

Citral-releasing active patches preserve the overall quality and extend strawberry shelf-life

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ABSTRACT

Strawberries are known for their high perishability and susceptibility to rapid deterioration. Conventional packaging methods often fall short in extending the shelf-life of strawberries while preserving their desirable overall quality. This study explores a novel way to extend strawberry shelf-life by incorporating plastic-based patches embedded with citral, a natural substance present in citrus essential oils. The doses of active citral for patch application were tested in vitro and in vivo using antifungal assays against *Botrytis cinerea*. Two citral concentrations (0.08 and 0.16 $\mu\text{L}/\text{mL}$) were used to prepare the active patches which were, prior to storage, attached to the packages containing the strawberries. Afterward, the fruit was stored for 9 d at 4 °C, and quality evaluated throughout storage. Results showed that the active packaging with citral-embedded patches, regardless of the concentration, maintained the consumer acceptability level significantly higher than controls with strawberries, exhibiting nearly 3 additional days of shelf-life. Furthermore, active packaging effectively mitigated respiration rate, weight loss, and microbial growth, thus delaying senescence. Overall, this study shows that the developed packaging containing citral-patches presents a promising strategy to extend strawberry shelf-life.

1. Introduction

Strawberry (*Fragaria × ananassa* Duch.) is globally recognised for its unique organoleptic characteristics, including colour, taste, and flavour, along with its abundant bioactive compounds. These bioactive compounds, encompassing vitamins C and E, carotenoids, minerals, anthocyanins, flavonoids, and other phenolic compounds, actively contribute to consumer health, offering antioxidant, anticancer, and anti-inflammatory properties (Miller et al., 2019; Van De Velde et al., 2013). Despite their nutritional richness, strawberries have a short shelf

life due to their susceptibility to mechanical injuries, softening, physiological disorders, and susceptibility to *B. cinerea* mould spoilage (El Ghaouth et al., 1991; Vu et al., 2011; Wang et al., 2016). Although refrigeration soon after harvest helps to mitigate metabolic reactions and microbial spoilage (Kader & Saltveit, 2002), various technologies, in conjunction with cold storage (Parvez & Wani, 2018), have been employed to extend the shelf-life of strawberry fruit.

Modified atmosphere packaging (Matar et al., 2020, 2021; Kahramanoğlu, 2019; Zhao et al., 2019), heat treatment (Caleb et al., 2019; Panou et al., 2021), UV irradiation (Forges et al., 2020) and edible

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coatings (Qamar et al., 2018; Quintana et al., 2021; Saleem et al., 2021) have been successful in extending strawberry postharvest life. Besides, an emerging technology with significant potential is the active packaging, in which antimicrobials, antioxidant substances, oxygen scavengers, CO₂ emitters or flavour absorbers are incorporated into the packaging materials. For example, the release of bioactive compounds into the package headspace can inhibit decay and oxidation, extending shelf-life, preserving freshness, and reducing the need for added preservatives (Yildirim et al., 2018; Kuai et al., 2021).

Citral, referring to a nearly equimolar mixture of E- stereoisomer (geranial, 2E-3,7-dimethylocta-2,6-dienal) and Z- stereoisomer (neral, 2Z-3,7-dimethylocta-2,6-dienal) is a bioactive terpenoid aldehyde present in essential oils derived from various plants, such as lemongrass, citrus fruit, and ginger. Citral is generally considered safe and extensively used as a flavouring and scenting agent in cosmetics (Api et al., 2020). The compound also possesses a spectrum of beneficial properties including antioxidant, antibacterial, antifungal, anti-inflammatory, anti-obesity, and anticancer (Sanahuja & García, 2021; Idrees et al., 2019; Sharma et al., 2021; e Silva et al., 2022). Therefore, its application in the food industry represents a promising approach to the storability and quality of fresh fruit and vegetables. For example, citral has been tested in various postharvest applications, including fumigation and dipping of oranges (Wuryatmo et al., 2014), kiwifruits (Wei et al., 2021a, 2021b), and peaches (Li et al., 2022). Other authors reported the use of citral as a component of edible coating for apples (Vieira et al., 2019), strawberries (Guerriero et al., 2015), and fresh-cut pineapple (Prakash et al., 2020). Additionally, citral nanoemulsion has been tested for its efficacy on melon and papaya (Luciano et al., 2023), demonstrating antimicrobial and antifungal properties (Wei et al., 2021a), respectively. Moreover, being volatile, citral has also been used in the development of active packaging (Laorenza & Harnkarnsujarit, 2021; Yoplac et al., 2021; Sarfraz et al., 2021; Siracusa et al., 2018).

Volatile agents can be incorporated into packaging materials like films or sachets, exhibiting antimicrobial activity without physical contact with the food (Mokarizadeh et al., 2017). Notably, applications of low concentrations of citral on strawberry fruit, directly or included in edible coatings, effectively reduced decay caused by *B. cinerea*, and extended the shelf-life of the fruit (Shen et al., 2024; Tančinová et al., 2022). Other studies focused on the efficacy of citral vapour to control grey mould rots on packed strawberries during storage (Wuryatmo et al., 2014). An emerging strategy consists in using adhesive patches impregnated with essential oil or volatile compounds (Ghoshal, 2018; Lucas-Gonzalez et al., 2023; Songtipya et al., 2021).

Recently, a biocontrol microneedle (BMN) patch utilizing epsilon-poly-L-lysine (ϵ -PL), a food-grade antimicrobial compound within a dissolving microneedle system, was developed (Jiang et al., 2023). Through a dissolvable microneedle format, this patch effectively delivered the preservative directly into the fruit's skin, providing targeted protection against postharvest pathogens. Despite the efficacy, this strategy is not suitable for fruit without peel like strawberries. By contrast, the ability of some antimicrobial volatile compounds to penetrate the first layers of the fruits or vegetables could be exploited to develop a less invasive and equally effective patch especially against fungi responsible for post-harvest rot (Zhang et al., 2023; Chen et al., 2023).

