agronomy

MDPI

Article

Bioactive Chitosan–Essential Oil Coatings for Strawberries: A Trade-Off Between Sensory Quality and Antimicrobial Activity

Ylenia Pieracci [1,†], Priscilla Farina [1,†], Pierina Díaz-Guerrero [1], Chiara Sanmartin [1,2], Diego Mencarini [1], Barbara Conti [1,2], Arianna Petrucci [1,*], Sabrina Sarrocco [1,2,*] and Francesca Venturi [1,2]

[1] Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy; ylenia.pieracci@agr.unipi.it (Y.P.); priscilla.farina@agr.unipi.it (P.F.); pierina.guerrero@phd.unipi.it (P.D.-G.); chiara.sanmartin@unipi.it (C.S.); diego.mencarini@agr.unipi.it (D.M.); barbara.conti@unipi.it (B.C.); francesca.venturi@unipi.it (F.V.)

[2] Nutrafood, Research Center Nutraceuticals and Food for Health-Nutrafood, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

* Correspondence: arianna.petrucci@phd.unipi.it (A.P.); sabrina.sarrocco@unipi.it (S.S.)

† These authors contributed equally to this work.

Abstract

Bio-based coatings enriched with essential oils (EOs) represent a promising alternative to synthetic preservatives to extend strawberries' shelf-life. This study evaluated the effects of chitosan (CHT) formulations containing three selected EOs (*Illicium verum*, *Citrus sinensis*, and *Citrus limon*) on the volatile profile, sensory quality, and antifungal activity of strawberry fruits. Volatile emissions were characterized by Headspace Solid Phase Micro-Extraction/Gas Chromatography-Mass Spectrometry, while sensory properties were assessed using Quantitative Descriptive Analysis. Antifungal activity was evaluated both in vitro and in vivo against *Botrytis cinerea*. Chitosan alone slightly modified the volatile profile, while EO-enriched coatings induced marked and concentration-dependent changes, reflecting the chemical composition of the incorporated EOs. Among the tested formulations, CHT combined with 1% *C. sinensis* EO provided the best balance between preservation of the characteristic strawberry aroma and overall sensory acceptance. In vitro assays showed that EO volatiles, particularly from *C. sinensis* and *I. verum*, significantly inhibited fungal growth, while diffusible compounds were less effective. In vivo, EO-containing coatings reduced disease incidence and severity by approximately 50%. These findings highlight the potential of CHT–EO coatings as sustainable options for postharvest preservation, although optimization of EO type and concentration is crucial to balance sensory quality and antimicrobial efficacy.

Keywords: postharvest technology; edible coatings; essential oils; strawberry quality; *Botrytis cinerea*; volatile compounds; sensory quality; sustainable agriculture

Check for updates

Academic Editor: Malgorzata Jedryczka

Received: 26 May 2026
Revised: 16 June 2026
Accepted: 18 June 2026
Published: 20 June 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

In recent decades, increasing attention has been directed toward the role of nutrition in promoting human health and supporting economic growth. Among various dietary components, fruit and vegetables have attracted particular interest due to their well-documented health benefits [1]. Consequently, global demand for these produce has increased markedly. Despite their recognized importance, a considerable proportion of fruit and vegetables is lost along the supply chain (food losses) and at the retail and

consumer levels (food waste), mainly because of their high perishability [2]. According to the 2011 FAO report [3], approximately 44% of global fruit and vegetable production is lost or wasted before reaching consumers [4]. This wastage generates serious environmental concerns and highlights the urgent need for sustainable management and valorization strategies [2,5]. Food contamination further exacerbates this issue, as it can occur at any stage of production and processing due to environmental exposure or inadequate handling and storage practices. This complication is particularly evident for *Botrytis cinerea*, causal agent of gray mold, recognized as one of the main pathogens responsible for postharvest losses in fresh fruits. Its infection becomes even more serious during storage, when the fruits are metabolically more active and vulnerable [6]. To prevent spoilage and extend shelf-life, the food industry has long relied on synthetic additives, valued for their stability and low cost. However, their widespread use raises concerns about potential risks to human health and environmental safety [7]. In recent years, technological advances, globalization, and rising health awareness have driven a shift in consumer behavior toward natural alternatives, which are perceived as safer and more sustainable [8].

In this context, essential oils (EOs) have attracted a significant interest in the food industry as alternatives to synthetic additives, in order to improve the shelf-life of perishable food, thus facilitating waste reduction [9]. Essential oils have been recognized to be good candidates as natural additives thanks to their relevant antioxidant and preservative efficacy and safety for human health [10], in addition to their antifungal activity [11]. However, their practical employment in the food system presents some limitation mainly due to their intense aroma that influences the organoleptic properties of the foodstuff involved [12,13]. Nowadays, different innovative systems able to reduce the EO drawbacks and improve their bio-efficacy and release have been developed [13,14]. Active packages and polymer-based coatings represent advanced, environmentally friendly technologies for food preservation [13]. Among these, chitosan (CHT), a multifunctional biopolymer obtained from arthropod chitin, has emerged as a promising candidate for bio-based packaging applications, due to its excellent film-forming ability, intrinsic antimicrobial properties, and good compatibility with other bioactive compounds such as EOs [15]. When incorporated into packaging films, EOs can diffuse to the foodstuff surface by direct contact, where they exert their preservative functions [16]. The development of advanced technologies aimed at extending the shelf-life of food products has become a major focus in the food industry. Although many volatile compounds used as flavoring agents are considered safe by the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA), their application in food processing is still subjected to strict regulations [17].

