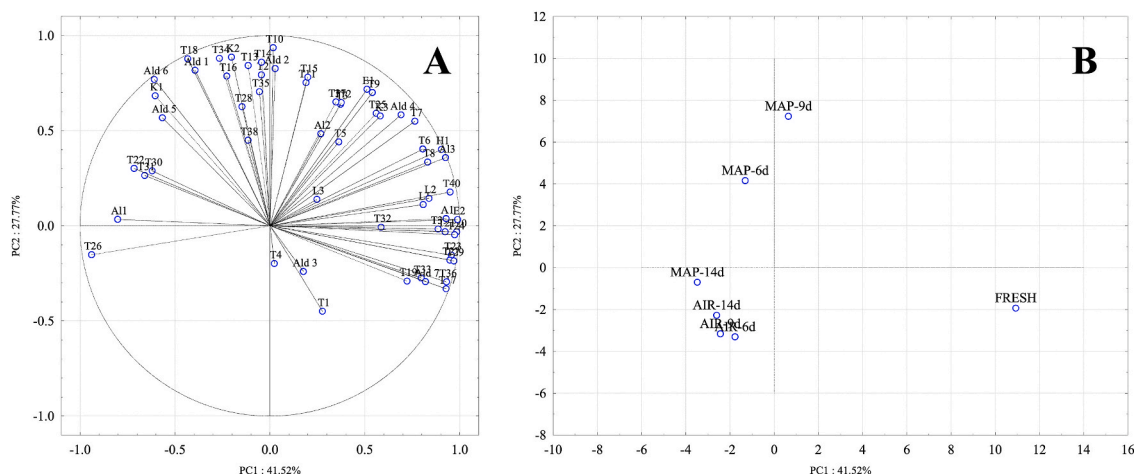


Fig. 2. PCA score scatter plot (A) and loading plot (B) carried out on the SPME GC-MS data of purple Polignano carrot stored at 4 °C passive modified atmosphere (pMAP) or in Air for 14 days. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Data in Table S3 demonstrate that in the fresh purple root, terpenes, distinguishable in monoterpenes (29.77%) and sesquiterpenes, the most abundant portion (70.23%), represented the 99.18% of the total VOCs. Furthermore, the most representative monoterpene was α -terpinolene (49.88%), while the most abundant sesquiterpene was myristicin (37.89%) (Table S3). Specifically, α -terpinolene, having a relatively high odour activity value (OAV) in refrigerated as well as frozen-stored carrots and already described important in imparting “citrus-like”, “fruity” and “sweet” notes to carrots (Kjeldsen, Christensen, & Edelenbos, 2003), could have a specific role in conferring “sweet” notes to the purple roots. These findings agree to Kreutzmann, Thybo, Edelenbos, and Christensen (2008), who have reported that the purple genotype is characterised by higher sweet flavour intensity respect to orange varieties. Purple roots, in fact, are richer in sugars which can mask some of the typical terpenes harsh flavour (Kreutzmann et al., 2008).

Compared to Fresh, in purple carrots stored in pMAP the terpenes total amount remained almost unchanged for all the storage time (Table S3); however, in pMAP-14d samples the monoterpenes were about 15% higher than in Fresh, while the sesquiterpenes decreased by about 15% (Table S3). Furthermore, while the most abundant monoterpene was α -terpinolene in all the pMAP carrots, among sesquiterpenes, myristicin was the most representative in the pMAP-9d samples, and *trans* γ -bisabolene the most abundant in pMAP-6d and pMAP-14d samples (Table S3).

For Air stored purple carrots, the total terpenes percentage remained nearly unaltered for the entire storage, compared to Fresh (Table S3). At 14 days, the monoterpenes content was about 33% higher, and the sesquiterpenes concentration was approximately 33% lower respect to the fresh product (Table S3). Finally, in all the Air stored purple samples the most abundant monoterpene was always α -terpinolene, while the most representative sesquiterpene was *trans* γ -bisabolene (Table S3).

Table S4, describing one-way Anova for orange carrot samples, shows that, 51 of the 61 VOCs identified are detectable during all the storage period and are shared by all the treated samples. Comparing the VOCs profiles of fresh and preserved orange carrots it was possible to deduce that 2-hexenal (Ald3), 2-nonanone (K3) and ethyl nonanoate (E2), never identified in Fresh, appeared both in pMAP and in Air at 6 days of storage. Instead, 2-decenal (Ald8), revealed in Fresh and in Air bags for the entire preservation, always missed in pMAP samples (Table S4).