Thus, our study aimed to first investigate the citral dose required to inhibit the in vivo and in vitro *B. cinerea* growth. Subsequently, cellulose patches with the most active citral concentrations were developed and inserted into packages containing strawberry fruit. The efficacy of this treatment was validated by assessing the changes in sensory, physical, chemical, microbial, and volatile organic compound profiles of strawberries during cold storage.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals, standards, and reagents for quality analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA), whereas microbiological media were obtained from Biolife Italiana s.r.l. (Milan, Italy). Methanol (HPLC grade), citral (C₁₀H₁₆O, CAS number: 5392-40-5) ($\geq 96\%$, Food Chemicals Codex, FCC, natural, food grade, FG), cellulose acetate (CA) powder (average Mn $\sim 50,000$, wt.% acetyl: 39.7) and triacetin (TA) were purchased from Sigma-Aldrich (Milan, Italy). The 2-methylpentanal ($\geq 98\%$) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Chemical standards (Hexanal, (E)-2-Hexenal, Benzaldehyde, Nonanal, Decanal, 2-Pentylfuran, Methyl acetate, Ethyl acetate, Methyl butanoate, Ethyl butanoate, Methyl hexanoate, Ethyl hexanoate, Hexyl acetate, Methyl heptanoate, Methyl octanoate, Ethyl octanoate, Ethyl decanoate, Methyl cinnamate, Ethyl 2-butenate, Ethyl nonanoate, Acetic acid, Acetone, 3-Octanone, γ -Caprolactone, Ethanol, 1-Hexanol, 2-Methyl 1-propanol, 1-Pentanol, (E)-2-Hexen-1-ol, 1-Octen-3-ol, 1-Octanol, 1-Nonanol, Phenylethyl Alcohol, Nerol, Limonene, Linalool, β -Farnesene, (6E)-Nerolidol, β -Myrcene, β -cis-Ocimene, β -Bisabolene) were purchased from Ultra Scientific Italia S.r.l. (Bologna, Italy). A mixture of normal alkanes (C5-C29) was purchased from o2si smart solutions (Charleston, SC, USA). Helium at a purity of 99.9995 % was provided by Sapio s.r.l. (Bari, Italy).

2.2. Minimal inhibitory concentration (MIC) determination

Three strains of *B. cinerea* (ITEM 5154, 17,199, and 17,200) obtained from the Agro-Food Microbial Culture Collection of the Institute of Sciences of Food Production (<http://www.ispa.cnr.it/Collection>, accessed January 10, 2024) were cultured on Potato Dextrose Agar (PDA) and incubated at 25 °C for 7 days to stimulate sporulation. At the end of incubation, the cultures were dispersed with 5 mL of sterilised 0.9 % NaCl containing 0.1 mL/L of Tween 80 by softly rubbing with a cotton swab. Afterwards, the conidial suspension was moved to a sterile Falcon tube and filtered (11 μ m, Nylon Net Filters, Millipore, Cork, Ireland). The concentration of conidia was estimated using a Thoma hemocytometer chamber. Serial dilutions (in 0.9 % NaCl solution) were made to obtain inocula for the experiments (ca. 1×10^6 conidia/mL). In vitro antimicrobial activity of citral against *B. cinerea* strains was evaluated by using both the microdilution broth method and the disc volatilisation method (Fancelli et al., 2020). The microdilution broth method and MIC determination were performed according to EUCAST (2020) using a citral concentration (v/v) ranging from 0.021 to 0.33 μ L/mL. The disc volatilisation method was performed following the procedure described by Fancelli et al. (2020). Briefly, PDA agar plates were first spread with an inoculum of fungal strains. Sterile paper discs were taped to the cover of each Petri dish and then soaked with 1.25, 2.50, 5.00, 10.00, and 20.00 μ L of citral to obtain final concentrations in the headspace of Petri dishes ranging from 0.021 to 0.33 μ L cm⁻³. Finally, the plates were covered with laboratory sealing film (Parafilm™ M, 5 cm, Zürich, Switzerland) and incubated at 25 °C for 7 days. The MIC was expressed as microlitres of citral per volume unit of head space (μ L cm⁻³), and defined as the lowest vapour concentration that did not produce visible growth. Each test was performed in triplicate, and the experiments were repeated twice.

2.3. Antimicrobial activity of gaseous citral on inoculated strawberries

Baskets with 500 g of strawberries (*Fragaria* \times *ananassa* Duch., Candonga var. Sabrosa), purchased from a local retail store (Apofruit Italia Soc. Coop, Scanzano Jonico, Italy), were transported to the laboratory and immediately processed as herein described. The strawberries were washed by immersion in sterile distilled water, let to dry for 30 min at room temperature in a sterile tray, and then dipped for 5 min in 100

ml of conidia suspension (1×10^6 conidia/mL) of selected *B. cinerea* kept at 25 °C. The counts of conidia attached to fruits were assessed by shaking 6 strawberries in 50 ml of sterile BPW at room temperature for 5 min. The resulting suspension was further diluted with sterile BPW and seeded in Petri dishes with Rose Bengal agar medium (VWR, Milan, Italy). After 3 days at 25 °C *B. cinerea* colonies were easily distinguishable from other contaminating moulds (Figure S1).

2.3.1. Assessment of gaseous citral efficacy in small scale trials

To assess the effect of gaseous citral on the growth of *B. cinerea* ITEM 5144, six reservoirs of 25 mL each (Sigma-Aldrich, Milan, Italy) containing 3 non-inoculated strawberries ("non-inoculated reservoir") and 6 reservoirs each containing 3 uninoculated strawberries ("non-inoculated reservoir") were prepared. Non-inoculated strawberries were tested to assess the effect of citral on the native microbial community. Three inoculated and three non-inoculated reservoirs were placed inside a 20-litre sealed polyethylene box equipped with a circulation fan and a heating system, which allowed citral (1.6 ml equivalent to the MIC of 0.08 µL/mL) to fill up the volume of the box (Fancello et al., 2020). The other 6 reservoirs (positive control) not exposed to citral vapour were placed inside plastic trays (separately inoculated and non-inoculated), covered with a lid (34 × 23 × 16 cm, Gensini, Florence, Italy), and maintained for 10 days inside a cold room with a temperature of approximately 5 °C and a relative humidity between 80 and 82 %. For both containers, relative humidity (RH) and temperature were checked using a thermo-hygrometer (Testo 635, Testo SE & Co. KGaA, Germany) at the beginning and at the end of the experiment. To ensure uniform distribution of citral inside a 20-litre sealed polyethylene box a ventilation system (12 V, Air 0.0283168 m³/min, speed 3000 rev min⁻¹) was used during the entire duration of the experiment.

2.4. Strawberry active packaging with citral patches

2.4.1. Active packaging development

Thermoplastic films were prepared by combining cellulose acetate powder (74 % wt/wt), triacetin (25 % wt/wt), and citral (1 % wt/wt). Subsequently, the mixture underwent thermoplasticization using a twin counter-rotating internal mixer (Rheomix 600, Haake, Germany) connected to a control unit (Haake PolyLab QC). The mixing parameters were set at a temperature of 150 °C a speed rotation of 50 rev min⁻¹, and a 5 min mixing time. After solidification, the blends were cut into uniform flakes and processed into films with a thickness of approximately 250 µm using a hotplate hydraulic press at 130 °C and 10 MPa. Following hot pressing, the films were cooled down to room temperature and maintained under controlled humidity and temperature (0 °C and >5 % RH) monitored using an environmental thermo-hygrometer (TROTEC TC100, Trotec GmbH, Austria). Films were used to determine their citral content or to obtain patches for using in bags. To determine the final citral content, ten film samples (10 × 10 cm) were placed inside a convection drying oven (UFE 500, Memmert, Germany) maintained at 80 ± 0.5 °C and with a continuous nitrogen flow rate of 0.5 ± 0.02 m/s. The weight loss after stabilisation (72 h) of the film samples was measured using an analytical balance (AT261 Delta range, Mettler, Switzerland) to determine the amount of citral released from the blended films. The final amount of citral was calculated by difference between the wet and dry samples. From the remaining films stored under controlled conditions, patches of dimensions approximately 5 and 10 cm² were obtained to ensure the desired citral content in the free space of the plastic bags filled with strawberries.

