VOLATILE METABOLITES, QUALITY AND SENSORY PARAMETERS OF "*FERROVIA*" SWEET CHERRY COLD STORED IN AIR AND HIGH CO_2 MODIFIED ATMOSPHERES

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Abstract

Volatile organic compounds, quality and sensory attributes of sweet cherry cv "Ferrovia", cold stored in Air or different modified atmospheres (Low-O_2 = 1% O_2/0.03% CO_2; High-CO_2 = 16% O_2/20% CO_2; Mix = 1% O_2/20% CO_2), were monitored until 21 days of conservation. Results showed that sweet cherry cv "Ferrovia" is sensitive to CO_2 accumulation (over 20%) in hypoxic condition, as showed by increase in respiration rate, biosynthesis of fermentative volatile metabolites and sensory perception of off-odours. However, High-CO_2 treatment seemed to preserve quality and sensory traits, presumably due to the high initial concentration of O_2 (16%) in gas composition that could limit the synthesis of ethyl esters and γ-butyrolactone, keeping the accumulation of off-flavours below their sensory perception threshold. Therefore, ethyl esters and γ-butyrolactone might be considered putative markers of sensory alterations related to fermentation. For γ-butyrolactone this result was confirmed by the correlation analysis between VOCs and sensory traits.

Keywords: Sweet cherry cv "Ferrovia"; Volatile organic compounds, Respiration activity, Sensory analysis; Correlation analysis; γ-Butyrolactone

1. Introduction

Sweet cherries (*Prunus avium* L.) are fleshy, non-climacteric stone fruit mainly grown in temperate climate countries. This fruit, particularly appreciated by consumers for its high-flavoured traits, sweetness and juiciness, presents high nutritional value and beneficial health properties (Karagiannis, Michailidis, Karamanoli, Lazaridou, Minas, & Molassiotis, 2018), being an important source of essential nutrients and bioactive components (Chockchaisawasdee, Golding, Vuong, Papoutsis, & Stathopoulos, 2016).

"Ferrovia" is a typical Italian sweet cherry cultivar (Girelli, De Pascali, Del Coco, & Fanizzi, 2016), characterized by a big, red, heart-shaped fruit with bright skin, firm consistency and very pleasant flavour of intermediate sweetness that makes this cultivar excellent for fresh consumption (Vavoura, Badeka, Kontakos, & Kontominas, 2015).

Sweet cherry is highly perishable fruit, owing to high respiratory activity, small carbohydrate reserve and elevated predisposition to mechanical injury (Wang, Bai, & Long, 2015; Chockchaisawasdee et al., 2016). Post-harvest cold temperatures and modified atmosphere (MA) packaging has been established to delay senescence of this fruit (Wang et al., 2015, Chockchaisawasdee et al., 2016). Vegetables and fruit reactions to CO_2 exposure during storage are largely conditioned by cultivar and postharvest treatment (Watkins, 2000). Depending on cultivar, in fact, sweet cherries can tolerate very low oxygen level (0.02% O_2 for 21-25 days) (Dangyang & Kader, 1992), whereas high CO_2 percentages (10-30%) can be effective in maintaining drupe firmness, ascorbic acid and titratable acidity levels, without the development of off-flavours (Wang & Vestrheim, 2002; Tian, Jiang, Xu, & Wang 2004). However, despite many successful uses of MA handlings, there is discrepancy about the optimum amount of CO_2 and/or O_2 to use in MA packages, as some studies report that sweet cherries develop off-flavours when kept in higher than 10% CO_2 and up to 5% O_2 (Goliáš, Němcová, Čaněk, & Kolenčíková, 2007).

Flavour and aroma have a crucial role in influencing consumer acceptance of fresh and processed food. Scientific evidence suggests that in fruit and vegetables the formation of odour and flavour sensations is directly affected by volatile organic compounds (VOCs) profiles (Cozzolino et al., 2016a). Since loss of flavour quality can happen before than loss of visual features, postharvest life of vegetable commodities can be determined based on flavour rather than appearance and textural attributes (Cozzolino, 2016b). Moreover, alterations of food distinctive aroma could also induce changes in nutritional quality, shortening product shelf life (Kader, 2013).

For these reasons, the definition of the most suitable conditions to preserve sweet cherry quality for the fresh markets is still a challenge. In addition, since fruit VOCs are determined by storage conditions (temperature and MA composition) (Zhang, Xi, Wei, Shen, Ferguson, & Chen, 2011), modifications of volatiles and flavour during postharvest storage can be evaluated, in order to establish suitable conditions able to maintain the characteristic flavour and nutritional aspect of fruit (Deza-Durand & Petersen, 2011).

Starting from these findings, the present study was designed to evaluate the effect of cold storage in different high CO_2 modified atmospheres until 21 days on the VOCs profile, quality and sensory attributes of sweet cherries cv "Ferrovia" and to identify putative volatile markers of sensory alteration.

To the best of our knowledge, this is the first report on the quality, volatile and sensory characterization of sweet cherry cv "Ferrovia" cold stored in Air and different high CO_2 MA.

The outcomes of this study could provide a better understanding of the postharvest behaviour of sweet cherry cv "Ferrovia" to different MA treatments, suggesting optimal conditions to preserve quality and sensory characteristics of this cultivar.

2. Materials and Methods

2.1. Plant material preparation

Sweet cherries (*Prunus avium* L., cv Ferrovia) at maturity stage (total soluble solid content and titratable acidity of about 18° Brix and 0.61 % malic acid, respectively), were provided by a local farm (Ermes snc, Noicattaro, Bari, Italy) and transported, within 2 h from harvest, to the Postharvest Laboratory of ISPA-CNR (URT c/o CS-DAT in Foggia, (Italy). Fruit was selected based on the absence of defects or diseases and were randomly distributed into four clusters, each one representative of the specific treatment used. In particular, three modified atmosphere treatments were applied, using different initial concentrations (%) of oxygen and carbon dioxide (O_2/CO_2) in nitrogen as follows: 1% O_2 + 0.03% CO_2 + 99% N_2 (Low-O_2), 16 % O_2 + 20% CO_2 + 64% N_2 (High-CO_2) and 1% O_2 + 20% CO_2 + 79% N_2 (Mix). Samples stored in unsealed bags were used as control (Air). For each treatment (Low-O_2, High-CO_2, Mix or Air), 18 packages (6 replicates × 3 storage times) were prepared by placing about 100 g of sweet cherries in polyethylene terephthalate (PET) trays (model CL1/135 Carton Pack, Italy), sealed (Boxer 50 Lavezzini Vacuum Packaging System, Italy) or unsealed in 30×40 cm polyamide/polyethylene (PA/PE) plastic bags (pCO_2 40 cm^3 m^{-2} 24 h^{-1} bar^{-1}, 140 µm thick, Orved, Italy). All samples were stored at 5 °C and were analysed at harvest and after 7, 14 and 21 days for the determination of VOCs profiles, quality and sensory parameters. Headspace gas composition (O_2 and CO_2) within each MA package was monitored daily using a gas analyser (CheckPoint, PBI Dansensor, Ringsted, Denmark).

2.2. Chemicals and reagents

Sodium chloride (NaCl) and 4-methy-2-pentanol were purchased from Sigma-Aldrich. Helium at a purity of 99.999% (Rivoira, Milan) was used as GC carrier gas, while ultra-pure water from a Milli-Q system (Millipore, Bedford, MA, USA) with a resistivity at 25 °C of 18 MΩ*cm was used throughout. SPME fibres and glass vials were from Supelco (Bellofonte, PA, USA); capillary GC-MS column HP-Innowax (30m×0.25 mm×0.5µm) was from Agilent J&W (Agilent Technologies Inc.). SPME fibres were conditioned prior to their first use as recommended by the manufacturer, but below the maximum suggested temperature. Before the initially daily analysis, fibres were conditioned for 5 min at the operating temperature of the GC injector port and the blank level was checked. Triplicate analyses were performed.

2.3. Quality analysis

2.3.1. Respiration rate, relative water content of peduncles and berry color

Respiration rate was measured initially (Fresh) and during storage (at 7, 14 and 21 days, just after the opening of the packages), using a closed system. About 100 g of cherries, for each storage treatment and replicate (n =3), were put into 6 L sealed plastic jars (one jar for each replicate), and CO_2 was allowed to accumulate up to 0.1% (standard concentration of CO_2). Time taken to reach this value was calculated by measuring CO_2 at regular intervals of time. For CO_2 analysis, 1 mL of gas sample was taken from the headspace of the plastic jars through a rubber septum and injected into a gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA, USA), equipped with dual columns and a thermal conductivity detector. Carbon dioxide was analysed with a retention time of 16 s and a total run time of 120 s on a 10 m porous polymer (PPU) column (Agilent, Santa Clara, CA, USA) at a constant temperature of 70 °C. Respiration rate was expressed as mL CO_2 kg^{-1} h^{-1}.

Relative water content (RWC) of peduncles was measured initially and during storage on peduncle pieces of about 1 cm each, for a total of 4 (±0.3) g of peduncle for each replicate (Rosales, Fernandez-Caballero, Romero, Escribano, Merodio, & Sanchez-Ballesta, 2013). Peduncle pieces were weighed fresh (Fw), after 24 h rehydration (Rw) in distilled water at ambient temperature and after drying (Dw) at 65 °C in oven, until constant weight. The RWC was calculated as percentage, using the following formula: RWC (%) = (Fw - Dw)/(Rw - Dw) x 100 (Sanchez-Ballesta et al., 2006).

