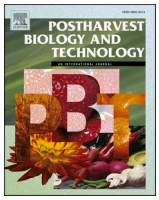
Postharvest Biology and Technology 204 (2023) 112430

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio



Innovative limonene and chitosan-based pads in improving shelf-life and preserving the volatile profile of fresh strawberries

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ARTICLE INFO

Keywords:
Natural active compounds
Innovative packaging
Antimicrobial activity
Fruit quality
Sustainability

ABSTRACT

Strawberries are extremely perishable fruit with a short shelf life due to its high susceptibility to mechanical injury, water loss, texture deterioration and microbiological decay, that rapidly reduces its marketability. The development of innovative and green strategies able to improve quality of strawberry is a current challenge. In this work, active adsorbent pads, consisted of a cellulose layer activated with either Chitosan (CH) or Limonene and Chitosan (CH-LO) solutions, were developed in order to preserve fresh strawberries. Results show that the strawberries stored at 8 °C in packaging containing CH or CH-LO coated-pads are marketable 3 d longer than controls. Furthermore, the CH pads allow for a reduction in respiratory activity and the mold counts. VOCs analysis carried out by HS-SPME/GC-MS revealed 123 compounds. PCA analysis, applied to the GC–MS dataset, to highlight cluster sampling, show that after 3 and 6 d of incubation, fruit stored with CH-LO are comparable to fresh strawberries. Although further experiments are needed to improve antimicrobial efficacy of the developed active pads, these results show a promising approach to preserve quality of strawberry during refrigerated storage.

1. Introduction

Strawberries are highly perishable and can be stored only for 1–2 d at room temperature during post-harvest (Garcia et al., 2012). Rapid cooling after harvest and storage at 0–4 °C extends shelf-life to about 5 d (Vargas et al., 2006) but even under such conditions growth of gray mold (*Botrytis cinerera*) can dramatically shorten the shelf-life of the fruits (Niu at al., 2021).

Synthetic fungicides during the crop growing cycle are used for the control of *B. cinerea* in postharvest. The fungicides active against the fungus *B. cinerea* used in preharvest strawberry are numerous and recently summarized by Feliziani and Romanazzi (2016). Nevertheless, *B. cinerea* is a 'high-risk' pathogen because the development of resistance to several classes of fungicides has been frequently reported worldwide (Myresiotis et al., 2007; Nielsen et al., 2022). For this purpose, Fungicide Resistance Action Committee (FRAC) have recently suggested the use of a limited number of fungicide applications per year. Therefore, the applying of an appropriate postharvest treatment to delay respiration, prevent physical damage and dryness, and to restrict microbial decay is a challenge for extending the shelf life of these fruit in postharvest. Indeed, in the last few decades, consumer demand is looking for products free in preservative or added with only when their presence is required (Realini and Marcos, 2014).

In this context, active packaging offers a good solution because it offers a protection to products, affecting their quality and health safety (Wyrwa and Barska, 2017). One of the most innovative and flexible applications of active packaging systems are the antimicrobial food pads (Corrales et al. 2014; Bovi et al. 2018); these are based on the classical cellulosic pads, commonly used to absorb moisture and liquid from fresh food packaged, that improve the esthetical and protect from unhealthy

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https://doi.org/10.1016/j.postharvbio.2023.112430
Received 26 January 2023; Received in revised form 7 June 2023; Accepted 8 June 2023
Available online 19 June 2023

exudate. The latter is generally responsible for unwanted smell, spoil food and spread of pathogens carried by food. The antimicrobial food pads present a multilayer structure in which the middle layer consists mostly of cellulose fibers and active ingredients that absorb excess fluid and inhibit the microbial growth (Ogunsona et al., 2020). They are classified into two major categories: contact and non-contact absorbents (Fernández et al., 2009). Cellulosic materials binding diverse molecules through electrostatic interactions (Fahma et al., 2010; Zhang et al., 2017), can be considered good carriers for antimicrobial materials such as silver nanoparticles (Fernández et al., 2010), essential oils (Oral et al. 2009), and carbohydrates (Bovi et al., 2018).

Chitosan, a non-toxic, non-antigenic, biocompatible polymer derived from shells of crustaceans, crab, lobster, and shrimp, has attracted a notable attention for its great potential of acting as an antibacterial and moisture absorber of natural origin (Santos et al., 2020). Chitosan isolated from shrimp is considered GRAS (Generally Recognized as Safe) in several food categories (GRAS Notice No. GRN 000443) (Raafat and Sahl, 2009; Garg et al., 2019). The integration of chitosan, as active substance, into the matrix of absorbing pad structures, is promising; indeed, it might absorb free water in the tray and prevents spread of pathogens in the package (Omer et al., 2022). Recently, Li et al. (2022) used a chitosan-natamycin pad to store fresh plums with positive effects on the reduction of browning index, and the polyphenol oxidase enzymatic activity, maintaining excellent fruit quality during the storage period. In addition, active film-pads produced with chitosan, green tea (GTE) and rosemary ethanolic extracts as natural antifungal agents to improve the shelf-life of fresh red raspberry were used (Vieira et al. 2022).

Essential oils (EO) are natural compounds, with potential antioxidant and/or antimicrobial activity, extracted from vegetables and fruit. They consist of a combination of terpenes, terpenoids and other aromatic and aliphatic constituents and with a composition that may change significantly varying on the specific oil (Bakkali et al., 2008). Lemon essential oil, which is extracted from *Citrus lemon*, is mainly composed of limonene, valencene and ocimene (Moufida and Marzouk, 2003). Limonene, has the GRAS status of the US FDA (US EPA, 1994), and is utilized as a food additive or flavoring agent, and is acknowledged for its fungicidal activity, including *Botrytis* spp. and *Aspergillus niger* (Sharma and Tripathi, 2008). The combination of chitosan and limonene for the development of active food packaging materials able to increase the safety and quality control of agricultural products is pursued (Maleki et al., 2018; Lan et al., 2020; Barbosa et al., 2022). However, despite the antimicrobial activity of limonene incorporated into chitosan films, these latter show poor mechanical properties that limit their applications as active packaging (Sánchez-González et al., 2011); in this regard, the development of antimicrobial pads, based on cellulose is encouraged.

Taking into account these considerations, in this paper, the susceptibility of *B. cinerea* to limonene and chitosan is in vitro evaluated; then, adsorbent pads were prepared with chitosan alone or chitosan and limonene and kept in packs of fresh strawberries for 9 d at 8 °C. During storage, the main quality and microbiological parameters of strawberries were assessed and a detailed evaluation of volatile organic compounds (VOCs) was carried out.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals, standards and reagents for postharvest quality analysis were from Sigma-Aldrich (Milan, Italy). Folin–Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Germany) whereas microbiological media were obtained from Oxoid (Milan, Italy).