2.4.2. Strawberries packaging and storage

About 10 kg of strawberries (*Fragaria × ananassa* Duch.) Candonga var. Sabrosa (Planitalia s.r.l., Policoro, Italy) were provided by Apofruit Italia Soc. Coop., a cooperative company of fresh fruit (Scanzano Jonico, Italy). The fruit, packed into PET trays (Carton Pack SpA, Rutigliano, Italy) were transported in refrigerated conditions (4 ± 1 °C) to the

National Research Council (CNR) postharvest laboratories. Strawberries were visually inspected, selected to choose fruit free from physical or biological damage. A total of 36 samples (3 treatments × 3 replicates × 4 storage times), containing each one 200 g of fruit, were prepared. In detail three replicated treatments were considered: two treatments of citral patches (prepared as reported above) at final citral concentrations of 0.08 and 0.16 µL/mL (CA and CB, respectively) and one control treatment (CTRL). All trays were finally packed into commercial polyethylene plastic bags (Cuki, Cuki Cofresco S.r.l., Volpiano, Turin, Italy) inside which patches (CA or CB) were applied with double-sided tape. The bags containing fruits are closed without being sealed. No patches were applied inside the control trays. All trays were left at room temperature for 4 hours to facilitate the release of citral from the patch before cold storage performed for 9 days at 4 °C and 85–90 % relative humidity. Strawberries were subjected to sensory, chemical, and physical evaluations at harvest (day 0) and after 3, 6, and 9 d of storage at 4 °C.

2.4.3. Determination of citral inside packages by GC–MS analysis

The determination of citral (sum of (E)- and (Z)- form) inside the bags containing both treated and untreated strawberries (two bags per each treatment and per day) was conducted using an HS-SPME/GC–MS on days 0, 3, 6 and 9 during storage at 4 °C, following the method described by Quintieri et al. (2022). The GC/MS system was an Agilent 6890 A GC (Agilent Technologies, Palo Alto, CA, USA) coupled with the Agilent 5973 N inert MSD mass spectrometer equipped with a triple-axis HED-EM detector. For citral extraction and desorption, a Headspace-Solid Phase Microextraction (HS-SPME) was performed using a manual SPME sampler holder (Supelco, Bellafonte, PA, USA). In the analyte extraction, a 50/30 mm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 1 cm fiber length; Sigma-Aldrich, Milan, Italy) was exposed to the plastic tray headspace for 30 min at 4 °C. After extraction, volatiles were thermally desorbed by exposing the fiber to the Split/Splitless Injection Port of the GC–MS system. The system was equipped with a 0.75 mm i.d. Ultra Inert liner Straight, maintained at 250 °C for 5 min in splitless mode. Citral analysis was carried out using the GC–MS system, which was equipped with a VF-WAXms (60 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies) fused-silica capillary column, and programmed temperature mode. The temperature program was initiated at 40 °C and was held for 5 min, then raised at a rate of 0.033 °C s⁻¹ to 140 °C, further raised at a rate of 0.083 °C/s to 170 °C, and finally raised at a rate of 0.833 °C/s to 230 °C, holding for 10 min. The helium flow was set to 0.016 × 10⁻³ L/s. The transfer line, ion source, and quadrupole temperatures were 280, 290, and 150 °C, respectively. Electron impact ionization (EI+) mode with an electron energy of 70 eV was utilized, and the mass spectra were recorded in the *m/z* range of 40–300 u. The total chromatographic run time was 72.2 min. The (E)- and (Z)-citral identification was conducted by comparing the sample mass spectra with spectra in the NIST/EPA/NIH Mass Spectral Database (National Institute of Standards and Technology, Version 2.4, 2020, USA) with a match quality higher than 80. To confirm the VOC identity, the linear retention index (LRI) was calculated for each volatile compound in relation to the retention times of C5–C29 n-alkanes and compared with those reported in the literature (Zellner et al., 2008; <https://www.nist.gov/>, National Institute of Standard and Technology). The amount of citral was determined by normalizing the measured peak areas to their peak areas at the first sampling (*t* = 0), which was assumed to be equivalent to 100 %.

2.4.4. Sensory analysis

A group of trained subjects (5 females and 4 males, ages of 30 and 60) evaluated at harvest (day 0) and after 3, 6, and 9 days of storage at 4 °C the appearance and odor of 3 strawberries from treated (CA and CB) and untreated samples (CTRL). The evaluation was performed in a single-blind manner, using alphanumeric casual codes to identify each sample. A first evaluation was performed on each sample, soon after opening

bags at each storing day. The visual quality of the fruit from each treatment was evaluated using a 5-point rating scale, developed by Nguyen, Nguyen and Nguyen (2020), where 5 = excellent (calyx is stiff and green, no sign of shrivelling, field-fresh), 4 = good (calyx is green but slightly stiff than at harvest, minor signs of shrivelling), 3 = acceptable (calyx may appear dry and wilted—limit of marketability), 2 = poor (fruit is dry and calyx is shrivelled, 1–5 % decay), 1 = very poor (calyx is very dry and yellowed, over 10 % decay). The characteristic odour was evaluated using a rating scale from 5 to 1 (5=excellent; 4=good; 3=fair, limit of sensory acceptability; 2=poor; 1=very poor, absent).

2.4.5. Respiration rate, weight loss and colour analysis

From each packaging type (including control samples) and replicates ($n = 3$), approximately 200 g of fruit were placed into a 3.6 L sealed plastic jar, using one jar per replicate. The CO_2 inside the jar was allowed to accumulate up to a standard concentration of 1 %, (SAPIO, Monza, Italy). The time to reach this value was determined by measuring the levels of CO_2 at regular intervals. The CO_2 analysis was conducted by injecting a 1 mL gas sample from the headspace of the plastic jars into a gas chromatograph (p200 micro GC-Agilent, Santa Clara, CA, USA). The respiration rate was reported as $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$.

Weight loss was calculated at each sampling day (0, 3, 6 and 9 days) as a percent of variation respect to the initial fresh weight as follows:

$$\text{Weight loss} = \left(\frac{\text{Final weight} - \text{Initial weight}}{\text{Final weight}} \right) \times 100.$$

The color of strawberry fruit was measured using a colorimeter (CR-400-Konica Minolta Co., Osaka, Japan) on four random points on the fruits' surface. The L^* , a^* , and b^* color values refer to the brightness, redness, and yellowness of each sample, respectively. The a^* and b^* color attributes were used to calculate chroma ($C = \sqrt{a^{*2} + b^{*2}}$) and hue angle ($h^\circ = \arctg \frac{b^*}{a^*}$) as reported by Cefola et al. (2012).