Colour parameters (L*, a* and b*) were measured, for each replicate, on 3 random points on peel surface of 10 cherry fruits using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in the reflectance mode and in the CIE L* a* b* colour scale. Colorimeter was calibrated with a standard reference having values of L*, a* and b* corresponding to 97.55, 1.32 and 1.41, respectively. Hue angle (h°) was calculated from a* and b* values.

2.4. Sensory analysis

Quantitative descriptive analysis (QDA) (Lawless & Heymann, 1998; Stone, Sidel, Oliver, Woolsey, & Singleton, 1974) was performed, for the evaluation of 22 sensory attributes (Table 1), by a panel composed by 8 judges (4 males and 4 females) that were trained as reported in Supplementary Material S1. QDA was achieved tasting fresh sweet cherries cv "Ferrovia" along with two cherry samples purchased at a local market, to make the trial anonymous. For the analysis of stored fruit, at each sampling day, all sweet cherries were placed at room temperature for 2 hours and MA bags were opened 30 minutes before tasting. In order to prevent the position and the carry-over effects, samples were coded with a three-digit number, following a presentation order generated by Williams Latin square's design (Williams, 1949). All samples were tasted 3 times (at 10:00 am, 4:00 pm and at 10:00 am of the day after) in the evaluation area conditioned at 20 (± 2) °C with 50 (± 5) % relative humidity and equipped with 8 booths lit with a red light at 192 Lux for olfactory, gustatory/tactile, retro-olfactory and after swallowing sensory properties, and with a white light at 850 Lux for visual qualities. Data were acquired and processed using FIZZ Forms software (Biosystemes, Couternon, France).

2.5. Volatile Organic Compounds analysis

2.5.1. Sample preparation and SPME procedure

Optimization of SPME extraction and desorption conditions was carried out by analysing commercial cherry samples purchased at a local supermarket. Volatiles profiling was performed according to the headspace SPME/GC-MS method described by Vavoura et al. (2015), but using DVB/CAR/PDMS (50/30 mm) fibre, the extraction temperature of 45°C and the extraction time of 20 min. Sample preparation procedure was the following: 1 g of cherry sample cv "Ferrovia" was mixed into a 20 ml screw-on cap HS vial (Supelco, Bellefonte, PA, USA) to 0.2 g of NaCl. In order to assure analytical reproducibility, in each sample 2.5 µL from a stock solution of 20 ppm of 4-methyl-2-pentanol, used as internal standard (IS), were added. After stirring, vials were sealed with a Teflon (PTFE) septum and an aluminium cap (Chromacol, Hertfordshire, UK) for the production of headspace and the consecutive analysis. The extraction and injection processes were automatically performed using an autosampler MPS 2 (Gerstel, Mülheim, Germany). The fibre was, then, automatically inserted into the vial's septum for 20 min, to allow volatile compounds adsorption onto the SPME fibre surface.

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

SPME fibre was inserted into the injector port of the gas chromatograph device, model GC 7890A, Agilent (Agilent Technologies, Santa Clara, USA) coupled with a mass spectrometer 5975 C (Agilent). Volatiles were thermally desorbed and transferred directly to a capillary column HP-Innowax (30 m × 0.25 mm × 0.5 µm Agilent J&W) and analyzed. Oven temperature program was initially set at 40 °C for 2 min, then increased to 160 °C at 5 °C min^{-1}, ramped to 250 °C at 10 °C min^{-1} and held at 250 °C for 2 min. The temperatures of ion source and quadrupole were held at 230 °C and 150 °C, respectively; helium was used as carrier gas with a flow of 1.5 mL min^{-1}; injector temperature was kept at 240 °C and the pulsed splitless mode was used for the analysis. Fibre was maintained in the injector for 10 min. Mass spectra were acquired at an ionization energy of 70 eV and metabolites were detected by mass selective detector. The detector operated in a mass range between m/z 30 and 300 with a scan rate of 2.7 scans/s. Each replicate was analyzed in triplicate in a randomized sequence where blanks, related to analyses of coating fibres not submitted to any extraction procedure, were run.

Volatile metabolites identification was based on mass spectra matching with the available database library (NIST, version 2005; Wiley, version 2007) and on the comparison of their retention times with an in-house developed retention time library based on reference commercial standards. Identification of volatile compounds was also accomplished by matching their retention indices (RI) (as Kovats indices), determined relative to the retention time of a C_8–C_{40} n-alkanes series with linear interpolation, with those reported in literature for similar chromatographic columns (Kovats, 1958).

Semi-quantitative data of each metabolite (Relative Peak Area, RPA%) were calculated in relation to the peak area of 4-methyl-2-pentanol, used as IS. Areas of the identified volatiles were measured from the total ion current (TIC).

2.6. Statistical data analysis

A multifactor Anova for $P \leq 0.05$ was performed to evaluate the effect of MA treatments (Low-O_2, High-CO_2, Mix and Air), storage time (7, 14, and 21 days) and their interaction on VOCs profiles quality parameters and sensory attributes. Sensory analysis data were subjected to one-way Anova in order to highlight significant differences ($P \leq 0.05$) among stored sweet cherries at 7, 14 and 21 days respect to fresh samples. Mean values (n = 3) for each parameter were separated using Least Significant Difference (LSD) test ($P \leq 0.05$). Moreover, correlation analysis between VOCs and sensory attributes was achieved using the software Statistica (version 6.0, StatSoft, Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Effect of modified atmosphere treatments on quality parameters of sweet cherry cv "Ferrovia" during cold storage

During storage, MA composition in sweet cherry bags changed due to product respiration and gas permeation through packaging material (Fig. 1A). In particular, in High-CO_2 bags the concentration of O_2 (initially 16%) gradually decreased, reaching the mean value of about 1% at the end of the conservation period. In Low-O_2 and Mix samples, initial O_2 concentration (1%) remained almost constant during the entire storage time. The amount of CO_2, on the other hand, increased during conservation, reaching the final concentration of 25.7% (±2.5), 45.3% (±2.10) and 42.4% (±0.87) in Low-O_2, High-CO_2 and Mix packages, respectively (Fig. 1A).

Table S1 reports that respiration activity was affected by MA treatments (A), storage time (B) and by the interaction of both factors (AxB). RWC of peduncle and hue angle were, instead, influenced only by MA treatments and storage time, separately (Table S1), but not by their interaction.

Sweet cherries showed an initial respiration activity of 8.2 ± 0.3 mL CO_2 kg^{-1} h^{-1} which did not change in Air samples, during storage (Fig. 1B). In MA fruit respiration activity picked at the 14^{th} day, reaching mean values with the following order High-CO_2 (81.1 ± 0.7 mL CO_2 kg^{-1} h^{-1}), Mix (64.6 ± 1.2 mL CO_2 kg^{-1} h^{-1}) and Low-O_2 (48.51 ± 2.5 mL CO_2 kg^{-1} h^{-1}). This behaviour might indicate a stress induced by high CO_2 concentration, as previously observed on table grape (Cefola, Damascelli, Lippolis, Cervellieri, Linsalata, Logrieco, & Pace, 2017). Although sweet cherries usually present a good tolerance to high CO_2 (Kader, Zagory, & Kerbel, 1989; Esturk, Ayhan & Ustunel, 2012), our quality data suggest that cv "Ferrovia" is sensitive to CO_2 accumulation. This physiological susceptibility, that seems to be also confirmed by VOCs profiles analysis described below, is probably cultivar-dependent, as generally detected for vegetables and fruit (Watkins, 2000).

RWC of peduncle was significantly higher in Low-O_2 than in Mix and High-CO_2 fruit, while Air samples showed the lowest mean value through the entire storage (Table S1). This result might denote a dehydration process in control samples, according to data reported by Cefola et al. (2017) on table grape rachis.

3.2. *Effect of modified atmosphere treatments on VOCs profile of sweet cherry cv "Ferrovia" during cold storage*

A total of 48 volatile compounds were identified by SPME GC-MS analysis of sweet cherry samples cv "Ferrovia". Metabolites can be grouped into seven distinct chemical classes: esters (11), alcohols (14), aldehydes (10), ketones (6), terpenes (4), and others (3), as reported in Table 2, which also shows VOCs abbreviation code, the experimental and literature reported Kovats index and the identification methods. Most of the volatiles listed in Table 2 have been already reported in fresh sweet cherries (Serradilla, Martín, Ruiz-Moyano, Hernández, López-Corrales, & Córdoba 2012; Vavoura et al., 2015). Compared to Vavoura et al. (2015), who also studied "Ferrovia" sweet cherry, it was possible to detect a larger number of VOCs, presumably owing to the different pedo-climatic environment and the diverse extraction method used. For the same reasons, perhaps, in contrast to previous data (Vavoura et al. 2015), predominant VOCs of fresh sweet cherries cv "Ferrovia", analyzed in the present study, were cis 2-hexen-ol (48.36%) followed by 2-hexenal (24.68%), 1-hexanol (11.17%) and hexanal (7.56%). These C6-aldehydes and alcohols have been identified among the principal odorants influencing flavour in sweet cherry (Vavoura et al., 2015).