Table S4 shows that the principal VOCs in the orange fresh product were, as expected, terpenes, (99.31%), with monoterpenes at 35.55% and sesquiterpenes, the most representative, at 64.45% (Table S4). Additionally, the most abundant monoterpene was the α -terpinolene (74.01%), while the principal sesquiterpene was β -cariophyllene

(40.98%) (Table S4). In this case, besides α -terpinolene, to the “sweet” notes of the orange root there is also the important contribute of β -cariophyllene, presenting this sesquiterpene a high OAV both in refrigerated and in frozen-stored carrots (Kjeldsen et al., 2003).

Compared to Fresh, the terpenes content in pMAP orange carrots was essentially unaffected during all the storage (Table S4); nevertheless, monoterpenes constantly increased, while sesquiterpenes decreased in the course of pMAP packaging (Table S4). Particularly, at the 14th day monoterpenes doubled their percentage (69.89%), and sesquiterpenes were almost halved (30.11%) (Table S4).

Moreover, as in purple carrots, α -terpinolene was the most abundant monoterpene in all the pMAP samples, while β -cariophyllene was the principal sesquiterpene in the pMAP-6d and pMAP-9d samples, and *trans* γ -bisabolene was the most abundant in the pMAP-14d (Table S4).

In fresh-cut orange carrot stored in Air, the terpenes concentration was almost unaltered, related to Fresh, for the entire storage time (Table S4). Further, also in this case, monoterpenes amount increased during the preservation reaching at the 14th day 51.26% (about 15% more than in Fresh), while sesquiterpenes content reduced of approximately 15% compared to Fresh (Table S4). Finally, in all the Air orange carrots the major monoterpene was α -terpinolene, while *trans* γ -bisabolene was the principal sesquiterpene (Table S4).

Due to the data complexity, multivariate statistical analysis was performed on the SPME GC-MS data, in order to identify volatiles putative markers associated to Fresh, Air and pMAP samples. In addition, PLSR models were built with the aim to predict volatiles by E-nose profiles.

3.3. Identification of volatile markers of freshness for purple and orange carrots

In order to identify the potential volatiles responsible of the discrimination of fresh purple or orange Polignano carrots from those cold stored in Air or pMAP at 6, 9, and 14 days, SPME GC-MS data were subjected to principal component analysis for both colored carrots, separately.

Particularly, Fig. 2A and B report for the purple carrot the loading plot and the corresponding score scatter plot of the PCA, respectively. The first and the second principal components (PC1 and PC2) explain 69.3% of the variance and illustrate that the purple carrots based on PC1 (accounted for 41.5%) can be discriminated into three clusters, related to the different storage conditions (Fig. 2A). In particular, Fresh product is well distinguished both from the Air and pMAP samples (Fig. 2A). Fresh-cut carrots stored in Air or pMAP can be additional discriminated into two clusters based on the PC2 (accounted for 27.8%), describing most of

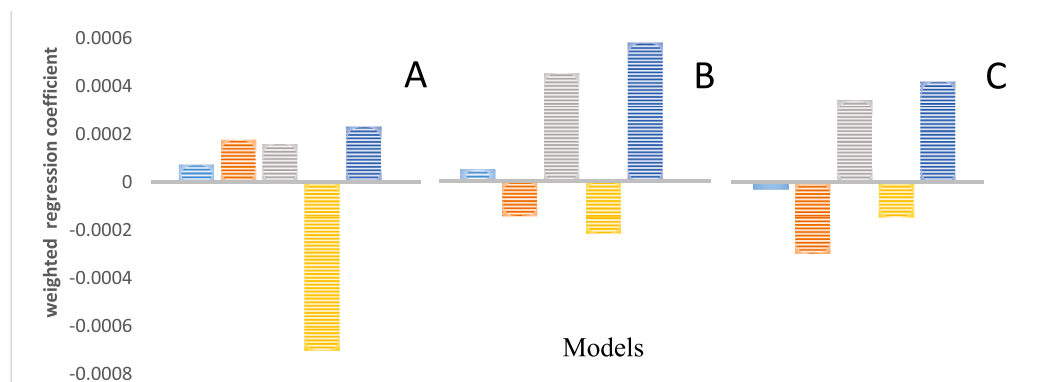


Fig. 4. Weighted regression coefficient for the predictors VOCs in Sensor 2- (A), Sensor 6- (B) and Sensor 8-model (C) for purple carrots. ■ γ -Terpinene; ■ α -Terpinolene; ■ *trans*- β -Cariophyllene; ■ *trans*- γ -Bisabolene; ■ Myristicin.. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the conservation their amount was not significantly different between the two treatments (Table S4). Finally, 5-nonanone, missing in the fresh orange carrot, appeared in both pMAP-6d and Air-6d samples. It was still identified even at lower level in Air-14d, while it was absent in pMAP-9d and pMAP-14d.