2.4.6. Soluble solids content (SSC), titratable acidity and pH

About three replicates of 100 g of strawberries each from each treatment and storage time were homogenised (T-25 digital Ultra-Turrax®-IKA, Staufen, Germany). The homogenate was used to assay soluble solids content (SSC), titratable acidity (TA), and pH. The SSC was determined using a digital refractometer (DBR35-XS Instruments, Carpi, Italy), and results were expressed in %. The TA expressed as citric acid was determined using an automatic titrator pH meter (pH-Burette 24-Crison Instrument, Barcelona, Spain) with 0.1 mol/L NaOH to the final pH of 8.1. The same instrument was used to measure the pH of the strawberry homogenate.

2.4.7. Antioxidant activity, total phenol content, and total sugars

An amount of 5 g of finely chopped strawberries, were homogenized in 20 mL methanol/water solution (80:20 v/v) for 2 min, (T-25 digital ULTRA-TURRAX®-IKA, Staufen, Germany) and then centrifuged (Prism C2500-R, Labnet, Edison, NJ, USA) at $15,000 \times g$ for 5 min at 4°C . The extracts were collected and stored at -20°C before the analysis.

The antioxidant activity (AA) was measured on the methanol extract using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay as described by Cefola et al. (2014). The absorbance was measured at 515 nm after 40 min in the dark, using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The results were expressed as g of Trolox per kg fresh weight (fw) using a Trolox calibration curve ($82 - 625 \mu\text{M}$; $R^2 = 0.999$)

The total phenol content (TPC) was determined according to the method reported by Fadda et al. (2016), where 100 μL of each extract was mixed with 1.58 mL of water, 100 μL of Folin-Ciocalteu's reagent, and 300 μL of sodium carbonate solution (200 g l^{-1}). The absorbance was measured at 765 nm after 2 h of incubation in the dark and the amount of TPC was expressed as g of gallic acid equivalent per kg of fresh fruit (g GAE/kg fw) after plotting gallic acid as a standard curve with the 5

concentrations ranging from 50 to 500 $\mu\text{g mL}^{-1}$ ($R^2 = 0.998$).

Total sugar content (TS) was determined using a phenol-sulfuric colorimetric method (Buysse & Merckx, 1993). Briefly, 5 g of strawberry sample was blended with 20 mL of 95 % ethanol for 2 min and centrifuged at $15,000 \times g$ for 5 min. The diluted extracts were used for determining the colour development at 490 nm. Glucose was used as standard ($y = 123.74x - 6.8529$ $R^2 = 0.9933$) and TS was expressed as g glucose per kg of fw.

2.4.8. Determination of volatile organic compounds (VOCs)

Strawberries (about 200 g) were manually ground into a homogeneous paste in a mortar, kept in an ice bath to slow down deterioration. The samples were then analysed using the HS-SPME/GC-MS method described by Cefola et al. (2023). Specifically, samples collected on day 0 and day 9 samples (from the three treatments (CA, CB, and CTRL) at three storage intervals (3, 6, and 9 d) were analyzed for volatile organic compounds (VOCs) in triplicate.

The samples' mass spectra were compared with those from the NIST/EPA/NIH Mass Spectral Database (National Institute of Standards and Technology, Version 2.4, 2020, USA) using a match quality of greater than 80 to identify volatile chemicals. To verify the identity of VOCs, the linear retention index (LRI) for each volatile was computed with respect to the retention times of C5-C29 n-alkanes and compared with those published in the literature (Zellner et al., 2008; www.nist.gov). Chemical standards were also used to confirm the identification of a set of 41 volatile compounds. Agilent Technologies (Santa Clara, USA) provided the MSD Chemstation, which was used to calculate the compounds' total ion peak area. The quantitative evaluation of the compounds was determined as ratio between peak areas and the 2-methyl-pentanal peak area used as the internal standard.

2.4.9. Microbiological analysis

Approximately 25 g of strawberries per treatment were taken from 3 sample trays at day 0, 3, 6, and 9 and homogenised for 2 min in 225 mL of sterilised peptone water (1 g/L) in a stomacher bag. Decimal serial dilutions of homogenates were spread in triplicate on Petri dishes on Plate count agar (PCA, Merck, Darmstadt, Germany) amended with cycloheximide (0.1 g/L) for the determination of total mesophilic aerobic bacteria (TBC) following ISO 21,527-1:2008. Total yeast and mould (Y/M) counts were enumerated on Petri dishes of potato dextrose agar (PDA, Oxoid Ltd, Basingstoke, UK) with chloramphenicol 0.1 g/L incubating at 25°C for 3–5 d TBC and Y/M counts were expressed as mean (\pm standard deviation) of decimal logarithmic of colony-forming unit per gram (CFU/g) in relation to citral treatments and days of storage. All fruit with visible mould growth or rots were considered infected and monitored for percentage of rot incidence in relation to the total number of fruit in each.

2.5. Statistical analysis

A two-way ANOVA for $p \leq 0.05$ was performed to evaluate the effects of active packaging type (CTRL, CA and CB), storage (3, 6, and 9 days), and their interaction with quality parameters of strawberry fruit. When the interaction between factors was significant, data were shown as graphs with mean values followed by letters (a, b, or c) to denote significant differences. The statistical analysis was performed using the software Statgraphics 19 – X64. Concerning the GC-MS data, Principal Component Analysis (PCA) was conducted as an unsupervised exploratory method to detect grouping and trends among different treatments. For each variable, the values of the three replicated samples were averaged, resulting in a working matrix with 10 rows (day 0, CA, CB, and CTRL at t1; CA, CB, and CTRL at t2; and CA, CB, and CTRL at t3) and 143 columns. The PCA was performed in the R environment (R Core Team, 2021) (<https://www.r-project.org/>) using in-house codes. The data matrix was auto-scaled before applying PCA. The correlation loadings were arbitrarily chosen with a cutoff value greater than +1 and less than



Fig. 1. Strawberries inoculated with *Botrytis cinerea* and treated with citral at 0.08 $\mu\text{L mL}^{-1}$ (A) and untreated (B) and internal sections of the same strawberries inoculated with *Botrytis cinerea* and treated with citral at 0.08 $\mu\text{L mL}^{-1}$ (C), and untreated (D) after 10 days of storage at 5 °C in air.

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3. Results and discussion

3.1. In vitro and in vivo antimicrobial activity of gaseous citral

A preliminary evaluation of citral antimicrobial activity was performed against three *B. cinerea* strains (ITEM 5154, 17,199, and 17,200) by applying both the microdilution broth and the disc volatilisation methods. Considering the first method, the liquid phase MIC of citral for all three tested strains was 0.039 $\mu\text{L mL}^{-1}$, and this concentration was

stable after 10 days of incubation at 25 °C.