Two-way Anova analysis (Table S2) demonstrated that variations in VOCs profile of esters, ketones, aldehydes and alcohols were significantly affected by the interaction (AxB) of the two factors (MA treatment, A and storage time, B), as illustrated in figure 2.

Regarding ester compounds, ethyl esters (E1, E2, E3, E6 and E8), never observed in Fresh and Air samples, increased their levels sharply at 14 days of storage in Low-O_2 and Mix. These volatiles were also detected at the end of the storage period in High-CO_2 fruit (Fig. 2A). The trend shown by ethyl esters, ethyl acetate (E1) being the most abundant, can be attributed to low-oxygen-induced conditions which favor the activation of fermentative metabolism, as previously reported for other fruit (Mattheis & Fellman, 2000). In addition, Mattheis & Fellman (2000) highlighted that ethyl esters in sweet cherries under air condition are present in trace or undetectable amounts. Even though in High-CO_2 bags the level of CO_2 reached the value of 30% at the 7th day of storage, products of fermentative metabolism have been only detected at 21 days, probably because the initial high O_2 percentage (16%) in the gas composition contributed to slow metabolism, avoiding anaerobic stress induction. On the contrary, in Low-O_2 and Mix packages a gas composition of about 1% O_2 and 20-30% CO_2 was measured at 14 days (Fig. 1A), allowing the detection of compounds due to anaerobic metabolism already at the 14th day.

It is to underline that, ethyl esters accumulation in fruit kept in anaerobic environments can not only modify aroma, but can also reduce the synthesis of other esters, generally produced during fruit ripening (Mattheis & Fellman, 2000). This is in agreement with the trend showed by all the other esters (E4, E5, E7, E10 and E11) statistically significant for the interaction (AxB) which, detected in Fresh and Air sweet cherries, reduced their levels in all MA treated fruit during the conservation period (Table S2, Fig. 2A).

All the six ketones (K1-K6) identified in SPME GC-MS analysis of "Ferrovia" sweet cherries were significantly influenced by the interaction (AxB) (Table S2, Fig. 2B). Similarly to ethyl esters, the detection of γ-butyrolactone (K5) at 14 days of storage in Low-O_2 and Mix and at 21 days in High-CO_2 samples (Fig. 2B) should be related to hypoxic conditions which induce anaerobic metabolism, as already reported in grapes (Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2013).

The compound 6-methyl-5-hepten-2-one (K4), an apocarotenoid volatile producing a fruit-like aroma, was present from the 14th day in Mix and only at 14th day in Low-O_2 (Fig. 2B). Biosynthesis of K4 has been reported to be favored by cold storage, proposing that low temperature preservation may specifically influence the activity of some enzymes responsible for the conversion of carotenoid precursors to this compound (Farneti et al., 2015; Cozzolino, 2016b). In view of that, findings of the present study suggest that enzymes involved in the production of K4 could be positively influenced not only by low temperature, but also by hypoxic conditions (Table S2, Fig. 2B).

Among aldehydes significantly affected by the interaction (AxB), 3-methyl butanal (Ald1), an important flavour compound in many food products, was present only from the 7th to the 14th day in High-CO_2 fruit (Fig. 2C). This metabolite derives from the catabolism of leucine, an amino acid normally released by cellular proteolysis (Smit, Engels & Smit, 2009). In has been suggested that the first step of Ald1 biosynthesis is catalyzed by branched-chain aminotransferases (BCATs), of which several isomers, identified in fruit and vegetables (Wang, Baldwin, Plotto, Luo, Raithore, Yu, & Bai, 2015), seem to be positively correlated to the production of branched-chain volatiles (Yang, Song, Fillmore, Pang & Zhang, 2011). In our study, the formation of Ald1 in High-CO_2 fruit could be due to the activation, at this storage condition, of specific BCAT isomers involved in the biosynthesis of this aldehyde.

Nonanal (Ald 6) and decanal (Ald 7), arisen from oleic acid hydroperoxide decomposition, were present in Fresh and in Air samples throughout the whole storage period (Fig. 2C). In addition, they have been observed in all MA treated sweet cherries during conservation (Fig. 2C). These results are similar to those reported by Argenta, Mattheis, Fan and Finger (2004), who have illustrated that the amount of volatile C8-C10 aldehydes decreased in Fuji apples held in high CO_2, respect to fresh fruit, depending on the time of storage. In this context, time of exposure, rather than atmosphere composition, could be crucial for C8-C10 aldehydes production (Argenta et al, 2004).

Table S2 shows that, among the identified alcohols, six of them resulted significantly influenced by the interaction between MA treatments and storage time (AxB). Accumulating evidence has shown that C5, C6 and C9 volatiles in vegetables can be formed by LOX pathway but, while the production of C6 and C9 odorants has been clarified, the synthesis of C5 compounds has not yet been fully explained (Contreras, Schwab, Mayershofer, González-Agüero & Defilippi, 2017). Nevertheless, as suggested by previous investigations, the production of C5 VOCs seems to be favored by O_2 reduction and CO_2 accumulation (Contreras et al., 2017; Mastrandrea, Amodio, Pati & Colelli, 2017). According to that, figure 2D reports that 1-pentanol (Al3), never found in Fresh and Air samples, was observed from the 7th day in Mix and High-CO_2 and from the 14th day also in Low-O_2 (Fig. 2B).

The synthesis of C9 volatiles, generally present in small amounts, occurs during the early stages of development, and reduces throughout fruit ripening (Contreras et al., 2017). In agreement with literature data, the trend of nonanol (Al12), registered in our experiments (Fig. 2D), showed that this volatile was detected in fresh cherries, but at the end of the storage it was only present in Low-O_2 and High-CO_2 samples.

3.3. *Effect of modified atmosphere treatments on sensory attributes of sweet cherry cv "Ferrovia" during cold storage*

Two-way Anova analysis carried out on sensory attributes of sweet cherry cv "Ferrovia" show that *Amaranth colour* was affected only by MA treatments (Table S3). *Brightness*, *Extraction steam*, *Olfactive intensity*, *Bitter* and *Aromas persistence* were influenced by storage, while *Cherry smell* was affected by MA treatments and storage time (Table S3). The remaining sensory attributes (*Color uniformity*, *Stains, Turgidity, Pulp colour, Herbaceous smell, Juiciness, Sweet, Acid, Herbaceous aroma, Cherry aroma, PAI, NAI* and *Sweet persistence*) were influenced by the interaction (AxB) of the two factors (storage atmosphere, A and storage time, B), as illustrated in Table S3. In order to compare sweet cherry samples preserved in MA and Air respect to Fresh, one-way analysis of variance and LSD procedure were performed. *Colour uniformity* showed that mean values took on a normal U-shape, since intensity at 14 days of storage was significantly lower compared to those at 7 and 21 days (Fig. 3A). However, at the end of the storage period, one-way Anova analysis did not show significant differences between each MA fruit respect to Fresh and Air sweet cherries.

Stains intensity showed, compared to Fresh, a gradual and significant increase during storage in all MA treatments ($F = 12.10$, $p < 0.0001$) which presented at 21 days a better appearance respect to Air sample (Fig. 3B). Indeed, fruit visual inspection, by panel leader, highlighted that some Air sweet cherries reported evident growth of mould from the 14^{th} day of storage.

The intensity of *Turgidity* decreased during storage in sweet cherries kept in Air compared to MA treatments (Fig. 3C). After 7 days of conservation, Air samples showed significant differences only respect to High-CO_2 treatment, while at 21 days Air fruit presented the lowest intensity value compared to Fresh and MA treatments ($F = 3.97$, $p < 0.001$) (Fig. 3C).

Pulp colour attribute significantly increased in all stored samples respect to Fresh, except for Mix fruit ($F = 26.43$, $p < 0.0001$). In particular, High-CO_2 sweet cherries showed at the end of preservation the highest value respect the other MA treatments (Fig. 3D).

Herbaceous smell significantly decreased during storage in Air and all MA treatments compared to fresh fruit ($F = 5.92$, $p < 0.0001$). At 21 days, the intensity of this sensory trait kept higher in Low-O_2 and High-CO_2 respect to Air, with a significant mean value only for High-CO_2 (Fig. 3E).

Concerning *Juiciness*, regardless the highest value scored for High-CO_2 samples at 7 days, only slight changes were perceived during storage in all other treatments (Fig. 3F). At the end of the preservation period, only Low-O_2 presented a significant high score compared to Fresh and Air ($F = 3.06$, $p < 0.001$).

One-way statistical analysis on Fresh and stored samples of *Sweet* attribute intensity showed significant differences during preservation ($F = 7.90$, $p < 0.0001$). Specifically, in the first week of storage the intensity of this attribute significantly increased in the Air and High-CO_2 respect to Fresh (Fig. 3G). At 14 days of conservation, values remained high in Air, while for High-CO_2 sweet cherries intensity reduced to the level of fresh sample, maintaining the same score till 21 days (Fig. 3G). In the third week, *Sweet* attribute intensity of the Mix fruit reduced, while the intensity of Low-O_2 sample significantly increased compared to all the other samples (Fig. 3G).