Methanol (HPLC grade), Limonene (Purity 97 %; Mw: 136.23 g mol^{-1}; CAS Number: 5989–27–5), Chitosan from shrimp shells (viscosity: 200 mPa.s, 1 % in acetic acid (20 °C); CAS Number: 9012–76–4) were purchased from Sigma–Aldrich (Milan, Italy). Amphotericin B 20 U disks were purchased from Condalab (Madrid, Spain). The 2-methylpentanal (≥98 %) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Chemical standards (Hexanal, (E)− 2-Hexenal, Benzaldehyde, 2-Pentylfuran, Methyl butanoate, Ethyl butanoate, Ethyl (E)− 2-butenoate, Methyl hexanoate, Ethyl hexanoate, Hexyl acetate, Methyl heptanoate, Methyl octanoate, Ethyl octanoate, Ethyl decanoate, Acetic acid, Acetone, 3-Octanone, Acetoin, γ-Caprolactone, Ethanol, 2-Methyl-1-propanol, 1-Pentanol, 1-Hexanol, (E)− 2-Hexen-1-ol, 1-Octen-3-ol, 1-Octanol, 1-Nonanol, Phenylethyl Alcohol, D-Limonene, Linalool, (6*E*)-Nerolidol) 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. In vitro evaluation of limonene and chitosan antimicrobial activity against Botrytis cinerea

2.2.1. Inoculum preparation

Three *Botritys cinerea* strains (ITEM 5154, 17199, and 17200), from the Agro-Food Microbial Culture Collection of the Institute of Sciences of Food Production (https://www.ispacnr.it/en/microbial-collection) were cultured on Petri dishes with Potato Dextrose Agar (PDA) and incubated at 25 °C for 7 d to stimulate sporulation. At the end of incubation, the obtained cultures were covered with 5 mL sterile saline (0.9 % NaCl) containing 0.1 mL L^1 Tween 80, softly rubbed with a cotton swab and the conidial suspension moved to a sterile Falcon tube. The spore suspension was filtered (11 μm, Nylon Net Filters, Millipore, Cork, Ireland) and spore counts determined using a Thoma hemacytometer chamber. Serial dilutions (in 0.9 % NaCl solution) to obtain inocula for the following experiments (ca.1 × 10^6 conidia mL^{-1}) were made.

2.2.2. In vitro antimicrobial activity of limonene

The in vitro antimicrobial activity of Limonene against the *B. cinerea* strains was tested using the disk diffusion and volatilization methods with slight modifications (Nedorostova et al., 2009; Fancello et al., 2020). In brief, the antifungal disk diffusion methods were carried out laying sterile paper disks (Whatman No. 1, Darmstadt, Germania, diameter 55 mm) impregnated with different amount of Limonene (ranging from 6.25 to 50 μL) on PDA Petri dishes previously seeded with 100 μL of the fungal inoculum as reported above. Blank disks were soaked with sterile ddH_2O for negative control, whereas Amphotericin B antibiotic disks 20 units (Condalab, Madrid Spain) were used as positive controls. After 2 d of incubation at 25 °C, the diameter of inhibition halos around the disks were measured by a caliber in relation to the assayed Limonene concentrations.

For disk volatilization methods the procedure described by Fancello et al. (2020) was followed, using a concentration range from 0.05 to 0.41 μL cm^{-3} of Limonene. In brief, a mycelial plug (5 mm diameter) was cut from the margin of actively growing 7-day-old colony, using a flamed cork borer, and placed on center of PDA Petri dish. Then, sterile paper disks impregnated with different concentrations of Limonene suitable to obtain a final concentrations range as above reported were taped to the cover of each Petri dish. Plates were then wrapped with Parafilm and incubated for 4 d at 25 °C. The diameter of growing mycelium was recorded at different time intervals (1, 2, 3.5 days).

2.2.3. Minimum inhibition concentration (MIC) determination of limonene and chitosan

For the determination of the minimum inhibition concentration (MIC) of the chitosan and Limonene the broth microdilution method was performed in flat-bottom well microplates (Primo® Cell Culture Plasticware, Euroclone, Milan, Italy), following a modified version of the protocol proposed by EUCAST (EUCAST antifungal microdilution method for molds, 2022). The double strength Roswell Park Memorial Institute 1640 (RPMI) medium 2 % glucose medium (RPMIG) was used

for the assay and it was added with increasing concentrations of Limonene (ranging from 0.039 to 20 μL mL^{-1}) and chitosan (ranging from 5000 μg mL^{-1} to 9.8 μg mL^{-1}). Stock solution (40 μL mL^{-1}) of Limonene was obtained by diluting it in 1 % of DMSO aqueous solution whereas chitosan (10,000 μg mL^{-1}) was dissolved in a solution of 1 % acetic acid, incubated overnight in agitation at 50 °C to complete dissolution and sterilized at 121 °C for 15 min. The inoculum, as previously prepared by spore counts and serial dilution determinations, was diluted in RPMIG to reach a final concentration of 2×10^5 conidia per mL; then 100 μL were dispensed in microplate wells previously filled with 100 μL of limonene or chitosan solution at each concentration (2× final concentration). Uninoculated medium and inoculated medium without antimicrobial compounds were included as controls. Plates were incubated at 25 °C for 3 d. Then, the MICs (μL mL^{-1} or μg mL^{-1}) values were obtained as the lowest limonene or chitosan concentration inhibiting a visible growth (indicated by absence of turbidity) of the *B. cinerea* strains analysed. Tests were done in quadruplicate and experiments repeated twice.

2.3. Preparation of active pads and strawberries storage

Absorbent pads (Sirane Ltd, Telford, UK) measuring 90 × 170 mm were used as active substrates. Pads consisted of a 5 mm layer of cellulose covered in thermoplastic polyethylene (about 200 μm thick). The PE layer is perforated to guarantee the absorption of liquid exudates and to avoid direct contact of the active and absorbent material with the packaged strawberries. A chitosan (CH) based solution was prepared by dissolving chitosan powder in 0.1 M acetic acid until a perfectly transparent 2 % (w/v) solution was obtained; then, chitosan solution was sterilized as reported above. An emulsion based on limonene (LO) was prepared by mixing limonene at 1.5 % w/v with distilled water and Tween 80 (approved additive E433; Commission Regulation UE No. 1129/2011) at a concentration of 0.1 % w/v. Twelve pads were spray-coated with 2.5 mL of CH solution and 2.5 mL 0.1 M acetic acid of on the non-PE coated side and allowed to dry at room temperature for six hours. In the same way, twelve pads were treated with a mixture of 2.5 mL of CH solution and 2.5 mL of LO solution (CH-LO). Six pads treated by spraying with 5 mL of acetic acid and water mixture were used as reference (Ctrl).

Strawberries (*Fragaria* x *ananassa* Duch.) cv Candonga were harvested at a commercial maturity stage (more than 75% full color of berries and total soluble solids of 9.5 ± 0.4 %) by Apofruit Italia Soc. Coop., (Scanzano Jonico, Italy) and transported in refrigerated conditions to the postharvest laboratory of CNR-ISPA located in Foggia. On arrival, damaged, diseased and defected strawberries were excluded and about 1 kg of fruit were used for the initial analysis in four replicates, while the remaining, about 9 kg, were packed into polyetileneterftalate (PET) trays (about 250 g for each one) put inside PE bags containing antimicrobial pads activated with chitosan (CH) or chitosan and limonene essential oil (CH-LO) or non-activated (Ctrl). A total of 36 trays (4 replicates × 3 treatments, CH, CH-LO or Ctrl, × 3 storage times, after 3, 6 and 9 d) were prepared and stored at 8 °C. At each storage time, strawberries belonging to each treatment were analysed for postharvest quality, microbial profile and for the detection of volatile organic compounds.

2.4. Postharvest quality evaluation of strawberry packed with the active pads

2.4.1. Visual quality and respiration rate

Visual quality of strawberries was performed by a group of 6 judges (3 males and 3 females) at each sampling day according the color chart with subjective quality ratings of visual quality deterioration of strawberry reported by Nunes (2015). In this rating scale, from 5 (excellent) to 1 (very poor), the score 3 was attributed to samples with acceptable quality for marketability.

The respiration rate of strawberries was measured at 8 °C at harvest and at each storage time using a closed system as reported by Kader (2002). For each replicate, 250 g of sample were put into 3.6-L sealed plastic jar where CO_2 was allowed to accumulate up to 0.1 % (CO_2 standard). The CO_2 analysis was conducted by taking 1 mL of gas sample from the head space of the plastic jars through a rubber septum, and injecting it 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 μmol CO_2 kg^{-1} s^{-1}.