Regarding the gaseous phase MIC, the active concentration was within the range of 0.021 to 0.33 $\mu\text{L mL}^{-1}$. In the first 24 hours at 25 °C, growth was observed in the control samples for all three strains, while no growth was observed for citral-containing samples, regardless of the concentration. The strain *B. cinerea* 17,199 did not grow, however the strains ITEM 17,200 and ITEM 5154 showed slight growth at a citral concentration of 0.021 $\mu\text{L cm}^{-3}$ (diameter of the CTRs 50.00 mm and 24.16 mm vs 20.41 mm and 19.82 mm of the treated, respectively). After 48 h at a citral concentration of 0.041 $\mu\text{L cm}^{-3}$, no growth was observed for all three strains. However, at this concentration, strains

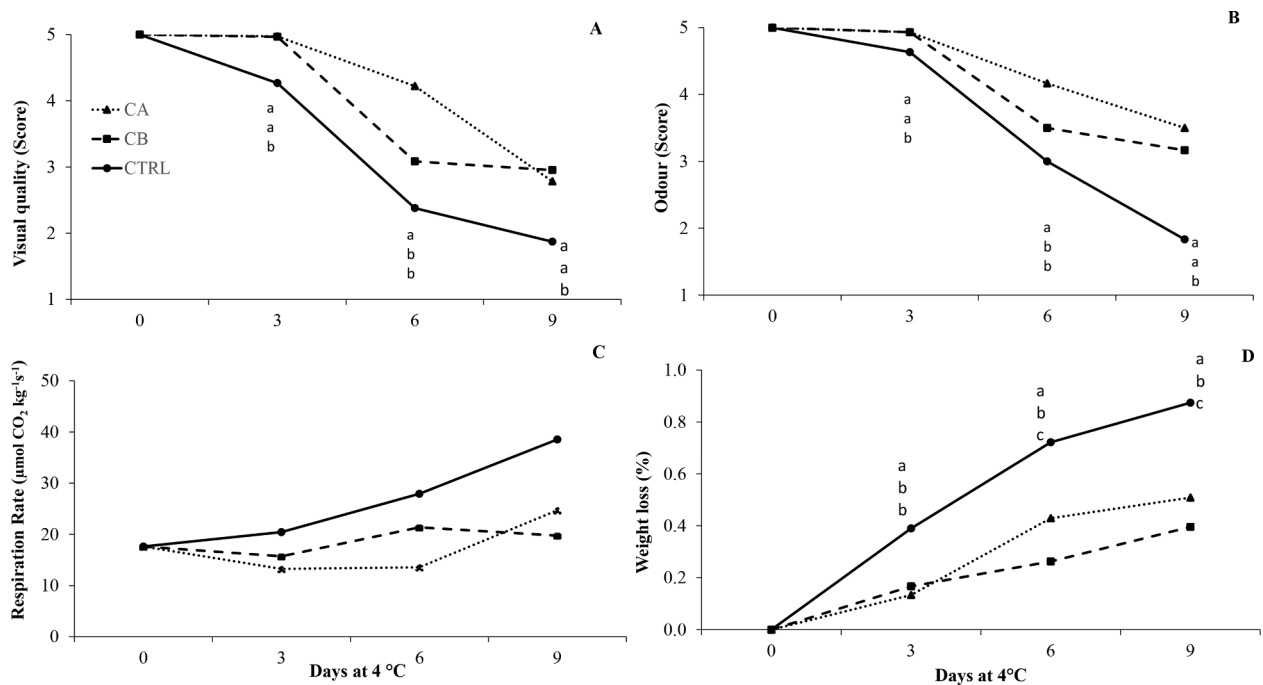


Fig. 2. Visual quality (A), odour (B), respiration rate (C) and weight loss (D) of strawberries packaged in activated bag with citral patches at two concentrations (CA= 0.08 $\mu\text{L mL}^{-1}$ or CB = 0.16 $\mu\text{L mL}^{-1}$) or in non-activated bag (CTRL) during storage at 4 °C.

ITEM 17,199 and ITEM 5154 showed growth after 6 days (diameter of each CTRs 90.00 mm vs. 63.49 and 83.47 mm of treated, respectively). Finally, no growth was observed at a citral concentration of $0.08 \mu\text{L cm}^{-3}$. Therefore, based on these results, the subsequent experiments were carried out considering this concentration and using ITEM 5154 as target strain. Inoculated and non-inoculated strawberries, kept inside the box, were then analysed and results showed that the concentration of citral used strongly reduced mould growth. As depicted in Fig. 1(A and B), untreated strawberries showed evident mycelium growth, while treated fruit remained unspoiled. Internally, there was also a significant difference between untreated and treated strawberries (Fig. 1C and Fig. 1D). These results confirmed the efficacy of the selected citral concentration in inhibiting fungal growth on the fruit (Quintieri et al., 2022; Shen et al., 2024) even after 10 d of storage at 5°C . Recent findings substantiate that the application of EOs in the gaseous phase is more effective than in the liquid phase because of its increased antimicrobial activity, efficacy at lower concentrations, and versatility which allows for being used in different environments (Reyes-Jurado et al., 2020). Several studies have been conducted using gaseous EOs during storage of fruits and other foods (Chrysargyris et al., 2021; Lin et al., 2022; Quintieri et al., 2022; Sumalan et al., 2020). Tancinová et al. (2022) reported that for strawberries, the lowest MICs among gaseous EOs were obtained with the application of litsea (*Laureaceae*) and lemongrass. These EOs, when applied to strawberries at concentrations of 250 or 500 $\mu\text{L/L}$ completely inhibited the growth of every strain of *B. cinerea* up to 14 days of storage at 22°C . It is important to note that after 10 days of storage, the treated strawberries were not decayed and did not show any microbial damage compared to the untreated fruit and were thus considered acceptable to consumers. Other studies using compounds from citrus derivatives showed that temperature plays a pivotal role. For example, Liu et al. (2016), reported that the storage time for strawberries was reduced to 3 days using higher temperatures (20°C), while Shehata et al. (2020), showed that the storage time was increased to 18 days when a lower temperature (2°C) was used.

3.2. Postharvest quality evaluation of fresh strawberries stored in active packaging

An increase of 160 % and 120 % of citral was observed in the CA samples after 3 and 9 d of storage, respectively (data not shown). However, for the same length of storage, the increase in citral in the CB samples was much higher (250 and 330 %, respectively). Therefore, depending on the initial concentration of citral in the patches, at least up to 3 d of storage these results were consistent with a dose-response pattern.