Acid attribute statistically declined in Air and High-CO_2 samples compared to Fresh during the entire storage period ($F = 7.34$, $p < 0.0001$). In the second week of conservation, *Acid* intensity significantly increased only in Low-O_2 bags (Fig. 3H), while at 21 days Mix sweet cherries showed significant higher intensity respect to Air. A large body of evidence has confirmed that sweet and acid are linked (Meheriuk et al., 1995). In our experiment the intensity of these two traits in all treatments presented a mirrorlike behavior, in fact when values of *Sweet* intensity increased those of *Acid* decreased and vice versa (Fig. 3G-3H).

Regarding *Herbaceous aroma*, the intensity generally reduced during the storage (Table 3S). Sweet cherries stored in Low-O_2 after 7 days and in Mix after 14 days showed mean values of intensity similar to Fresh. At 21 days of storage, significant intensity significant decrease in Air and Mix samples was observed ($F = 7.32$, $p < 0.0001$) (Fig. 3I). These findings suggest that High-CO_2 and Low-O_2 preserve better this sensory trait until the end of the storage (Fig. 3I).

The intensity of *Cherry aroma*, a very important sensory parameter that contributes to determine the typical flavor and overall acceptability of sweet cherry, presented, at the end of the first week of conservation, a significant increase in Air, High-CO_2 and Low-O_2 fruit ($F = 10.53$, $p < 0.0001$) (Fig. 3J). At 14 days, the intensity of *Cherry aroma* was significantly high only in Air and High-CO_2 samples respect to Fresh (Fig. 3J). At 21 days, *Cherry aroma* intensity maintained significant high values respect to Fresh only for High-CO_2, while Mix treatment induced a significant reduction (Fig. 3J).

Mean values of intensity of *PAI* and *Cherry aroma* attributes followed a similar trend (Fig. 3J and 3K, respectively). Mean values of *PAI* score significantly declined during storage (Fig. 3K), with the significant lowest intensities observed for Low-O_2 at 14 and for Mix at 21 days of storage, while the highest scores were detected at 7 and 14 days of storage for High-CO_2, and at 21 days for Low-O_2 sample ($F = 13.20$, $p < 0.0001$).

One-way Anova analysis on *NAI* attribute intensity showed significant differences during preservation ($F = 34.41$, $p < 0.0001$). *NAI* intensity displayed the highest value at 21 days of storage in Mix, suggesting that, in this MA condition, off-flavours could develop (Fig. 3L).

Finally, *Sweet persistence* sensory trait revealed mean values of intensity similar to *Sweet* (Fig. 3M). At 7 days High-CO_2 sample showed significantly higher values than Fresh, while at 14 and 21 days Low-O_2 and Mix fruit presented, respectively, intensity mean values significantly lower than Fresh ($F = 14.07$, $p < 0.0001$).

According to these findings, among all MA treatments, High-CO_2 appears to preserve better than Low-O2 and Mix packaging most of the sensory parameters of sweet cherry cv "Ferrovia" throughout storage, probably thanks to the higher concentration, respect to the other MA treatments, of O_2 (16%) in the initial gas composition.

3.4. Correlation between sensory attributes and VOCs of sweet cherries cv "Ferrovia" during cold storage in MA or Air

Statistical analysis on sensory profiles of sweet cherry cv "Ferrovia" over storage period allowed to detect significant modifications of the intensity of *Herbaceous Smell*, *Cherry Smell*, *Herbaceous Aroma*, *Cherry Aroma*, *PAI*, *NAI* and *Aroma Persistence*. In order to correlate these data with the volatile metabolites identified by SPME GC-MS, a correlation analysis was accomplished and results are illustrated in Table 3.

Specifically, cis 2-hexen-1-ol (Al10), nonanol (Al12), hexanal (Ald2) and 2-hexanal (Ald3), all characterized by green and grassy odour notes, were positively correlated with the sensory attributes associated to freshness, *Herbaceous smell* and *Herbaceous Aroma*. Al10, Ald2 and Ald3, among the most abundant VOCs in fresh sweet cherry, are C6-aldehydes and C6-alcohols biosynthesized in green leaves from α-linolenic and linoleic acids via their respective hydroperoxides (Hatanaka, 1996). Consequently, these odorants could be considered putative markers of "Ferrovia" fresh sweet cherry. This result was corroborated by the fact that Ald2 and Ald3 resulted also directly related to *PAI* sensory trait and, together with Al10, negatively associated with *NAI* attribute (Table 3). 1-Penten-3-ol (Al1) and 1-hexanol (Al7) displayed a similar trend, as being positively associated to *PAI* and negatively to *NAI*. Al1 was also directly correlated to *Herbaceous Smell*, while Al7 was positively associated to *Cherry Aroma* (Table 3).

NAI sensory trait resulted directly associated to 1-pentanol (Al3), described as pungent, fermented and solvent-like flavour. This volatile, in fact, is negatively related to *PAI* along with 6-methyl-5-hepten-2-one (K4) and γ-butyrolactone (K5). In particular, K4 is also negatively correlated to *Aroma Persistence*. In this context, scientific evidence has shown that 6-methyl-5-hepten-2-one increases in low-temperature fruit storage (Farneti et al., 2015; Cozzolino et al., 2016b), causing scald-like symptoms development in peel tissue of susceptible fruit kept at 0-5 °C. This superficial disorder, induced by oxidative stress, intensifies with the duration of storage (Whitaker & Saftner, 2000).

Beside the over mentioned volatiles, *Herbaceous Smell* was positively associated to 1-penten-3-one (K3), ocymene, (T2) and 2-methyl-furan (O1), which were all negatively correlated to *Cherry Smell*. Moreover, *Herbaceous Smell* was inversely related with two isopentenols, 3-methyl-3-buten-1-ol (Al4) and 3-methyl-2-buten-1-ol (Al6), both associated with fruity flavour. These fusel alcohols, produced via the mevalonate pathway, are by-products of alcoholic fermentation (Chung, Lee, Seo, & Kim, 2017; Vyviurska, Matura, Furdìkovà, & Spanik, 2017).

Finally, *Cherry Smell* was directly related to 1-octanol (Al11) and *Cherry aroma* resulted negatively associated to linalool (T3), characterized by a citrus-like odor. T3 is synthesized in a single step reaction by linalool synthase, using geranyl diphosphate as a precursor. Linalool synthesis has been suggested to be finely controlled at the level of enzyme expression, since geranyl diphosphate is a central intermediate of many pathways (Gomes, Fabi, & Purgatto, 2016). In particular, some studies have reported that the timing of ripening-related conditions and potential depletion of some constituents of linalool synthesis can elucidate possible change of linalool amount in stored fruit, even if volatile regulation production during postharvest handling has been not yet completely clarified (Gomes et al., 2016).

4. Conclusion

VOCs profile, quality and sensory parameters were assessed on sweet cherry cv "Ferrovia" stored in AIR and different MA treatments for 21 days at 5 °C. All samples analyzed showed significant modifications during the conservation on respiration activity, VOCs pattern and sensory traits of "Ferrovia" sweet cherry, demonstrating that this cultivar is sensible to high CO_2 treatments. In particular, when CO_2 concentration accumulated in packaged fruit over 20% and O_2 reached values around 1% (as in Low-O_2 and Mix bags at 14 days) a fermentative metabolism occurred, with the consequent increase of ethyl esters and γ-butyrolactone amount. Indeed from a sensory point of view, Mix sweet cherries were negatively perceived by panelist for the development of off-flavours.

On the other hand, High-CO_2 treatment appears to preserve quality and sensory traits, probably thanks to the higher concentration of O_2 (16%) in the initial gas composition, that may prevent the accumulation of ethyl esters and γ-butyrolactone, avoiding the development of off-flavours, actually not perceived by the sensory panel.

Therefore, ethyl esters and γ-butyrolactone might be considered possible markers of sensory alterations related to fermentation. For γ-butyrolactone this result was also confirmed by the correlation analysis between VOCs profiles and sensory traits which has highlighted that this volatile was negatively related to *PAI*. Furthermore, the same analysis has demonstrated that C6-aldehydes and C6-alcohols, being positively correlated to *Herbaceous smell* and *Herbaceous Aroma*, can be assumed putative markers of freshness.

In conclusion, considering the sensibility of sweet cherry cv "Ferrovia" to high CO_2 in hypoxic condition, further investigations are needed in order to establish specific CO_2 and O_2 percentages able to induce fermentative metabolism.

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Supplementary Material, S1

1. Sensory panel training

During the training period, judges tasted 8 samples of cherries purchased from different markets, with the aim to select specific traits and to develop a vocabulary of the sensory quality of cherries (Table 1). Twenty-two attributes were selected and included in the score sheet for the quantitative evaluation, using an intensity scale from 0 to 10. Regarding attributes *Amaranth colour*, *Colour uniformity*, *Brightness* and *Pulp colour*, the colours were printed on paper (Corollaro et al., 2013) and the extremes of the scale were anchored with bipolar words. For others attributes we used a unipolar intensity scale with words (none, strong), as well as bipolar words (soft, hard or small, great). Concerning the evaluation of judges' performance, sweet, acid and bitter solutions references were tasted and the subjects bias and variability were evaluated. Moreover, performances of each subject and of entire panel on the selected attributes were evaluated by the mean and the standard deviation of the data, obtained from tasting 4 sweet cherry samples (2 kept in the refrigerator for two weeks and 2 new fresh samples), 3 times in different sessions.