2.4.2. Total soluble solids, titratable acidity, pH, antioxidant acidity, total phenols

Total soluble solids (TSS), titratable acidity (TA) and pH were determined from approx. 100 g of homogenized strawberries per replicate. The TSS content was determined using a digital refractometer (DBR35-XS Instruments, Carpi, Italy) and expressed in %, while TA was measured using a semiautomatic titrator and pH meter (PH-Burette 24 -Crison Instrument, Barcelona, Spain) with 0.1 M NaOH to the final pH 8.1, and results were expressed as percentage of citric acid. As for pH measurement, the same instrument and the same strawberry juice were used.

The antioxidant activity was measured according the procedure described by Cefola et al. (2014) 2,2-diphenyl-1-picryhydrazyl radical (DPPH) assay. The absorbance at 515 nm was read after 40 min using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The results were expressed as g kg^{-1} of Trolox per fresh weight (fw) using a Trolox calibration curve (82 – 625 μM; $R^2 = 0.999$).

The total phenol content was determined according to the method described by Fadda et al. (2016). Briefly, 100 μL of each extract was mixed to 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 at 765 nm was measured after 2 h of incubation in the dark and the results were reported as g kg^{-1} of gallic acid equivalent (GAE) per fw. The calibration curve of gallic acid was prepared with five points, from 50 to 500 μg mL^{-1}, with $R^2 = 0.998$.

2.4.3. Microbial evaluation

Total aerobic mesophilic bacteria (TBC), and total yeast and mold (Y/M) counts were monitored in triplicate in treated (CH and CH-LO) and control strawberries (Ctrl) stored at 8 °C. at 0, 3, 6 and 9 d of storage. Under aseptic conditions, 25 g of each sample was added to 225 mL of sterilized peptone water (1 g L^{-1}) in a stomacher bag and homogenized for 2 min. Ten-fold serial dilutions were done and plated by standard microbiological pour plate technique. All the microbiological counts were carried out in triplicates. TBC counts were determined using plate count agar (PCA) amended with cycloheximide (0.1 g L^{-1}) at 30 °C for 24–48 h (ISO 21527–1.2008), whereas Y/M counts were determined both by dichloran rose bengal chloramphenicol agar (DRBC agar base), chloramphenicol, 0.1 g L^{-1} (ISO, 4833.2003) and Potato dextrose agar (PDA); counts were enumerated after 3–5 d of incubation at 25 °C (Bugatti et al., 2020).

All berries with visible molds were considered infected and registered in relation to total number of berries of each tray at 0, 3, 6 and 9 d of storage.

2.5. Determination of volatile organic compounds (VOCs)

Aliquots of strawberries (about 100 g) cut into small pieces, were manually homogenized in a mortar keeping the samples in ice bath to reduce degradation processes. A set of 20 strawberry samples was analysed by an optimized method based on headspace solid phase micro extraction and gas-chromatography mass-spectrometry (HS-SPME/GC–MS). In particular, Volatile Organic Compounds (VOCs) analysis were performed on 2 fresh samples (five replicates) and during storage

at 8 °C on 18 samples [2 samples; 3 treatments (CH, CH-LO and Ctrl); 3 storage times (3, 6 and 9 d)] analysed in triplicate for a total number of 64 analyses. A GC/MS system composed by an Agilent 7890 A GC (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975 C inert MSD mass spectrometer equipped with Triple-Axis HED-EM detector, was used for VOC analysis. For VOCs extraction and desorption, a HS-SPME procedure was performed using a multi-purpose autosampler MPS 2 (Gerstel, Mülheim an der Ruhr, Germany), fitted with headspace incubation chamber and SPME sampling unit and heating block. Specifically, in order to generate the sample headspace, a 10-mL headspace vial with magnetic screw cap and a pierceable PTFE/silicon septa (Agilent Technologies, Palo Alto, CA, USA) containing 2.5 g of homogenized sample, added with 2.5 μL of 2-methyl-pentanal (10 g L^{-1} in methanol), used as internal standard, was placed into the incubator of the MPS 2 autosampler at 50 °C for 15 min. Before application, the fiber was inserted into the GC injector for conditioning at the temperature of 270 °C for 60 min. Subsequently, for analyte extraction, a SPME divinylbenzene/carboxen/polydimethylsiloxane fiber (SPME-Fast Fit Fiber Assembly-FFA-DVB/CAR/PDMS, 50/30 μm film thickness 1 cm fiber length, Supelco, Bellafonte, PA, USA) was placed in the sample headspace at 50 °C for 30 min by using the autosampler. After extraction, the fiber was placed into the CIS-4 Programmed Temperature Vaporization (PTV) injection (Gerstel) port of the GC-MS system in order to thermally desorbed headspace volatiles. The injection port was set at 250 °C and kept for 5 min in splitless mode and was fitted with a 0.75 mm i.d. Ultra Inert Liner Straight (Agilent Technologies).

The strawberry VOCs analysis was carried out by the GC/MS system fitted with a VF-WAXms (60 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies) fused-silica capillary column and using programmed temperature ramp. Temperature program was applied as follows: 40 °C for 5 min, 2 °C min^{-1} up to 140 °C, 5 °C min^{-1} up to 210 °C, 20 °C min^{-1} up to 230 °C and held for 10 min. The helium flow was set to 1 mL min^{-1}. The ion source, transfer line and quadrupole temperatures were 230, 280 and 150 °C, respectively. An electron impact Ionization (EI+) mode with an electron energy of 70 eV was used. The mass spectra were recorded in the *m/z* range of 40–300 u and the total chromatographic run time was 80 min

To identify volatile compounds, samples mass spectra were compared with spectra in the NIST/EPA/NIH Mass Spectral Database (National Institute of Standards and Technology, Version 2.0f, 2008, USA) applying a match quality higher than 80. 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 literature in order to confirm the VOC identity (Zellner et al., 2008; www.nist.gov) (National Institute of Standard and Technology, 2019). For a group of volatile compounds (n = 31) the identification were also confirmed using chemical standards. Total ion peak area of the compounds was determined by using MSD Chemstation (Agilent Technologies, Santa Clara, USA). The quantitative evaluation of the compounds was determined as ratio between their peak areas and the 2-methyl-pentanal peak area used as internal standard (2-methyl-pentanal).

2.6. Statistical analysis

Two-way ANOVA procedure was conducted to compare the main effects and their interaction of storage time and kind of treatments on the log-transformed TBC and Y/M counts from strawberry samples using IBM SPSS Statistics 21.0 (SPSS, IBM corp., Armonk, NY, USA). Tukey post hoc comparison test was performed to determine the statistical difference ($P < 0.05$) between means: If homogeneity variance was not met (Levene's test, $P < 0.05$) non-parametric Kruskal-Wallis H test was performed to evaluate differences ($P < 0.05$) between the mean ranks of experimental microbial counts. Post hoc Dunn pairwise test was carried out to compare medians within each microbial group.

Concerning the GC-MS data a pre-processing was carried out before the chemometric analysis. Specifically, the VOC peak areas, determined in relation with internal standard, were used to obtained a matrix of 64 rows (HS-SPME/GC–MS analyses) and 123 columns (variables, VOCs).

Permutational multivariate analysis of variance (PerMANOVA; $\alpha \leq 0.05$) was carried out on prepocessed data comparing VOC profiles of fresh strawberry (F) with VOCs profiles of treated (CH-LO and CH) and control (Ctrl) samples during storage. The post-hoc approach Mann–Whitney U test ($\alpha \leq 0.01$) was applied specifically to treated samples (CH-LO and CH) and Ctrl samples after 6 days of storage to highlight VOC having median values of peak area ratios significantly different with respect to those of fresh samples. PerMANOVA and Mann–Whitney U test were performed using Real Statistics Resource Pack software (Release 7.6; Copyright 2013 – 2021 Charles Zaiontz; www.real-statistics.com) and Statistica 8.0 (StatSoft Italia srl, Padova, Italy), respectively.