Results from the two-way ANOVA showed a significant effect of citral x storage time interaction on the sensory quality (visual quality and characteristic odour), respiration rate and weight loss of strawberry fruit; moreover, the storage period significantly affected titratable acidity, sugars, and chroma ($p < 0.05$). No significant effects of the main factors and their interaction with the remaining quality parameters (total soluble solid, pH, antioxidant activity, total phenols, and hue angle) were found (Table S1). Significant differences in visual quality (Fig. 2A) were found after 3 days of storage at 4°C in the samples stored in active packaging at both citral concentrations showing a mean score of 4.97 ± 0.06 , significantly higher than the control (4.27 ± 0.25). As expected, all strawberry samples experienced a decline in visual quality throughout storage, although strawberries kept in packs with low concentration citral patches ($0.08 \mu\text{L/mL}$) displayed better visual quality than the others. After 6 d of storage, average visual quality values of 4.22 ± 0.35 , 3.08 ± 0.63 , and 2.38 ± 0.4 were registered for samples packed with CA patches, CB patches, and untreated (CTRL), respectively. However, at the end of storage time, only the strawberries subjected to citral treatments obtained visual quality scored close to the limit of marketability (score 3), suggesting that citral embedded patches can delay visual quality deterioration of strawberries. Similarly, panellist

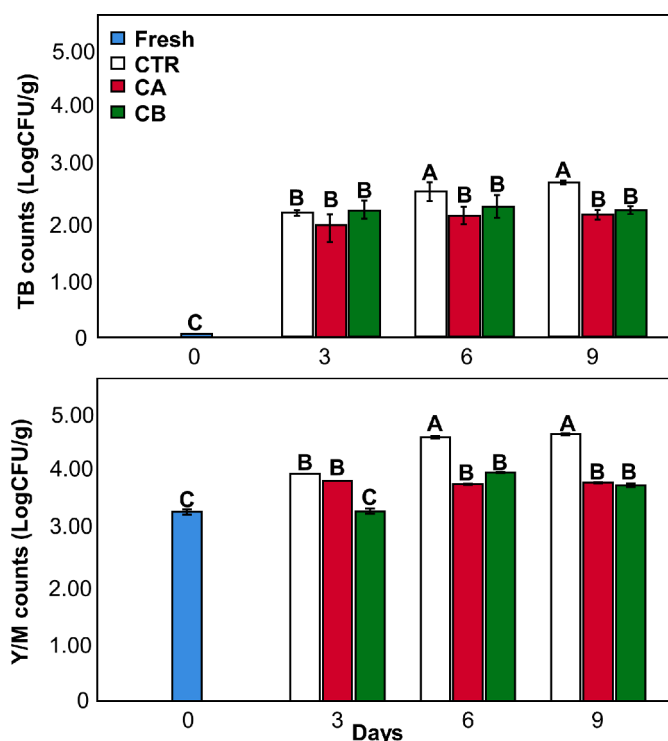


Fig. 3. Total aerobic mesophilic bacterial counts (TBC, upper frame) and total counts of yeast and moulds (Y/M, lower frame) of strawberry samples packed with patches soaked with different amounts of citral and stored at 4°C for 9 days. Bars represent the average \pm standard deviation ($n = 3$). Bars with different uppercase letters represent significantly different values $F(9, 20) = 169.534$, $p = 6.90 \times 10^{-17}$ and $F(9, 20) = 969.263$, $p = 2.13 \times 10^{-24}$, respectively.

assigned the higher score for the perception of characteristic odour to strawberries stored in active packages with the lowest citral concentrations (Fig. 2B). Moreover, at the end of storage, fruit packaged with citral patches were scored above the marketable limit, while control samples presented an objectionable odour (Fig. 2B). In comparison, applying citral-containing essential oils directly on the fruit or through edible coatings significantly altered the flavour of fresh-cut apples, pineapples, and strawberries, making them unpalatable to consumers; however, this negative effect depended on the EO concentration used (Azarakhsh et al., 2014; Gurriero et al., 2015; Rojas-Graü et al., 2007). Our results, however, showed that strawberries treated with citral patches retained a rather high overall characteristic odour score until the end of storage, extending sensory acceptability by 3 days compared to the control.

Our study also showed, for the first time, the effect of citral treatments on the respiration rate of strawberry fruit during cold storage. Thus, during storage, the respiration rate (Fig. 2 °C) of control samples was significantly higher than that of strawberries treated with both citral patch concentrations. Specifically, after 3 d at 4°C , the respiration rate was 14.94 ± 0.61 , 9.67 ± 0.50 , and $11.48 \pm 0.05 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$, for untreated samples (CTRL), and packaged with citral patches at $0.08 \mu\text{L/mL}$ (CA) and at $0.16 \mu\text{L/mL}$ (CB), respectively. Furthermore, after 9 d at 4°C , strawberries treated with different citral concentrations presented a similar respiration rate with values around $16 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$, significantly lower than the untreated samples, which showed a value of $28.16 \pm 2.50 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$.

In general, the weight loss of control samples was statistically higher than that of strawberries treated with both citral patch concentrations (CA and CB; Fig. 2D). Also, during the storage, there were significant differences between samples treated with different citral concentrations; Thus, after 6 days of storage weight loss of CA samples was significantly

Table 1

List of volatile compounds ($n = 144$) identified by HS-SPME/GC-MS analysis of treated and untreated strawberry samples.