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426 **Table 1**. Sensory attributes of sweet cherry cv “Ferrovia” reported on the evaluation sheet, their definitions and references.

Sensory Attributes	Definition	References
Amaranth colour	Measuring amaranth colour intensity of cherry peel from light to dark.	Light (R229, G0, B28); Dark (R89, G0, B11)
Colour uniformity	Evaluating homogeneity of colour distribution on samples peel	Non-uniform; Uniform
Brightness	Evaluating intensity of the light reflected from peel surface	Dull; Bright
Stains	Evaluating numerosity of dark spots on the peel.	No stain (0); 10 dark spots per peace (10).
Turgidity	Measuring feeling of fullness of the cherry tightened between thumb and forefinger.	Soft; Hard
Extraction stem	Measuring the force to applied to pull away the stem from the cherry	Small; Great
Pulp colour	Measuring intensity of the amaranth colour from very light to moderate.	Very light (R255, G153, B165); Moderate (R178, G25, B43).
Olfactive intensity	Measuring whole volume of positive and negative odours perceived by the nose.	None (0); Strong (10)
Herbaceous smell	Measuring intensity of typical odour of freshly cut green grass.	10% alcohol solution (0); 100 ppm cis-3-Hexen-1-ol in 10% alcohol solution (10)
Cherry smell	Evaluating intensity of typical odour of ripe fruit.	10% alcohol solution (0); 100ppm γ-decalactone in 10% alcohol solution (10)
Juiciness	Measuring the amount of liquid released during chewing.	Banana (0); cucumber (6); watermelon (10)
Pulp texture	Evaluating the degree of the resistance of the pulp structure during chewing.	Soft; Hard
Sweet	Measuring intensity of the specific sensation of sugar.	4%, 8% and 15% sucrose solutions, intensity scale values 2, 5, and 10, respectively
Acid	Measuring intensity of the specific sensation caused by acidic substances	0.05% and 0.16% tartaric acid solutions, intensity scale values 2 and 8, respectively
Bitter	Measuring intensity of the bitterness caused by specific substances.	0.06%, 0.10% and 0.18% caffeine solutions, intensity scale values 2, 5, and 10, respectively
Herbaceous aroma	Measuring typical odour of freshly cut green grass retro-nasally perceived.	See *Herbaceous smell* attribute
Cherry aroma	Evaluating intensity of typical odour of fruit retro-nasally perceived.	See *Cherry smell* attribute
PAI	Measuring intensity of positive aromas retro-nasally perceived.	None (0); Strong (10)

NAI	Measuring intensity of negative aromas retro-nasally perceived.	None (0); Strong (10)
Aromas persistence	Measuring intensity of aromas, retro-nasally perceived, 1 minute from swallowing.	None (0); Strong (10)
Sweet persistence	Measuring intensity of the specific sensation of sugar, 1 minute from swallowing.	See *Sweet* attribute
Bitter after-taste	Measuring intensity of the bitterness perceived 1 minute after swallowing.	See *Bitter* attribute

Table 2. Volatile organic compounds (VOCs) detected in sweet cherry cv "Ferrovia" and their identification codes.

Metabolite	Code	[a]Ri_t/RI_{sp}	[b]ID	Metabolite	Code	[a]Ri_t/RI_{sp}	[b]ID
Esters				**Aldehydes**			
Ethyl acetate	E1	869/870	RI/MS/S	Butanal 3-methyl	Ald1	899/899	RI/MS/S
Ethyl 2-butenoate	E2	1183/1180	RI/MS	Hexanal	Ald2	1084/1086	RI/MS/S
Ethyl hexanoate	E3	1252/1251	RI/MS/S	2-Hexenal	Ald3	1242/1248	RI/MS/S
Hexyl acetate	E4	1289/1289	RI/MS/S	Octanal	Ald4	1309/1308	RI/MS/S
2-Hexen-1-ol acetate	E5	1346/1342	RI/MS/S	2-Heptenal	Ald5	1343/1342	RI/MS/S
Ethyl caprylate	E6	1440/1440	RI/MS/S	Nonanal	Ald6	1401/1401	RI/MS/S
2-Hexenyl butyrate	E7	1479/1475	RI/MS/S	Decanal	Ald7	1506/1505	RI/MS/S
Ethyl benzoate	E8	1671/1670	RI/MS/S	Benzaldehyde	Ald8	1530/1532	RI/MS/S
trans 2-Hexenyl hexenoate	E9	1676/1669	RI/MS/S	Dodecanal	Ald9	1716/1713	RI/MS/S
2-Hexenyl tiglate	E10	1694/1672	RI/MS	Tetradecanal	Ald10	1935/1935	RI/MS/S
Isopropyl laurate	E11	1840/1845	RI/MS/S	**Ketones**			
Alcohols				3-Pentanone	K1	980/980	RI/MS/S
1-Penten-3-ol	Al1	1188/1189	RI/MS/S	2-Pentanone-4-methyl	K2	1011/1012	RI/MS/S
3-Hexanol	Al2	1201/1203	RI/MS/S	1-Penten-3-one	K3	1026/1026	RI/MS/S
1-Pentanol	Al3	1223/1222	RI/MS/S	6-Methyl-5-hepten-2-one	K4	1350/1348	RI/MS/S
3-Methyl-3-buten-1-ol	Al4	1235/1236	RI/MS/S	γ-Butyrolactone	K5	1631/1632	RI/MS/S
cis 2-Penten-1-ol	Al5	1272/1272	RI/MS/S	2-Dodecanone	K6	1712/1709	RI/MS/S
3-Methyl-2-buten-1-ol	Al6	1336/1334	RI/MS/S	**Terpenes**			
1-Hexanol	Al7	1340/1339	RI/MS/S	dl-Limonene	T1	1214/1215	RI/MS/S
trans 3-Hexen-1-ol	Al8	1367/1366	RI/MS/S	Ocymene	T2	1269/1259	RI/MS/S
cis 3-Hexen-1-ol	Al9	1374/1374	RI/MS/S	Linalool	T3	1549/1549	RI/MS/S
cis 2-Hexen-1-ol	Al10	1394/1394	RI/MS/S	α-Terpineol	T4	1703/1702	RI/MS/S
1-Octanol	Al11	1498/1499	RI/MS/S	**Others**			
Nonanol	Al12	1565/1565	RI/MS/S	2-Methyl furan	O1	855/856	RI/MS/S
Benzene methanol	Al13	1881/1882/	RI/MS/S	Formammide N,N-dibutyl	O2	1779/1773	RI/MS/S
Dodecanol	Al14	1882/1881	RI/MS/S	Benzothiazole	O3	1964/1969	RI/MS/S

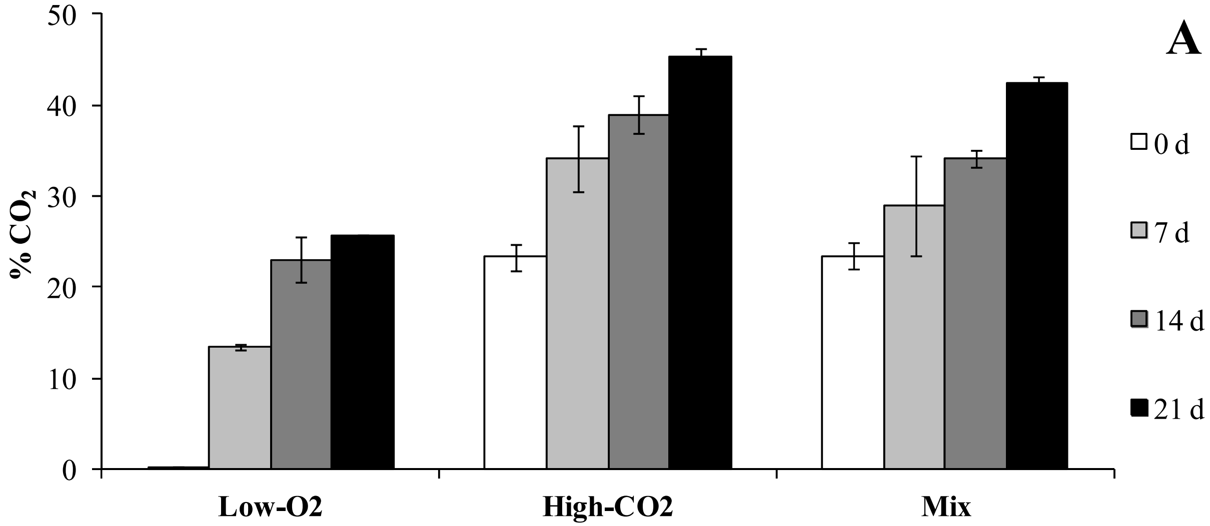
[a]RI_t: Relative retention indices on polar column reported in literature by www.pherobase.com; www. flavornet.org; www.ChemSpider.com; webbook.nist.gov; RI_{sp}: Relative retention indices calculated against n-alkanes (C_8-C_{40}) on HP-Innowax column; [b]Identification method as indicated by the following: RI: Kovats retention index on a on HP-Innowax column; MS: NIST and Wiley libraries spectra; S: co-injection with authentic standard compounds on the HP-Innowax column

Table 3. Pearson correlation matrix among VOCs and sensory attributes detected in sweet cherry cv "Ferrovia".