Moreover, before performing Principal Component Analysis (PCA), for each variable were averaged values across replicates of the same sample and within the samples of the same treatments, for the fresh samples and for each treatments obtaining a matrix of 10 rows and 123 columns. Average values were normalized by performing Probabilistic Quotient Normalization (PQN). After pre-processing, GC–MS data were analysed using multivariate statistical analysis technique. Specifically, PCA (unsupervised statistical method) was applied with the aim to visualize sample clustering among the different treatments (CH, CH-LO, Ctrl) of strawberries. Samples were coded as F-0d (fresh samples at t = 0), CH_3d, CH_6d and CH_9d (strawberries packed in PET trays with pads activated with chitosan essential oil (CH) at t = 3, 6 and 9 d of storage, respectively), CH-LO_3d, CH-LO_6d and CH-LO_9d (strawberries packed in PET trays with pads activated with a mixture chitosan and limonene essential oils (CH-LO) at t = 3, 6 and 9 d of storage, respectively), CTRL_3d, CTRL_6d and CTRL_9d (control strawberries at t = 3, 6 and 9 d of storage, respectively). The VOCs that most greatly contributed to the separation in PCA were selected by applying an arbitrary cut-off, for the loading values, equal to 0.2 in absolute value (i.e. VOCs with loading values smaller than −0.2 and greater than 0.2). PCA analysis were carried out by using V-Parvus 2010 (Forina et al., 2010).

3. Results and discussion

3.1. In vitro antimicrobial activity of chitosan and limonene

The results, obtained by disk diffusion method, confirm the ability of Limonene (LO) to counteract the growth of spoilage microorganisms and, as reported by other authors (Vega-Vásquez et al., 2021; Amiri et al., 2022), to inhibit all *B. cinerea* strains in a concentration-dependent manner (Table S1). In particular, the highest antifungal activity against ITEM 5154 and ITEM 17200 compared to Amphotericin B (AMPH) was registered starting from 12.5 μL of Limonene (Table S1). Similar results were obtained when LO was assayed by disk volatilization method as showed in Table S2; the inhibition of *B. cinerea* strains tested was in function on the concentration of gaseous LO in the head space of petri dishes (Table S2). Except for the highest assayed concentration, where 0.41 μL cm^{-3} of LO registered a slightly inhibition also after 84 h of incubation, the inhibition activity of limonene reduced its activity until disappearing completely after 24 h. Finally, regarding the statistical analyses, for the effect of different gaseous limonene concentrations the ANOVA analysis highlighted a significant effect of concentration ($P < 0.001$), strain ($P < 0.001$) and time ($P < 0.001$) on the diameter of inhibition. The MIC of LO was the same (1.25 μL mL^{-1}) for all *B. cinerea* strains tested. The MIC of Chitosan was tested at the required concentrations (see Materials and Methods) against the strains of *B. cinerea* ITEM 5154, ITEM 17200 and ITEM 17199, but no antimicrobial activity was observed at the concentration used. This last result, mostly confirmed the results obtained in the in vivo experiments (see below).

Chitosan did not show antimicrobial activity regardless the assayed concentrations; thus, the final concentration of 1 % was selected by

following previous studies addressed on the development of chitosan and essential oil composite films (Sánchez-González, et al., 2011; Castanheiro, 2021).

LO belongs to the family of terpenes which are characterized by a high volatility (vapor pressure of limonene equal of 1.33 × 102 Pa at 20 °C) and are prone to oxidative degradation (CLH report, 2018); by considering that residence time of terpenes in environment could be too low to exhibit antimicrobial efficacy over strawberries storage times, we preferred to not use LO solely. Not for nothing, the research of innovative coating with polymeric materials or the incorporation in microcapsules or microspheres is constantly pursued to preserve the quality and prolong the longevity of highly volatile compounds (Shah et al., 2016, Lopes et al., 2019; Akhavan-Mahdavi et al., 2022; Zhang, et al., 2022).

A careful study of published works based on the application of EO-CH based coatings showed that the effective concentration of EOs, containing ca. 37–50 % of LO, was usually not lower than 0.5 % to preserve fruit quality (Wang et al., 2022a, Chetia et al., 2023). In addition, Ibáñez et al. (2020) highlighted that MIC concentrations of limonene are variable according to its stereochemistry and the target strain. Thus, both taking these considerations into account and the effect due to the interaction of LO with the food system (Antunes and Cavaco, 2010; Zheng et al., 2023), we decided to apply a higher concentration of LO (ca. 6-fold the MIC value) in comparison to that determined by in vitro assays.

3.2. Quality and microbial traits of strawberry packed with the antimicrobial pads

During storage at 8 °C the strawberries packed in PET trays with active pads (CH or CH-LO) and control samples were analysed for the main quality parameters.

Regardless treatments, the total soluble solids (TSS), titratable acidity and pH registered constant values of 9.5 (± 0.5) %, 0.76 (± 0.02) % citric acid and 3.7 (± 0.04), respectively throughout the storage. Also the antioxidant activity and total phenol content did not change over the storage by registering values of (3.23 ± 0.12) g kg^{-1} Trolox (per fw) and 2.12 ± 0.34 g kg^{-1} gallic acid (per fw), on average, in all packaging conditions.

Visual quality scores, showed a reduction in all packaging conditions (Fig. 1A), due to mold development, which affected strawberries appearance. Anyway, a significant effect of packaging was showed. In particular, fruit packed with CH and CH-LO pads reported higher scores than control samples, after 3 and 6 d in storage. The use of the active pads caused an increase in strawberries visual shelf-life of about 3 d in respect of the control sample, resulting marketable for about 6 d at 8 °C. Then, after 6 d of storage, panelists assigned lower visual quality scores to CH and CH-LO samples (Fig. 1A), probably because of the mold development occurred.

Fresh strawberries showed an initial respiration rate of about 11 μmol CO_2 kg^{-1} s^{-1}, in line with previously observation (Cozzolino et al., 2021). During storage, significant increases were measured in all samples, even if fruit packed with CH or CH-LO pads showed reduced respiration rate at each storage day, in comparison with control (Fig. 1B). The increase in respiration rate during storage is related to fruit damage occurring during storage as also reported by microbial analysis. As regards the effect of CH pads on respiration rate, the results reported in this article, confirmed the action of chitosan in reduce respiration rate of strawberry, if it is used as active pads, and this effect is probably due to the antioxidant proprieties of chitosan (USFDA, 2001; EU, 2012).

Moreover, the chitosan action is probably due to the slowdown of the volatilization of LO while maintaining the active concentration of LO in strawberry packs (Barbosa et al.; 2022).