Volatile compound	Code	LRI _{lit} /LRI _{sp} ^a	Volatile compound	Code	LRI _{lit} /LRI _{sp} ^a
Aldehydes			Carboxylic acids		
Acetaldehyde ^d	Ald1	710/709	Acetic acid ^b	C1	1456/1456
Hexanal ^{b,d}	Ald2	1080/1080	Butanoic Acid ^d	C2	1625/1625
(E)-2-Pentenal ^d	Ald3	1130/1130	2-Methylbutanoic acid	C3	1665/1665
(E)-2-Hexenal ^{b,d}	Ald4	1196/1198	2-Methylpentanoic acid	C4	1764/1764
(Z)-2-Heptenal ^d	Ald5	1318/1317	Hexanoic acid ^d	C5	1840/1840
(E,E)-2,4-Hexadienal	Ald6	1397/1397	Heptanoic acid ^d	C6	1950/1950
(E)-2-Octenal	Ald7	1420/1420	Octanoic acid ^d	C7	2056/2056
(E,E)-2,4-Heptadienal ^d	Ald8	1461/1461	2-Methyl-Propanoic acid ^d	C8	1566/1566
Benzaldehyde ^{b,d}	Ald9	1517/1518	Dodecanoic acid ^d	C9	2471/2472
(E)-2-Nonenal ^d	Ald10	1525/1526	Ketones		
(E,E)-Decadienal ^d	Ald11	1762/1760	Acetone ^b	K1	818/818
Nonanal ^{b,d}	Ald12	1389/1389	2-Pentanone ^d	K2	976/976
Decanal ^{b,d}	Ald13	1494/1494	2-Heptanone	K3	1178/1178
Benzeneacetaldehyde ^d	Ald14	1641/1641	3-Octanone ^b	K4	1242/1243
(E)-Cinnamaldehyde ^d	Ald15	2043/2043	Acetoin ^d	K5	1283/1283
Neral ^d	Ald16	1676/1676	Lactones		
Geranial ^d	Ald17	1730/1729	γ-Caprolactone ^b	L1	1696/1696
2-Methyl pentanal ^c	Ald18	-/1007	γ-Octalactone	L2	1912/1913
Furans			γ-Decalactone	L3	2153/2153
2-Pentyl-furan ^{b,d}	F1	1234/1234	γ-Dodecalactone	L4	2381/2381
Esters			Mesifuran	L5	1587/1587
Methyl acetate ^{b,d}	E1	828/828	Alcohols		
Ethyl acetate ^b	E2	890/890	Ethanol ^{b,d}	Alc1	935/935
Methyl propionate ^d	E3	908/908	1-butanol ^d	Alc2	1146/1146
Methyl butanoate ^{b,d}	E4	985/985	2-Methyl-1-pentanol ^d	Alc3	1297/1300
Ethyl butanoate ^{b,d}	E5	1034/1034	2-Heptanol	Alc4	1315/1315
Isopropyl butanoate ^d	E6	1036/1037	1-Hexanol ^b	Alc5	1352/1351
Butyl ethanoate ^d	E7	1070/1070	(E)-2-Hexen-1-ol ^{b,d}	Alc6	1402/1402
Methyl pentanoate ^d	E8	1083/1083	1-Octen-3-ol ^{b,d}	Alc7	1458/1458
3-Methyl-1-butanol acetate ^d	E9	1118/1118	1-Octanol ^{b,d}	Alc8	1551/1551
Ethyl pentanoate ^d	E10	1127/1127	1-Nonanol ^{b,d}	Alc9	1652/1652
Methyl 4-methylpentanoate ^d	E11	1136/1134	6-Nonen-1-ol ^d	Alc10	1714/1710
Pentyl acetate ^d	E12	1162/1162	Benzyl alcohol ^d	Alc11	1873/1873
Esters			Alcohols		
Methyl hexanoate ^{b,d}	E13	1182/1182	Phenylethyl alcohol ^{b,d}	Alc12	1906/1906
2-Methylpentyl acetate ^d	E14	-/1203	Nerol ^{b,d}	Alc13	1795/1795
Butyl butanoate ^d	E15	1201/1203	2-Methyl-1-propanol ^{b,d}	Alc14	1098/1098
Ethyl hexanoate ^{b,d}	E16	1221/1221	1-Pentanol ^{b,d}	Alc15	1256/1256
Hexyl acetate ^{b,d}	E17	1260/1260	1-Heptanol ^d	Alc16	1450/1450
Methyl heptanoate ^b	E18	1273/1274	3-Phenylpropanol ^d	Alc17	2040/2041
Methyl 2-Hexanoate ^d	E19	1272/1281	3-Phenyl-2-propan-1-ol ^d	Alc18	1941/1936
(Z)-3-Hexen-1-ol acetate	E20	1306/1306	(E)-2-Nonen-1-ol	Alc19	1703/1706
2-Hexen-1-ol acetate ^d	E21	1327/1329	Terpenes		
Heptyl acetate	E22	1369/1368	Linalool ^{b,d}	T1	1540/1540
Methyl octanoate ^{b,d}	E23	1377/1377	β-Farnesene ^{b,d}	T2	1657/1657
Butyl hexanoate ^d	E24	1399/1399	α-Muurolene ^d	T3	1712/1712
Hexyl butanoate ^d	E25	1405/1403	(6E)-Nerolidol ^{b,d}	T4	2028/2028
Ethyl octanoate ^{b,d}	E26	1428/1428	β-Myrcene ^{b,d}	T5	1156/1156
Hexyl 3-methylbutanoate ^d	E27	1430/1430	Limonene ^{b,d}	T6	1169/1170
3-Hexenyl butanoate ^d	E28	1452/1448	β-cis-Ocimene ^b	T7	1246/1245
2-Hexenyl butanoate ^d	E29	1463/1463	β-Bisabolene ^{b,d}	T8	1711/1713
Octyl acetate ^d	E30	1469/1469	α-Curcumene ^d	T9	1766/1766
Hexyl hexanoate ^d	E31	1597/1597	Terpene 1 ^d	T10	-/1827
Octyl butanoate ^d	E32	1605/1605	Terpene 2 ^d	T11	-/1849
Ethyl decanoate ^{b,d}	E33	1630/1630	Terpene 3 ^d	T12	-/1933
Octyl 3-methylbutanoate ^d	E34	1654/1636	Terpene 4 ^d	T13	-/1994
(E)-2-Hexenyl hexanoate ^d	E35	1660/1658	Pyrans		
Phenylmethyl acetate ^d	E36	1720/1723	Nerol oxides ^d	P1	1464/1464
2-Phenylethyl acetate ^d	E37	1805/1807	Unknown		
Benzenepropyl acetate ^d	E38	1941/1936	Unknown 1 ^d	U1	-/961
Methyl cinnamate ^{b,d}	E39	2080/2081	Unknown 2 ^d	U2	-/1255
Ethyl Cinnamate ^d	E40	2135/2135	Unknown 3 ^d	U3	-/1260
3-Methylbutyl butanoate ^d	E41	1258/1259	Unknown 4 ^d	U4	-/1311
Isopentyl hexanoate ^d	E42	1452/1452	Unknown 5 ^d	U5	-/1351
2-Methyl-Octyl-butanoate ^d	E43	1620/1623	Unknown 6 ^d	U6	-/1492
Esters			Unknown		
Citrenolol acetate ^d	E44	1656/1657	Unknown 7 ^d	U7	-/1496
Nerol Acetate ^d	E45	1722/1722	Unknown 8 ^d	U8	-/1549
Geranyl acetate ^d	E46	1754/1754	Unknown 9 ^d	U9	-/1660
Geranyl propanoate ^d	E47	1813/1814	Unknown 10 ^d	U10	-/1667
Geranyl isobutyrate ^d	E48	1855/1871	Unknown 11 ^d	U11	-/1700
Geranyl butyrate ^d	E49	1889/1889	Unknown 12 ^d	U12	-/1759
Geranyl 2-Methylbutyrate ^d	E50	1889/1889	Unknown 13 ^d	U13	-/1768

(continued on next page)

Table 1 (continued)

Volatile compound	Code	LRI _{lit} /LRI _{sp} ^a	Volatile compound	Code	LRI _{lit} /LRI _{sp} ^a
Geranyl hexanoate ^d	E51	2089/2075	Unknown 14 ^d	U14	-/1810
Ethyl 2-butenate ^{b,d}	E52	1158/1158	Unknown 15 ^d	U15	-/1851
Methyl salicylate ^d	E53	1771/1771	Unknown 16 ^d	U16	-/2057
Ethyl nonanoate ^{b,d}	E54	1537/1537	Unknown 17 ^d	U17	-/2450
Ester 1	E55	-/2208			
Ester 2 ^d	E56	-/2212			

^a LRI_{lit}: Linear Retention Indices reported in literature by www.nist.gov; LRI_{sp}: Linear Retention Indices calculated against n-alkanes (C5–C29) on VF-WAXms column.