VOCs	Code	*Herbaceous smell*	*Positive Aroma Intensity (PAI)*	*Negative Aroma Intensity (NAI)*	*Cherry smell*	*Herbaceous aroma*	*Cherry aroma*	*Aroma persistence*
trans 2-Hexenyl hexenoate	E9	*0.58* *	-	-	-	-	-	-
Isopropyl laurate	E11	-	-	-	*-0.75* **	-	-	-
1-Penten-3-ol	Al1	*0.59* ***	*0.61* *	*-0.59* *	-	-	-	-
1-Pentanol	Al3	-	*-0.64* *	*0.59* *	-	-	-	-
3-Methyl-3-buten-1-ol	Al4	*-0.60* *	-	-	-	-	-	-
cis 2-Penten-1-ol	Al5	*0.82* ***	-	-	-	-	-	-
3-Methyl-2-buten-1-ol	Al6	*-0.56* *	-	-	-	-	-	-
1-Hexanol	Al7	-	*0.62* ***	*-0.69* **	-	-	*0.60* *	-
cis 2-Hexen-1-ol	Al10	*0.57* *	-	*-0.57* ***	-	*0.60* *	-	-
1-Octanol	Al11	-	-	-	*0.69* *	-	-	-
Nonanol	Al12	*0.67* *	-	-	-	*0.59* *	-	-
Hexanal	Ald2	*0.81* ***	*0.65* ***	*-0.60* ***	-	*0.62* *	-	-
2-Hexenal	Ald3	*0.83* ***	*0.57* ***	*-0.61* *	-	*0.78* ***	-	-
3-Pentanone	K1	*0.64*	-	-	-	-	-	-

		***						
1-Penten-3-one	K3	*0.79* ***	-	-	*-0.59* *	-	-	-
6-Methyl-5-hepten-2-one	K4	-	*-0.71* ***	-	-	-	-	*-0.66* *
γ-Butyrolactone	K5	-	*-0.55* *	-	-	-	-	-
Ocymene	T2	*0.76* ***	-	-	*-0.61* *	-	-	-
Linalool	T3	-	-	-	-	-	*-0.58* *	-
2-Methyl furan	O1	*0.72* **	-	-	*-0.60* *	-	-	-

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.



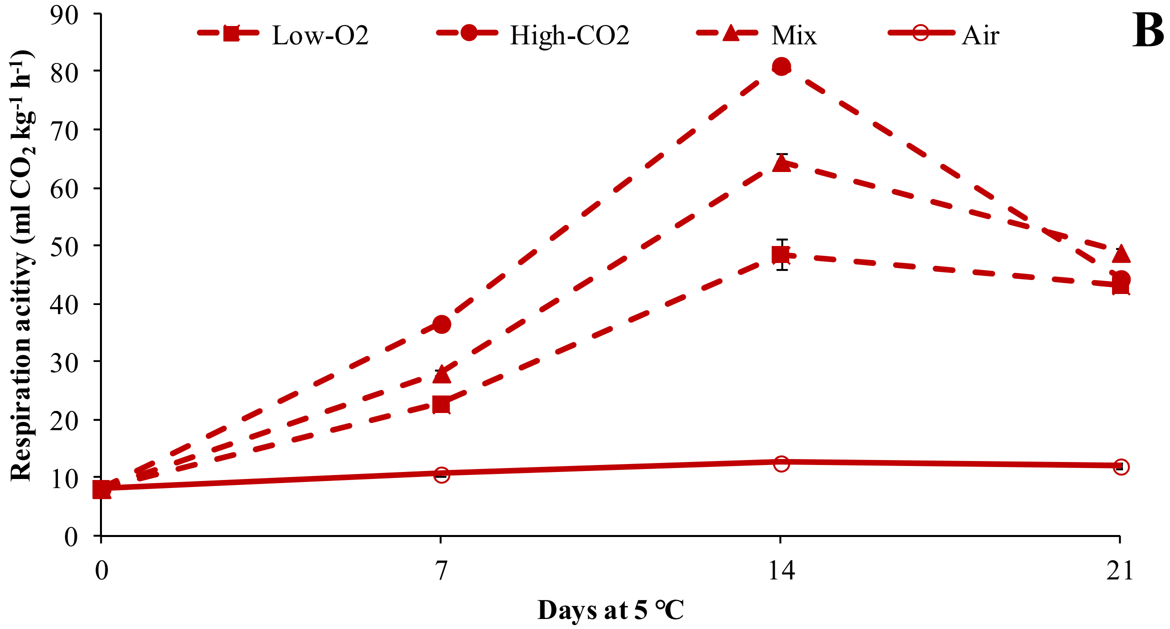


Figure 1. CO_2 concentration (A) and respiration activity (B) of sweet cherry cv "Ferrovia" cold stored in different MA treatments (Low-O_2 = 1% O_2 + 0.03% CO_2 + 99% N_2; High-CO_2 =16 % O_2 + 20% CO_2 + 64% N_2; Mix = 1% O_2 + 20% CO_2 + 79% N_2; Air = Control). Data represent mean value ± standard deviation.

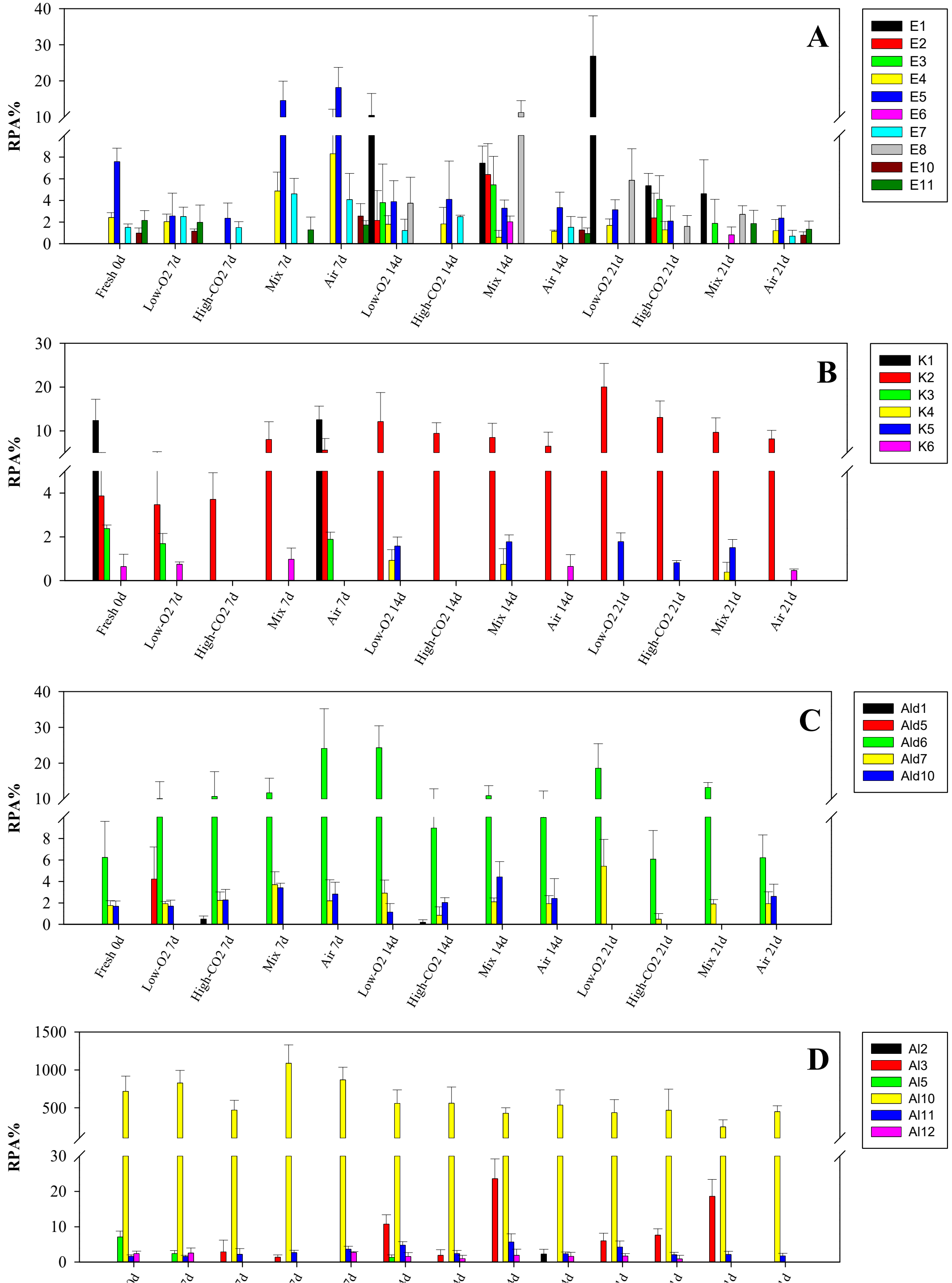

A
B
C
D
RPA%
E1
E2
E3
E4
E5
E6
E7
E8
E10
E11
K1
K2
K3
K4
K5
K6
Ald1
Ald5
Ald6
Ald7
Ald10
Al2
Al3
Al5
Al10
Al11
Al12
Fresh 0d
Low-O2 7d
High-CO2 7d
Mix 7d
Air 7d
Low-O2 14d
High-CO2 14d
Mix 14d
Air 14d
Low-O2 21d
High-CO2 21d
Mix 21d
Air 21d

Figure 2. Changes in esters (A), ketones (B), aldehydes (C) and alcohols (D) of sweet cherry cv "Ferrovia" cold stored in different MA treatments (Low-O_2 = 1% O_2 + 0.03% CO_2 + 99% N_2; High-CO_2 =16 % O_2 + 20% CO_2 + 64% N_2; Mix = 1% O_2 + 20% CO_2 + 79% N_2; Air = Control). Data represent mean value of Relative Peak Area (RPA%) ± standard deviation. VOCs codes are reported in Table 2.