With regard to microbiological analyses no inference can be drawn in relation to the effect of time and treatment level on microbial data

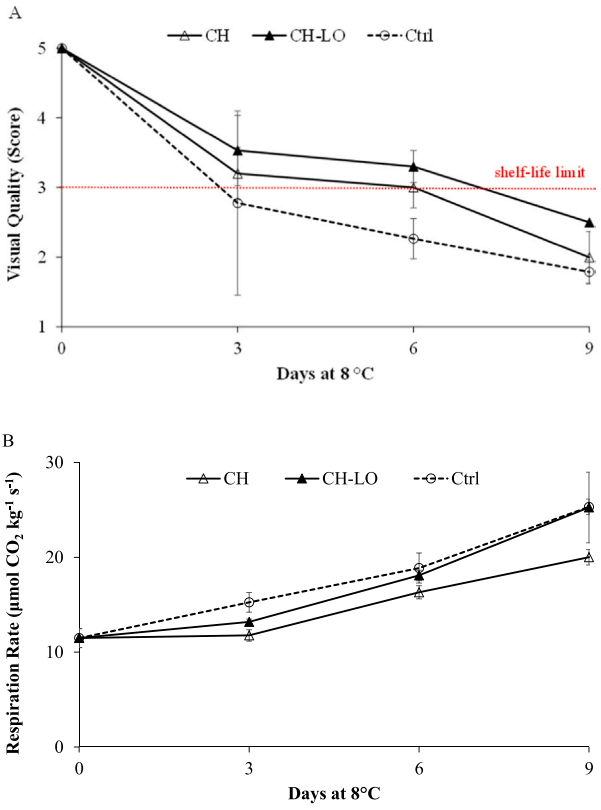

Fig. 1. Changes in visual quality score (panel A) and respiration rate (RR) (panel B) during the storage of strawberries packed in PET trays with pads activated with chitosan (CH) or chitosan and limonene essential oil (CH-LO) or non-activated (Ctrl).

because variance did not meet homogeneity. By using non parametric Kruskal-Wallis H test, statistically significant differences in mean ranks of TBC and TM/Y were found among the tray treatments, ($\chi^2(9) =$ 69.227–59.973, $p < 1.357 \times 10^{-9}$), with mean ranks ranging 5.94–80.61 and 5.00–73.78, respectively.

Results of microbiological analyses (Fig. 2) put in evidence that at day 3 of storage TBC loads in CH treated samples significantly increased ($p < 0.01$) by 1.0 log-cycle in median in comparison to those registered at the early time of storage; by contrast, compared to the time 0 of sampling, CH-LO treated samples and control ones showed significant increases in TBC loads starting from 6 days of storage. No inhibitory effect on TM/Y counts was instead registered in strawberry packs treated with CH and CH-LO. Recently, the inhibitory effect of chitosan based films, incorporated with EO of *Perilla frutescens* leaves, against *B. cinerea* was evaluated during the storage of strawberries (Wang et al., 2022b). Pure chitosan based film attached to the container lid did not show any antimicrobial effect; by contrast chitosan based films incorporated with EO reduced *B. cinerea* infections (Wang et al., 2022b). Despite what was observed in the in vitro assays and the higher concentration assayed in active pads, limonene did not affect TM/Y load over strawberries storage. No clear explanation for this finding was found, although interactions between the essential oils and the fruit surface can lead to divergent behavior with respect to that observed in the in vitro studies as also reported by other authors (Antunes and Cavaco, 2010; Perdones et al., 2012; Zheng et al., 2023).

As regards decay, strawberries in treated trays displayed percentages (5.80%, on average) similar ($P > 0.05$) to those of control samples (15.04%, on average) up to day 6 of storage, whereas at day 9 the strawberries in CH treated packs showed a sharp reduction in infection by means of 30% in comparison to those of control samples (Fig. 3). Contrary to the extensive use of chitosan as a coating on various fruits

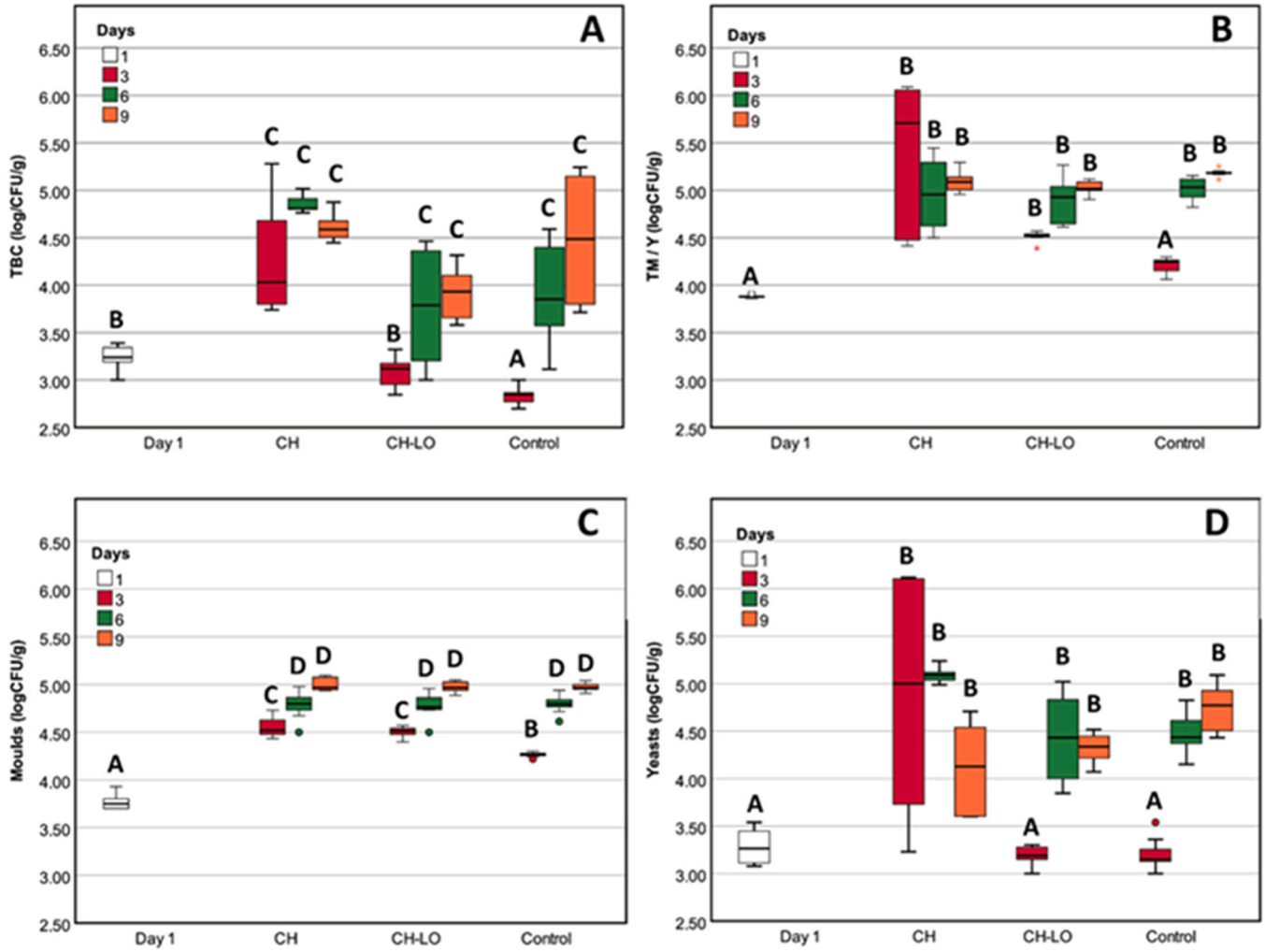

Fig. 2. Box plots of total bacterial counts (TBC), total counts of molds and yeasts (TY/M) on DBRC-agar medium (B) and molds (C) and yeasts (D) counts on PDA medium registered on strawberries samples packed with chitosan (CH), chitosan + limonene (CH-LO) or nothing (Ctrl) and stored at 11 °C for 9 d. Day 1: early time of storage. N = 9. Boxplots show the median, first and third quartiles as boxes, with whiskers representing the 5% and 95% intervals. Significant differences ($p < 0.01$) are denoted with different letters according to Kruskal–Wallis ANOVA with Dunn's test and Bonferroni correction.