^b VOCs identified by chemical standards.

^c Internal standard (I.S.).

^d VOCs with loading values smaller than -1 and greater than $+1$.

higher than that registered for CB (0.43 vs 0.26 %, on average; $P < 0.05$), increasing after 9 days to 0.51 and 0.40 %, respectively. Similar results were reported for kiwifruit, where lower respiration rate and weight loss were found for fruit subjected to citral treatment compared to untreated samples, during entire refrigerated storage (Wei et al., 2021b). The positive effect of citral on the respiration rate and weight loss might be due to the preservation of the cellular membrane integrity as previously reported for kiwifruit treated with citral (Wei et al. (2021b)

3.3. Microbial evaluation of fresh strawberries stored in patch-activated packaging

The overall fungal counts on stored strawberries were, on average, much higher than those of mesophilic bacteria, which were undetected in the samples at the beginning of treatment (Fig. 3). After 3 days of storage, all samples showed similar increases in TBC by an average of 2 log cycles. Unlike the samples treated with citral (CA and CB) that showed no change in TBC until the end of storage ($P > 0.05$), the CTRL samples showed substantial increases in bacterial concentrations after 9 days (Fig. 3). On day 3, substantial ($P < 0.05$) increases in counts were obtained for the total yeast and mould (Y/M) population of CTRL and CA samples when compared to the untreated ones, whereas they were similar ($P > 0.05$) in CB samples. These results were consistent with the low rot incidence that became detectable on control samples only after 6 days of storage. Recently, a strong antifungal activity on strawberries inoculated with three strains of *B. cinerea* has been shown when paper patches impregnated with different essential oils were used (Tančinová et al., 2022). Nevertheless, the concentrations used were much higher than those applied in the present study. A strong efficacy (5.20 % of decay rate after 7 days) has also been reported for strawberries dipped in a water solution of a citral-cyclodextrin inclusion complex, packed in plastic airtight boxes containing the same complex in powder (sustained-release treatment), and stored at room temperature. Thus, the authors suggested that citral vapour could be used as a potential preservative for controlling postharvest decay of fresh fruit (Shen et al., 2024). Recently, we have also shown the effectiveness of citral treatments in controlling moulds and related rot incidence on strawberry during storage (Quintieri et al., 2022).

3.4. Volatile organic compounds of fresh strawberries stored in active packaging

A total of 144 volatile compounds were detected in strawberry samples from the control treatment and in those treated with citral patches, of which 23 were unknown (6 out of 23 VOCs were identified only for their chemical classes). These volatile compounds were grouped in the following classes: aldehydes ($n = 17$), ketones ($n = 5$), carboxylic acids ($n = 9$), esters ($n = 56$), furan ($n = 1$), pyran ($n = 1$), lactones ($n = 5$), alcohols ($n = 19$), terpenes ($n = 13$) and unknown compounds ($n = 17$; Table 1).

PCA was calculated on the averaged and auto-scaled data to assess any sample grouping due to the different packaging typology. The PCA

model describes 71.8 % of the total explained variance with 3 PCs. The biplots for PC1-PC2 and PC1-PC3 are shown in Fig. 4, while Tables S2 and S3 display the variables with correlation loadings values greater than $+1$ and smaller than -1 , arbitrarily chosen as cut-off values. Although no specific pattern was observed in the distribution of samples for the two PCA biplots, some potential correlations between selected VOCs and relevant samples were observed.

Specifically, in the PC1-PC2 biplot, fresh samples (day 0) are placed in the quadrant with negative PC1 and PC2 values, along with CTRL and CB samples that were stored for 3 days. The results for fresh samples also aligns with negative PC1 and positive PC3, enriched in methyl 4-methyl pentanoate, isopentyl hexanoate, 3-methyl butyl butanoate, isopropyl butanoate, and some common strawberries volatiles such as methyl butanoate (Yan et al., 2018), methyl pentanoate (Ulrich et al., 2018) and methyl hexanoate (Du et al., 2011; Neri et al., 2015; Schwieterman et al., 2014; Ulrich et al., 1997, 2018; Yan et al., 2018). After 6 and 9 days of storage, CTRL samples, with negative PC1 and positive PC2, stand out for β -farnesene, characterized by woody green odour (Abouelenein et al., 2023), Hexyl butanoate, preferred by consumers for sweetness-like odour (Fan et al., 2021), methyl cinnamate (ester considered key volatile compound in strawberries for Urrutia et al., 2017), 1-methylhexyl butanoate, along with 2-hexenyl butanoate (Abouelenein et al., 2023), and hexanoic acid (Abouelenein et al., 2023; Ulrich et al., 2018) contributing to the strawberries' aroma profile. After 9 days, the CTRL samples, with negative PC1 and PC3, features octanoic acid with fatty, waxy, rancid, vegetable oil odour (Abouelenein et al., 2023), hexanoic acid with sweet-cheesy odour (Abouelenein et al., 2023), γ -caprolactone, that increases with ripening (Neri et al., 2015), together with dodecanoic acid, hexyl 3-methyl butanoate, α -muurolene and 2-nonenal. After 3 and 6 days, γ -dodecalactone and linalool were detected in CA samples, associated with fruity and sweetness odour (Fan et al., 2021; Ikeura et al., 2008), and characteristics of strawberries aroma profiles (Ulrich et al., 2018; Yan et al., 2018) 2-methylpentyl hexanoate, evident in the plot with positive PC2. After 9 days, CA and CB samples were positioned in the quadrant with positive PC1 and negative PC2. These samples were characterized by compounds derived from neral and geranial (nerol, nerol oxide, citronellol acetate, nerol acetate, geranyl acetate), as confirmed by the undetectability of these analytes in fresh samples (data not shown).

Interestingly, after 3 and 6 days in storage, the samples that were treated with a lower concentration of citral (CA) displayed a VOC profile similar to that of ripe strawberries. After 9 days, both the citral-treated samples (CA and CB) had elevated levels of citral and its derivatives, though to a lesser extent in the CA samples.

These results agree with those from the sensory evaluation carried out by panellists, who preferred strawberries packaged with the lowest concentration of citral, which were considered marketable for 9 days. On the other hand, after 9 days of storage, CTRL samples were well separated from the fresh ones by PC2 and PC3, and from CA and CB samples by PC1 and PC2. Indeed, these samples were judged not acceptable after 6 days of storage due to the development of aroma compounds related to advanced senescence. These results might be also

curation, Conceptualization.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2025.100903](https://doi.org/10.1016/j.afres.2025.100903).

Data availability

Data will be made available on request.

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