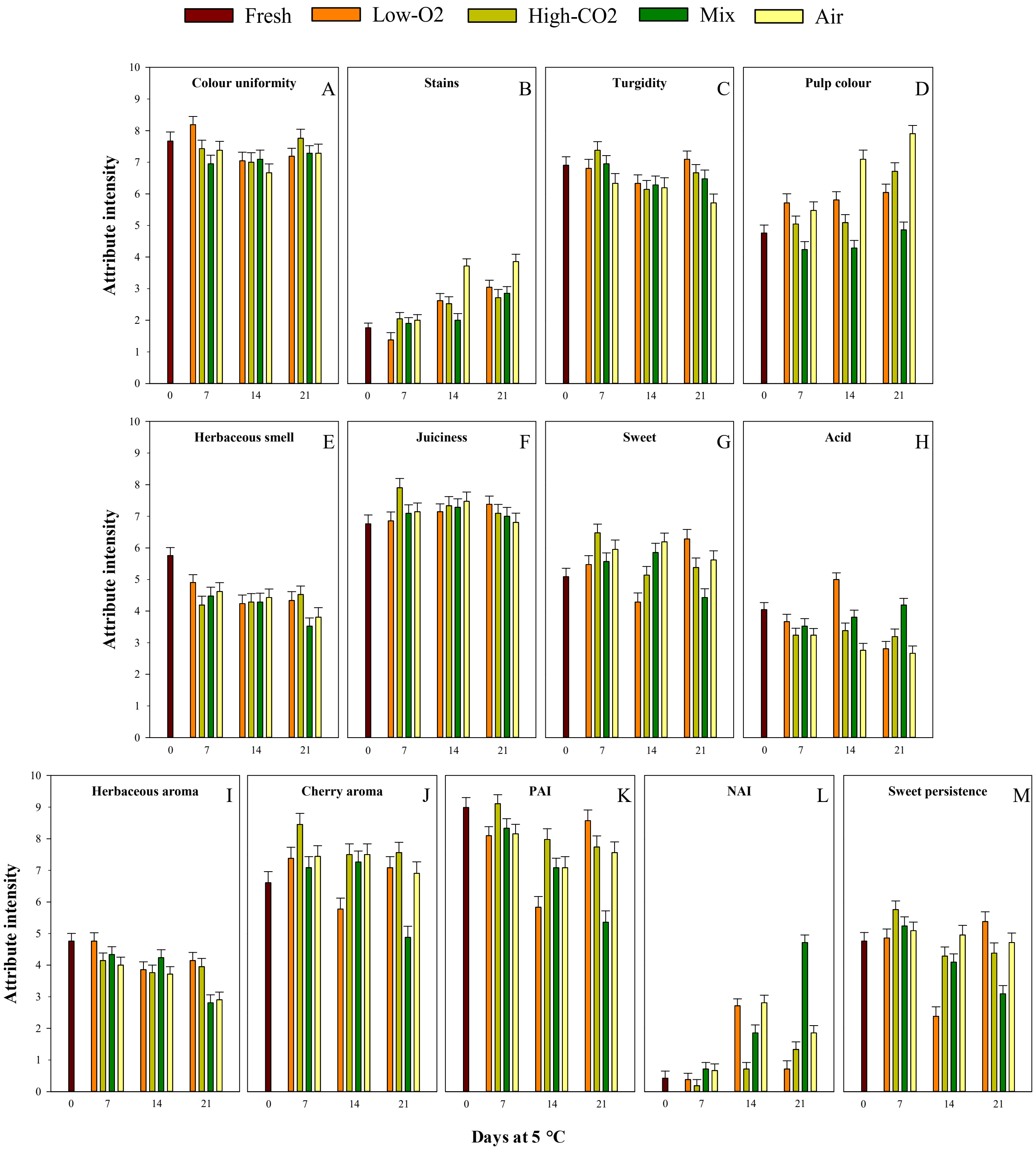


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Figure 3. Changes of sensory attributes of sweet cherry cv

“Ferrovia” cold stored in different MA treatments (Low-O_2 = 1% O_2 + 0.03% CO_2 + 99% N_2; High-CO_2 =16 % O_2 + 20% CO_2 + 64% N_2; Mix = 1% O_2 + 20% CO_2 + 79% N_2; Air = Control). For each attribute, data represent mean value (n=3) of intensity ± error standard.

Table S1. Effect of MA treatments (Low-O_2 = 1% O_2 + 0.03% CO_2; High-CO_2 = 16% O_2 + 20% CO_2 + 64% N_2; Mix = 1% O2 + 20% CO_2 + 79% N_2; Air = Control), storage time (7, 14 and 21 day at 5 °C) and their interaction on quality parameters of sweet cherry cv "Ferrovia".

	Respiration activity (mL CO_2 kg^{-1} h^{-1})	**Hue angle**	**RWC of peduncle (%)**
MA treatments (A)			
Low-O_2	38.2	16.6	64.3 a
High-CO_2	54.1	15.6	56.9 bc
Mix	47.2	16.3	59.3 b
Air	11.8	15.7	52.6 c
Storage time (B)			
7	24.5	16.8 a	57.4
14	51.7	15.5 b	58.1
21	37.2	15.9 ab	59.3
A	****	ns	***
B	****	*	ns
A x B	****	ns	ns

For MA treatment, data are mean values of 9 samples (3 replicates x 3 storage time); for storage, data are mean values of 12 samples (3 replicates x 4 treatments).
Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; * $P \leq 0.05$; *** $P \leq 0.001$; **** $P \leq 0.0001$).
Different letters indicate statistical differences within storage conditions and storage time, according to LSD test ($P \leq 0.05$).

Table S2. Effect of MA treatments (Low-O_2 = 1% O_2 + 0.03% CO_2 + 99% N_2; High-CO_2 = 16% O_2 + 20% CO_2 + 64% N_2; Mix = 1% O_2 + 20% CO_2 + 79% N_2; Air = Control), storage time (7, 14 and 21 day at 5 °C) and their interaction on VOCs from sweet cherries (*Prunus avium* cv. Ferrovia) analyzed by SPME GC-MS.

VOCs	Code	MA Treatments (A)				Storage Time (B)			A	B	A x B
		Low-O_2	High-CO_2	Mix	Air	7	14	21			
Esters											
Ethyl acetate	E1	12.5 a	1.8 bc	4.0 b	0.0 c	0.0 c	4.5 b	9.2 a	****	****	****
Ethyl 2-butenoate	E2	0.7 b	0.8 b	2.1 a	0.0 b	0.0 b	2.1 a	0.6 b	*	**	***
Ethyl Hexanoate	E3	1.3 ab	1.4 ab	2.4 a	0.0 b	0.0 b	2.3 a	1.5 a	*	**	**
1-Hexyl acetate	E4	1.8 b	1.0 b	1.8 b	3.6 a	3.8 a	1.3 b	1.0 b	**	****	****
2-Hexen-1-ol acetate	E5	3.2 b	2.9 b	6.0 a	8.0 a	9.4 a	3.7 b	1.9 b	**	****	****
Ethyl caprylate	E6	0.0 b	0.0 b	1.0 a	0.0 b	0.0 b	0.5 a	0.2 b	****	***	****
2-Hexenyl butyrate	E7	1.2 -	1.3 -	1.5 -	2.1 -	3.2 a	1.3 b	0.2 c	ns	****	**
Ethyl benzoate	E8	3.2 b	0.5 c	4.7 a	0.0 c	0.0 b	3.8 a	2.5 a	****	****	****
trans 2-hexenyl hexenoate	E9	0.3 -	0.3 -	0.0 -	0.7 -	0.7 a	0.2 b	0.0 b	ns	**	ns
2-Hexenyl tiglate	E10	0.4 b	0.0 b	0.0 b	1.5 a	0.9 a	0.3 b	0.2 b	****	**	*
Isopropyl laurate	E11	0.7 ab	0.0 b	1.1 a	1.3 a	1.3 a	0.2 b	0.8 ab	**	**	*
Alcohols											
1-Penten-3-ol	Al1	4.2 -	2.6 -	3.3 -	5.1 -	7.1 a	2.4 b	2.0 b	ns	****	ns
3-Hexanol	Al2	0.0 b	0.0 b	0.0 b	0.8 a	0.0 b	0.6 a	0.0 b	***	**	****
1-Pentanol	Al3	5.6 b	4.2 b	14.5 a	0.0 c	1.1 b	9.1 a	8.1 a	****	****	****
3-Methyl-3-buten-1-ol	Al4	4.3 -	4.3 -	5.4 -	5.3 -	5.6 -	3.9 -	5.0 -	ns	ns	ns
cis 2-Penten-1-ol	Al5	1.3 a	0.0 b	0.0 b	0.0 b	0.6 a	0.3 a	0.0 b	****	***	****
3-Methyl-2-buten-1-ol	Al6	6.3 -	5.5 -	6.7 -	9.1 -	7.5 -	5.3 -	8.0 -	ns	ns	ns
1-Hexanol	Al7	108.9 -	166.9 -	137.9 -	159.3 -	195.9 a	131.8 b	102.0 b	ns	*	ns
trans 3-Hexen-1-ol	Al8	6.2 -	6.1 -	6.2 -	5.6 -	7.1 -	4.6 -	6.3 -	ns	ns	ns
cis 3-Hexen-1-ol	Al9	3.1 -	2.6 -	2.7 -	3.0 -	3.1 -	2.2 -	3.1 -	ns	ns	ns
cis 2-Hexen-1-ol	Al10	607.6 -	500.2 -	588.5 -	617.5 -	813.6 a	520.9 b	400.9 b	ns	****	*
1-Octanol	Al11	3.6 a	2.2 a	3.5 b	2.6 ab	2.6 b	3.8 a	2.6 b	*	*	**
Nonanol	Al12	2.0 a	0.6 b	0.6 b	1.5 ab	1.3 -	1.5 -	0.7 -	*	ns	*
Benzene methanol	Al13	13.8 -	9.4 -	10.8 -	10.7 -	11.4 -	11.7 -	10.4 -	ns	ns	ns
1-Dodecanol	Al14	4.4 -	5.3 -	8.2 -	5.9 -	7.2 -	6.1 -	4.6 -	ns	ns	ns
Aldehydes											