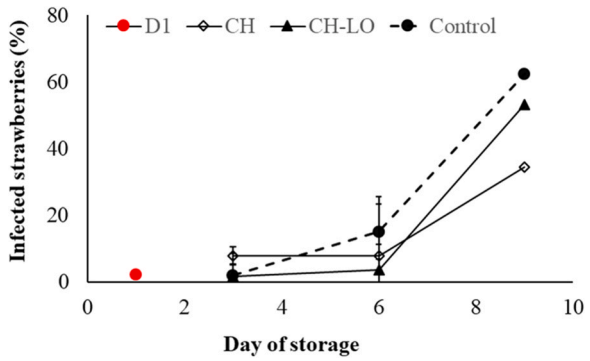

Fig. 3. Percentage of strawberries with visible molds assessed during the storage in PET trays with pads activated with chitosan (CH) or chitosan and limonene essential oil (CH-LO) or non-activated (Control). Day 1 (D1): early time of storage. Values represent mean + standard deviation (N = 3).

thus extending their shelf life, to our knowledge there are no examples of the use of chitosan-based strips or pads for the same purposes. A possible explanation of CH pad efficacy can be given by the strong affinity of chitosan for water uptake (Akter et al., 2014), controlling humidity and condensation inside the strawberry trays. The use of moisture absorbers is increasingly gaining ground in food packaging (Gaikwad et al., 2019); in addition, this strategy has proved effective in counteracting the increase in decay on strawberry as well as several fruits (Bovi et al., 2019; Deshwal et al., 2021). The remaining autocomes were partially agreed with those registered by Vu et al., 2011 reporting that mold decay of strawberries treated with CH-LO were significantly lower than that of control sample; however, the treatment was efficient for preserving the quality of strawberries for 12 days. This effect could be due to the type of application; indeed, CH-LO was applied by spraying directly suspensions on strawberries to form an edible coating; this could also explain the low concentration of LO assayed (0.02 %) by Authors. The interaction between the antimicrobial compounds (CH and CH-LO) and fruit surfaces also suggested the higher antifungal and/or anti-*Botrytis* activity registered for pure chitosan coatings or chitosan-EOs coatings reported by several Authors (Vargas et al., 2006; Ghosh and Katiyar, 2019; Zhang, et al., 2021). However, it is noteworthy that the application mode of essential oils and related pure component in coating and polymeric films to obtain active packaging showed different levels of efficacy in foods preservation (Antunes and Cavaco, 2010; Casalini and Giacinti Baschetti, 2023).

3.3. Analysis of volatile organic compounds (VOCs)

Analysis of the VOCs of the strawberries packed in PET trays with active pads (CH or CH-LO) and control samples were performed by means of HS-SPME/GC–MS analysis. A total of 123 compounds were found, of which 106 were identified and 17 were unknowns. These volatile compounds were grouped in the following classes: the aldehydes (n = 14), ketones (n = 5), carboxylic acids (n = 8), esters (n = 47), furans (n = 2), lactones (n = 4), alcohols (n = 19), terpenes (n = 7) and unknown compounds (n = 17). The name, abbreviation code, the literature and the experimental Linear Retention Indices for identified volatile compounds are listed in Table 1.

PCA was applied to the GC–MS dataset (data matrix composed by 10 rows and 123 columns) to highlight cluster sampling among the samples stored using different PET trays with active pads (CH, CH-LO) and control samples (Ctrl), showing potential effects of the two tested treatments to preserve the freshness of the product. As shown in Fig. 4 by plotting the scores of the samples in the sub-space PC1 vs. PC2, accounting for 91.1 % and 3.6 % of the total variance, respectively, a differentiation between three groups of samples was observed. The samples close to fresh strawberries are CH-LO after 3 and 6 d and Ctrl after 3 d of storage. In the same quadrant there is a group formed by the samples CH at 3 and 6 d of storage as well as Ctrl samples after 6 d. Moving along PC2 in the upper-right quadrant there is a more scattered group forming by the samples CH, CH-LO and Ctrl after 9 d of storage. This result was in agreement with the presence of high percentages (more than 30 %) of visible molds in strawberries at 9 d of storage as reported in Fig. 3.

The molecular basis of strawberry aroma has been widely studied for several years, but the qualitative composition of the strawberry volatile component remains controversial though considerable progress has been made during the past decades (Ulrich et al., 2018). Fig. 4 shows a PCA biplot obtained by plotting selected VOCs with loading values smaller than − 0.2 and greater than 0.2 (used as arbitrary cut-off values) with scores values. As reported in Table 1, the selected 10 VOCs, were Methyl hexanoate (E17), Hexanoic acid (C6), (E)− 2-Hexenal (Ald5), Methyl butanoate (E4), γ-Decalactone (L3), Butyl ethanoate (E9), (E)− 2-Hexen-1-ol (Alc9), 1-Hexanol (Alc8), Mesifurane (F2) and Benzaldehyde (Ald10). All these compounds but Alc8 and Ald10 were reported in the review edited by Ulrich et al. (2018) as the VOCs most frequently found in strawberries.

Interpretation of the VOCs shown in Fig. 4 allow understanding which are the most greatly contributed to the separation of the three groups identified. The VOCs with high positive loading values on PC2 (Ald10, Alc8, F2, E9 and Alc9) were the molecules mainly contributing to separate samples after 9 days of storage from those at 3 and 6 days of storage as well as from fresh samples. These results were in agreement with those reported in literature. In particular, the ester Butyl ethanoate (E9) was correlated negatively with consumer acceptance (Ulrich and Olbricht, 2016). Furthermore, Neri at al. (2015) observed an increase of the alcohol (E)− 2-Hexen-1-ol (Alc9) of about 13-fold in wounded strawberries in comparison to whole undamaged fruit. Concerning the furan Mesifurane (F2), it is reported to have a negative correlation with consumer acceptance (Ulrich and Olbricht, 2016). Similar results were obtained also by Perez et al. (1996) that observed an increase of Mesifurane (2.5-fold) in strawberries storage for 7 d at 17 °C as compared to fruit stored at 1 °C. In addition, the alcohol 1-Hexanol (Alc8) and aldehyde Benzaldehyde (Ald10) were also reported as molecules present in ripening stages of strawberry (Cozzolino et al., 2021; Li et al., 2021).

On the contrary, VOCs with higher loading values on PC1 and negative loading values on PC2 (i.e. E17, E4, C6, Ald5 and L3) seem to be associated with the freshness of strawberries and acceptability for consumers. In particular, in a similar study was observed an increase of the carboxylic acid Hexanoic acid (C6) and the ester Methyl butanoate (E4) during refrigerated storage of “Sweet Charlie” strawberries due to the continuing activity of enzymes and biosynthesis of precursor compounds (Ozcan and Barringer, 2011). As reported by Padilla-Jiménez et al. (2021) the esters Methyl butanoate (E4) and Methyl hexanoate (E17) could be volatile markers for harvesting strawberry fruit acceptable to consumers. In addition, Mishra and Kar (2014) observed a comparable result showing an increase of the ester E17 during cold storage of “Chandler” cultivar of strawberry. Furthermore, was showed a negative correlation with the taste sensation “sweet” for the aldehyde (E)− 2-Hexenal (Ald5) and a positive correlation with consumer acceptance and taste sensation "sweet" for the lactone γ-Decalactone (L3) (Ulrich and Olbricht, 2016). Concerning aldehyde Ald5 Ozcan and Barringer (2011) observed an increase of this VOC during refrigerated storage of “Sweet Charlie” strawberries. In addition, Ménager et al. (2004) reported that L3 was the main lactone found in strawberry samples that is known to provide an important contribute to the relevant aroma. The PCA results reported herein have shown that the use of pads activated with chitosan and limonene essential oils (CH-LO) during storage at 8 °C of strawberries was the best approach to preserve the freshness of the product with respect to the use of chitosan essential oil (CH).