3.4. PLSR models to predict volatiles by electronic nose

The PLSR analysis carried out on orange carrots allowed to obtain one significant model for the S2 sensor of the E-nose (Table S5), using as predictors α -terpinolene (T14), *trans*- β -cariophyllene (T21), *trans*- γ -bisabolene (T31) and myristicin (T40), all terpenoids among the major VOCs detected in raw carrot roots (Güler et al., 2015). The volatiles T31 and T40, both characterized by “spicy” and “sweet” notes, showed the highest weighted regression coefficient (β) values (0.008 and 0.14 respectively). Consequently, T31 and T40 could be considered markers of the S2 sensor for the orange variety.

In purple carrots, three significant models for the sensors S2, S6 and S8 were obtained (Table S5, Fig. 4), using as predictors γ -terpinene (T12), α -terpinolene (T14), *trans*- β -cariophyllene (T21), *trans*- γ -bisabolene (T31) and myristicin (T40). For S2 models, T31 showed the highest β (−0.0006), while for S6 and S8 models T21 and T40 might be considered putative markers (Fig. 4). In addition to T31 and T40, *trans*- β -cariophyllene (T21) can be considered as possible marker of the “sweet” notes of the purple root, in line with the major volatile constituents of this ecotype (Table S3).

The E-nose signals were, then, used to predict the presence of some specific terpenes. In orange carrots six significant models were obtained for β -pinene (T4), δ -elemene (T17), γ -curcumene (T26), *cis*- α -bisabolene (T33), *cariophyllene oxide* (T37) and α -cedrol (T39) (Table S6). In the case of purple carrots, E-nose signals were able to predict only two terpenes, *trans*- β -cariophyllene (T21) and myristicin (T40), mainly estimated by E-nose signals of the S6 and S8 sensors (Table S6). Particularly, myristicin, previously reported as having a high OAV for carrots (Fukuda et al., 2013), is a phenylpropanoids with antifungal activity (Güler et al., 2015). As it constitutes 26% of the total VOCs, myristicin could prove to be an important component in purple Polignano carrot.

4. Conclusion

The present study demonstrated that orange and purple Polignano carrots, although extremely perishable for their high respiration rate, could be stored as fresh-cut products until 14 days at 5 °C in pMAP, a treatment that resulted able to significantly reduce the respiration rate, preserving the antioxidant activity and total phenol content. Moreover, pMAP allowed to enhance the volatiles responsible of the aromatic

peculiar notes of these carrots. To the best of our knowledge, this is the first report on the VOCs profile of fresh-cut Polignano carrots during the MAP storage. PCA and PLSR analysis allowed to select the VOCs mainly associated with Fresh, Air or pMAP samples. Particularly, fresh purple carrots were correlated to many terpenes, to γ -Octalactone, γ -Nonalactone and γ -Decanolactone, to 2-hexenal and 2-decenal, which can be, then, considered markers of freshness for this root. Similarly, fresh orange carrots resulted associated to numerous terpenes, to heptanal, nonanal, 2-nonenal and 2-decenal and to 6-methyl-5-hepten-2-one.

PLSR analysis accomplished on the VOCs and the E-nose sensors allowed to obtain predictive models using E-nose signals to estimate the presence of some specific terpenes. In particular, considering orange carrots, six significant models were obtained for β -pinene, δ -elemene, γ -curcumene, *cis*- α -bisabolene, *cariophyllene oxide* and α -cedrol, while for purple carrots, E-nose signals were able to significantly predict only two terpenes (*trans*- β -cariophyllene and myristicin).

These PLSR models might allow to evaluate the main volatile metabolites of these two carrots by E-nose analysis.

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CRediT authorship contribution statement

R. Cozzolino: Writing - original draft, conceived and designed the study and wrote the paper, acquired and processed data. **B. De Giulio:** performed the experiments, acquired and processed data. **M.P. Pellicano:** Formal analysis. **B. Pace:** Formal analysis. **I. Capotorto:** performed the experiments. **A. Martignetti:** performed the experiments. **M. D’Agresti:** performed the experiments. **C. Laurino:** performed the experiments. All authors contributed to the discussion of the data and critically revised the manuscript. **M. Cefola:** Writing - original draft, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2020.110408>.

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