Butanal 3-methyl	Ald1	0.0	b	0.2	a	0.0	b	0.0	b	0.1	a	0.1	ab	0.0	b	****	*	**
Hexanal	Ald2	52.3	-	40.7	-	37.9	-	67.1	-	75.5	a	40.8	b	32.1	b	ns	**	ns
2-Hexenal	Ald3	205.7	-	145.8	-	159.8	-	184.9	-	268.0	a	154.4	b	99.7	b	ns	***	ns
Octanal	Ald4	0.8	-	1.0	-	1.1	-	0.8	-	1.3	a	1.1	b	0.5	b	ns	**	ns
2-Heptanal	Ald5	1.4	a	0.0	b	0.0	b	0.0	b	1.1	a	0.0	b	0.0	b	**	**	***
Nonanal	Ald6	17.7	a	8.6	b	11.9	b	13.4	ab	14.2	-	13.5	-	11.0	-	*	ns	**
Decanal	Ald7	3.4	a	1.2	c	2.6	ab	2.0	bc	2.5	-	2.0	-	2.4	-	**	ns	*
Benzaldehyde	Ald8	24.6	-	21.3	-	30.3	-	12.1	-	27.8	-	23.7	-	14.8	-	ns	ns	ns
Dodecanal	Ald9	12.5	-	8.6	-	17.1	-	10.5	-	12.1	-	10.4	-	14.0	-	ns	ns	ns
Tetradecanal	Ald10	0.9	b	1.4	b	2.6	a	2.6	a	2.6	a	2.5	a	0.7	b	***	****	*
Ketones																		
3-Pentanone	K1	0.0	b	0.0	b	0.0	b	4.2	a	3.1	a	0.0	b	0.0	b	****	****	****
2-Pentanone-4-methyl	K2	11.9	a	8.7	ab	8.7	ab	6.7	b	5.2	c	9.1	b	12.7	a	*	****	*
1-Penten-3-one	K3	0.6	a	0.0	b	0.0	b	0.6	a	0.9	a	0.0	b	0.0	b	****	****	****
6-Methyl-5-hepten-2-one	K4	0.3	a	0.0	b	0.4	a	0.0	b	0.0	b	0.4	a	0.1	b	*	**	*
γ-Butyrolactone	K5	1.1	a	0.3	b	1.1	a	0.0	c	0.0	c	0.8	b	1.0	a	****	****	****
2-Dodecanone	K6	0.3	a	0.0	b	0.3	a	0.4	a	0.4	a	0.2	b	0.1	b	**	**	****
Terpenes																		
dl-Limonene	T1	0.3	b	0.0	c	0.2	bc	0.7	a	0.5	a	0.2	b	0.2	b	****	**	ns
Ocimene	T2	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	-	-	-
Linalool	T3	3.1	ab	1.3	c	3.8	a	1.9	bc	2.1	-	2.5	-	2.9	-	**	ns	ns
α-Terpineol	T4	1.9	a	0.5	c	1.7	ab	1.1	bc	1.1	-	1.1	-	1.6	-	***	ns	ns
Others																		
2-Methylfuran	O1	0.0	-	0.0	-	1.3	-	0.0	-	1.0	-	0.0	-	0.0	-	ns	ns	*
Formammide N,N-dibutyl	O2	1.4	-	1.0	-	1.6	-	1.0	-	1.6	-	1.0	-	1.2	-	ns	ns	ns
Benzothiazole	O3	1.3	a	1.0	b	1.4	a	1.5	a	1.4	-	1.4	-	1.2	-	*	ns	**

For MA treatments, data are mean values of 9 samples (3 replicates x 3 storage time); for storage, data are mean values of 12 samples (3 replicates x 4 treatments).
Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$).
Different letters indicate statistical differences within storage conditions and storage time, according to LSD test ($P \leq 0.05$).

Table S3. Effect of different treatments (Low-O_2 = 1% O_2 + 0.03% CO_2 + 99% N_2; High-CO_2 =16 % O_2 + 20% CO_2 + 64% N_2; Mix = 1% O_2 + 20% CO_2 + 79% N_2; Air = Control), storage time (7, 14 and 21 day at 5 °C) and their interaction on sensory attributes of sweet cherries (*Prunus avium* cv Ferrovia).

Sensory attributes	**Treatments (A)**				**Storage Time (B)**			**A**	**B**	**A x B**
	Low-O_2	**High-CO_2**	**Mix**	**Air**	**7**	**14**	**21**			
Amaranth colour	7.7 b	7.8 b	7.1 c	8.2 a	7.8	7.5	7.9	****	ns	ns
Colour uniformity	7.5	7.4	7.1	7.1	7.5 a	7.0 b	7.4 a	ns	**	*
Brightness	6.4	6.3	6.5	6.1	6.7 a	6.4 a	5.9 b	ns	****	ns
Stains	2.3 b	2.4 b	2.3 b	3.2 a	1.8 c	2.7 b	3.1 a	****	****	**
Turgidity	6.7 a	6.7 a	6.6 a	6.1 b	6.9 a	6.2 b	6.5 b	**	***	*
Extraction stem	5.5	5.1	5.2	5.1	5.8 a	5.1 b	4.8 b	ns	****	ns
Pulp colour	5.9 b	5.6 b	4.5 c	6.8 a	5.1 c	5.6 b	6.4 a	****	****	****
Olfactive intensity	6.5	6.3	6.5	6.5	6.1 b	6.7 a	6.5 a	ns	**	ns
Herbaceous smell	4.5	4.3	4.1	4.3	4.5 a	4.3 ab	4.0 b	ns	**	*
Cherry smell	5.5 a	5.2 ab	5.1 b	5.0 b	4.8 b	5.9 a	4.8 b	*	****	ns
Pulp texture	6.4	6.5	6.2	6.2	6.3	6.3	6.3	ns	ns	ns
Juiciness	7.1	7.4	7.1	7.1	7.3	7.3	7.1	ns	ns	**
Sweet	5.3 b	5.7 ab	5.3 b	5.9 a	5.9 a	5.4 b	5.4 b	**	**	****
Acid	3.8 a	3.3 b	3.8 a	2.9 b	3.4 ab	3.7 a	3.2 b	****	**	****
Bitter	0.9	0.6	1.0	0.6	0.5 b	1.0 a	0.8 ab	ns	*	ns
Herbaceous aroma	4.3 a	4.0 ab	3.8 bc	3.5 c	4.3 a	3.9 b	3.5 c	***	****	***
Cherry aroma	5.4 c	6.3 a	5.1 c	5.8 b	6.1 a	5.6 b	5.3 c	****	****	****
PAI	6.0 b	6.6 a	5.5 c	6.1 b	6.7 a	5.6 b	5.8 b	****	****	****
NAI	1.3 c	0.7 d	2.4 a	1.8 b	0.5 b	2.0 a	2.2 a	****	****	****
Aromas persistence	5.8	6.0	5.7	5.9	6.1 a	5.6 b	5.9 a	ns	**	ns
Sweet persistence	4.2 b	4.8 a	4.1 b	4.9 a	5.2 a	3.9 b	4.4 b	****	****	****
Bitter after-taste	0.9	0.8	1.1	1.0	0.9	1.1	0.9	ns	ns	ns

For each treatment, data are mean values of 9 samples (3 replicates x 3 storage time); for storage, data are mean values of 12 samples (3 replicates x 4 treatments).
Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$).
Different letters indicate statistical differences within storage conditions and storage time, according to LSD test ($P \leq 0.05$).