To confirm these results PerMANOVA was applied to compare each VOCs profile (CH-LO, CH and Ctrl during storage) with VOCs profile of fresh strawberry samples.

As shown in Table 2, PerMANOVA showed that after 3 days of storage, only VOCs profiles of Ctrl samples were significantly different from those of fresh samples indicating that both treatments (CH-LO and CH) were able to preserve VOCs profiles. However, after 6 days of storage, only VOCs profiles of samples treated with CH-LO were not significantly different with respect to those of the fresh samples. On the other hand, all samples stored for 9 days were significantly different from fresh samples. The PerMANOVA results obtained at 6 and 9 d of storage, in particular those for samples treated with CH-LO, were in agreement either with PCA (Fig. 4) and visual quality evaluation (Fig. 1A). Specifically, samples stored for 6 days (CH_LO_6d) were close to the fresh sample in the PCA biplot and the same samples had a sample score above shelf-life limit as observed by the visual quality evaluation. Moreover, concerning samples stored for 9 days, in the PCA biplot all samples were cauterized in the upper quadrant well separated form fresh samples and visual quality scores for these samples were under shelf-life limit. Therefore, PerMANOVA results confirm that the use of pads activated with CH-LO was the best approach to preserve the freshness of strawberries. Mann–Whitney U test ($p \leq 0.01$), carried out at 6 days of storage, permitted to identify compounds having a role in the differentiation of VOCs profiles of strawberries treated with CH-LO and CH and control samples with respect to fresh samples. Significant differences in median values of peak area ratios were observed for 3, 6 and 34 VOCs, respectively, for samples treated with CH-LO and CH and control samples in comparison with fresh samples (Table 1). Among these 43 VOCs, 8 were within the 10 molecules found as the most greatly contributed to the separation of groups in PCA biplot (Fig. 4). In particular, the VOC Alc9 had median values in samples treated with CH-LO statistically higher than those measured in fresh samples. On the contrary, Ald5 showed, in the samples treated with CH, median values significant lower than in fresh strawberries. Concerning Ctrl samples, median values of Ald10, F2, E17, C6, L3 and Alc8 were statistically higher than in fresh samples.

4. Conclusion

In this work it has been shown that the use of active pads functionalized with either chitosan or chitosan and limonene coatings allow to extend the shelf-life of strawberries under cold storage conditions. Indeed, the loss of fresh appearance and the microbial development was delayed by approximately three days in the samples packaged with the activated pads. The activation with chitosan resulted in a significant reduction of the respiratory activity of the fruit, while the addition of limonene also preserves the volatile component responsible for the

Table 1
List of volatile compounds (n = 123) identified by HS-SPME/GC–MS analysis of strawberries as fresh samples and samples packed in PET trays with active pads (CH, CH-LO) and control samples and stored at 8 °C.

Volatile compound	Code	LRI_{lt}/LRI^{a}_{sp}	Volatile compound	Code	LRI_{lt}/LRI^{a}_{sp}
Aldehydes			***Carboxylic acids***		
Acetaldehyde	Ald1	710/709	Acetic acid[b]	C1	1456/1456
2-methyl-Pentanal[c]	I.S.	-/1007	2-Methylpropanoic acid[g]	C2	1566/1566
Hexanal[b]	Ald2	1080/1080	Butanoic acid	C3	1625/1625
(E)− 2-Pentenal	Ald3	1130/1130	2-Methylbutanoic acid	C4	1665/1665
(Z)− 2-Hexenal	Ald4	1196/1198	2-Methylpentanoic acid	C5	1764/1764
(E)− 2-Hexenal[b,f]	Ald5[d]	1216/1216	Hexanoic acid[g]	C6[d]	1840/1840
2-Heptenal	Ald6	1318/1317	Heptanoic acid	C7	1950/1950
(E,E)− 2,4-Hexadienal[f]	Ald7	1397/1397	Octanoic acid[g]	C8	2056/2056
(E)− 2-Octenal	Ald8	1420/1420	***Ketones***		
(E,E)− 2,4-Heptadienal	Ald9	1461/1461	Acetone[b]	K1	818/818
Benzaldehyde[b,g]	Ald10[d]	1517/1518	2-Pentanone	K2	976/976
(E)− 2-Nonenal	Ald11	1525/1526	2-Heptanone	K3	1178/1178
Benzeneacetaldehyde	Ald12	1641/1641	3-Octanone[b]	K4	1242/1243
(E,E)− 2,4-Decadienal	Ald13	1762/1760	Acetoin[b]	K5	1283/1283
(*E*)-Cinnamaldehyde	Ald14	2043/2043	***Lactones***		
Furans			γ-Caprolactone[b,e,f,g]	L1	1696/1696
2-Pentylfuran[b]	F1	1211/1211	γ-Octalactone[g]	L2	1912/1913
Mesifurane[g]	F2[d]	1587/1587	γ-Decalactone[g]	L3[d]	2153/2153
Esters			γ-Dodecalactone[g]	L4	2381/2381
Methyl acetate[f,g]	E1	828/828	***Alcohols***		
Ethyl acetate[g]	E2	890/890	Ethanol[b,g]	Alc1	935/935
Methyl propanoate[g]	E3	908/908	2-Methyl-1-propanol[b]	Alc2	1098/1098
Methyl butanoate[b]	E4[d]	985/985	1-Butanol	Alc3	1146/1146
Methyl isovalerate	E5	1017/1017	1-Pentanol[b]	Alc4	1256/1256
Ethyl butanoate[b]	E6	1034/1034	2-Methyl-1-pentanol	Alc5	1297/1300
Isopropyl butanoate	E7	1036/1037	2-Heptanol[g]	Alc6	1315/1315
Ethyl 3-methylbutanoate	E8	1064/1064	2-Penten-1-ol	Alc7	1318/1316
Butyl ethanoate	E9[d]	1070/1070	1-Hexanol[b, g]	Alc8[d]	1352/1351
Methyl pentanoate	E10	1083/1083	(E)− 2-Hexen-1-ol[b, e]	Alc9[d]	1402/1402
2-Methyl-1-butanol acetate	E11	1116/1116	1-Octen-3-ol[b]	Alc10	1444/1444
3-Methyl-1-butanol acetate	E12	1118/1118	1-Heptanol	Alc11	1450/1450
Ethyl pentanoate[g]	E13	1127/1127	1-Octanol[b, g]	Alc12	1551/1551
Methyl 4-methylpentanoate	E14	1136/1134	1-Nonanol[b, g]	Alc13	1652/1652
Ethyl (E)− 2-butenoate[b, g]	E15	1158/1158	(E)− 2-Nonen-1-ol	Alc14	1703/1706
Pentyl acetate[g]	E16	1162/1162	6-Nonen-1-ol	Alc15	1714/1710
Methyl hexanoate[b, g]	E17[d]	1182/1182	Benzyl alcohol	Alc16	1873/1873
2-Methylpentyl acetate	E18	-/ 1203	Phenylethyl Alcohol[b]	Alc17	1906/1906
Butyl butanoate	E19	1201/1203	3-Phenylpropanol	Alc18	2040/2041
Ethyl hexanoate[b]	E20	1221/1221	Cinnamic alcohol	Alc19	2274/2278
Hexyl acetate[b, f, g]	E21	1260/1260	***Terpenes***		
Methyl heptanoate[b]	E22	1273/1274	D-Limonene[b]	T1	1169/1170
Methyl 2-Hexenoate[g]	E23	1272/1281	Linalool[b]	T2	1540/1540
(E)− 3-Hexen-1-ol acetate	E24	1298/1297	β-Farnesene[g]	T3	1657/1657
(Z)− 3-Hexen-1-ol acetate	E25	1306/1306	α-Muurolene	T4	1712/1712
2-Hexen-1-ol acetate	E26	1327/1329	β-Bisabolene	T1	1711/1713
Heptyl acetate	E27	1369/1368	α-Curcumene	T2	1766/1766
Methyl octanoate[b]	E28	1377/1377	(6*E*)-Nerolidol[b, g]	T3	2028/2028
(E)− 2-Hexen-1-ol propanoate	E29	1392/1388	***Unknown compounds***		
Butyl hexanoate	E30	1399/1399	Unknown	U1	-/1286
Hexyl butanoate	E31	1405/1403	Unknown	U2	-/1292
Ethyl octanoate[b]	E32	1428/1428	Unknown	U3	-/1335
Hexyl 3-methylbutanoate	E33	1430/1430	Unknown	U4	-/1344
3-Hexenyl butanoate[g]	E34	1452/1448	Unknown[g]	U5	-/1384
2-Hexenyl butanoate	E35	1463/1463	Unknown	U6	-/1529
Octyl acetate	E36	1469/1469	Unknown[g]	U7	-/1534
Hexyl hexanoate[g]	E37	1597/1597	Unknown	U8	-/1699
Octyl butanoate[g]	E38	1605/1605	Unknown	U9	-/1708
Ethyl decanoate[b]	E39	1630/1630	Unknown	U10	-/1746
Octyl 2-methylbutanoate or Octyl 3-methylbutanoate	E40	1634/1636 or 1654/1636	Unknown	U11	-/1799
(E)− 2-Hexenyl hexanoate	E41	1660/1658	Unknown	U12	-/1810
Phenylmethyl acetate	E42	1720/1723	Unknown	U13	-/1897
Methyl salicylate	E43	1771/1771	Unknown[g]	U14	-/2187
2-Phenylethyl acetate	E44	1805/1807	Unknown[g]	U15	-/2190
Benzenepropyl acetate	E45	1941/1936	Unknown	U16	-/2268
Methyl cinnamate[g]	E46	2080/2081	Unknown	U17	-/2273
Ethyl cinnamate[e,f, g]	E47	2135/2135			

[a] LRI_{lt}: 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 − 0.2 and greater than 0.2.
[e] VOCs selected by the Mann–Whitney U test statistically different ($\alpha \leq 0.01$) from fresh samples and samples packed in PET trays with active pads (CH-LO) and stored for 6 d.

[f] VOCs selected by the Mann–Whitney U test statistically different ($\alpha \leq 0.01$) from fresh samples and samples packed in PET trays with active pads (CH) and stored for 6 d.
[g] VOCs selected by the Mann–Whitney U test statistically different ($\alpha \leq 0.01$) from fresh samples and control samples (Ctrl) for 6 d.

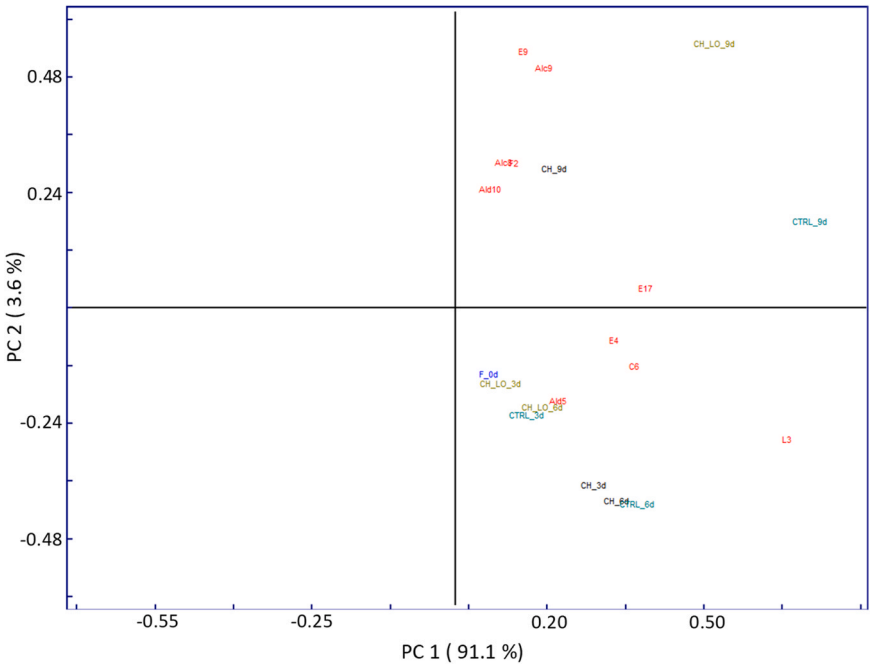

Fig. 4. Biplot of HS-SPME/GC-MS data for the fresh strawberries (F_0d) and for the different treatments (CH, CH-LO, Ctrl) of strawberries samples storage for 3, 6 or 9 d. It shows VOCs with loading values smaller than − 0.2 and greater than 0.2.

Table 2
P-value obtained by statistical PerMANOVA analysis comparing VOCs profiles of fresh strawberry (F) with VOCs profiles of treated (CH-LO and CH) and control (Ctrl) samples during storage.

		Days of storage		
VOCs profiles analyzed using PerMANOVA		3	6	9
F	CH-LO	0.307	0.478	0.001*
F	CH	0.360	0.049*	0.001*
F	Ctrl	0.019*	0.002*	0.002*

* groups significantly different with $\alpha \leq 0.05$

freshness of the strawberries. In conclusion, the use of active packaging based on chitosan and limonene could represent a valid solution to extend the shelf-life of strawberries and preserving their freshness. It is a practical solution, easy to apply, and which offers numerous advantages such as protection against mechanical damages, effective absorption capacity, high permeability and excellent product presentation. The use of bioactive molecules from natural compounds has the dual benefit, on one side it addresses to limit the presence of synthetic additives in food and on the other it promotes the valorization of sustainable raw materials. In a more sustainable and eco-friendly approach, also the cellulose-based adsorbent pads could be obtained as secondary products from the waste generated by the agro-industrial processing. The bio-based nature of all the components of this packaging solution represents an environmentally responsible opportunity for the modern food packaging industry.

Funding

This work was supported by the Project Prin 2017 "MultIFunctional poLymer cOmposites based on groWn matERials (MIFLOWER)" from the Italian Ministry of Education University and Research (grant number: 2017B7MMJ5_001). This research was funded by Italian Ministry of University and Research (MUR), project "Conservabilità, qualità e sicurezza dei prodotti ortofrutticoli ad alto contenuto di servizio - ARS01_00640 – POFACS", D.D. 1211/2020 and 1104/2021.

CRediT authorship contribution statement

Maria Cefola: Conceptualization, Methodology, Funding acquisition, Formal analysis, Writing - Original Draft, Writing - Review & Editing; **Leonardo Caputo:** Conceptualization, Investigation, **Laura Quintieri:** Conceptualization, Methodology, Writing - Original Draft; **Salvatore Cervellieri:** Investigation, Writing - Original Draft; **Francesco Fancello:** Investigation, Writing - Original Draft, **Thomas Netti:** Investigation; **Vincenzo Lippolis:** Writing - Original Draft; **Michela Palumbo:** Validation, Writing - Original Draft, Formal analysis, Visualization; **Ilde Ricci:** Investigation, Visualization; **Andrea Sorrentino:** Conceptualization, Funding acquisition Writing - Original Draft, **Bernardo Pace:** Funding acquisition, Project administration, Writing - Review & Editing; **Severino Zara:** Conceptualization, Investigation, Funding acquisition, Writing - Original Draft, Review & Editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank Massimo Franchi of CNR-ISPA for the technical

support in the laboratory.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2023.112430.

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