The aim of the present study was to investigate the effect of CHT coatings enriched with EOs on the volatile emission and sensory acceptance of strawberry fruits, so to evaluate their suitability as active packaging options for fresh fruit. Additionally, the selected EOs were tested for their antifungal efficacy against *B. cinerea*, one of the most damaging postharvest pathogens of strawberries. Strawberries belong to the group of "soft fruits," characterized by high perishability and short shelf-life, which are largely affected by physiological and pathological deterioration. They are among the most extensively cultivated and economically important fruit crops worldwide [18]. Their characteristic color and aroma make them highly appreciated by consumers and contribute to their widespread popularity in global markets [19]. The EOs employed for the treatments, selected by trained panelists according to their sensory profile, were *Illicium verum*, *Citrus sinensis*, and *Citrus limon*. These volatile oils are all classified either as food additives authorized for direct use in human food or as substances generally recognized as safe (GRAS) by

the U.S. FDA (21 CFR Parts 170–186) [20]. Therefore, they can be considered promising alternative preservatives.

2. Materials and Methods

2.1. Strawberry Fruits Selection

Strawberries (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier, Rosaceae) were purchased from a local grocery store in May 2025. The commercial product, named Fragola Matera®, is packaged and distributed by Fruttera Growers, Matera, Italy and certified as Zero Residue (meaning that the residues of synthetic pesticides are less than or equal to 10 ppb).

Fruits with no external flaws and that were consistent in size, color, and ripeness were selected for the following evaluations.

2.2. Essential Oils Selection

The essential oils (EOs) used for strawberry treatment were selected from a set of 17 commercially available options (Table 1) based on their sensory profile, by following a two-step sensory approach.

Table 1. The 17 essential oils evaluated by a trained sensory panel and their sample codes.

Essential Oil Number	Plant Species	Family	Sample Code
1	*Anethum graveolens* Ucria	Apiaceae	Ag
2	*Citrus aurantium* L.	Rutaceae	Ca
3	*Citrus limon* (L.) Osbeck	Rutaceae	Cl
4	*Citrus paradisi* Macfad.	Rutaceae	Cp
5	*Citrus sinensis* (L.) Osbeck	Rutaceae	Cs
6	*Foeniculum vulgare* Mill.	Apiaceae	Fv
7	*Illicium verum* Hook.f.	Schisandraceae	Iv
8	*Laurus nobilis* L.	Lauraceae	Ln
9	*Mentha arvensis* L.	Lamiaceae	Ma
10	*Myristica fragrans* Houtt.	Myristicaceae	Mf
11	*Ocimum basilicum* L.	Lamiaceae	Ob
12	*Origanum vulgare* L.	Lamiaceae	Ov
13	*Piper nigrum* L.	Piperaceae	Pn
14	*Rosmarinus officinalis* L.	Lamiaceae	Ro
15	*Salvia sclarea* L.	Lamiaceae	Ss
16	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Myrtaceae	Sa
17	*Thymus vulgaris* L.	Lamiaceae	Tv

The evaluation was conducted by a trained sensory panel from the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa, consisting of six assessors (four females and two males, aged 28–64 years). Panelists were selected from a larger pool of experienced judges regularly collaborating with the laboratory and were trained according to ISO 8586:2023 guidelines (ISO/CD 8589—Sensory analysis—Selection and training of sensory assessors. Geneva, Switzer land, 201027). All participants had prior experience in the sensory evaluation of produce and EOs and provided informed consent before participation. Training and calibration included sessions focused on descriptor recognition, reference standard alignment, scale use, and reproducibility assessment, following established protocols [21].

In the first phase, a consensus panel was conducted to define the sensory descriptors and their corresponding definitions. The number of assessors was consistent with recommendations for *consensus*-based sensory profiling, which typically involves small groups (6–12 assessors) to facilitate discussion and agreement. This phase was carried out

in accordance with ISO 13299:2016 guidelines (Sensory analysis—Methodology—General guidance for establishing a sensory profile. Geneva, Switzerland, 201027) for sensory profile development. The outcome of this step was the development of a structured evaluation sheet including 11 olfactory descriptors and one hedonic parameter. In the second phase, the same evaluation sheet was applied to the sensory characterization of the 17 EO samples.

The EO samples were prepared at a concentration of 2% (*v*/*v*) in an aqueous solution containing 2% (*v*/*v*) Tween 80 as the emulsifier. This concentration was chosen to provide a clearly perceivable but not excessive olfactory stimulus during orthonasal evaluation and to allow standardized comparison among all EO samples. The evaluation was carried out at room temperature in a well-ventilated ISO 8589 compliant sensory laboratory under controlled conditions. Aliquots of 100 µL of each EO solution were applied onto fragrance tester strips and evaluated orthonasally. This volume was selected to ensure homogeneous impregnation of the fragrance tester strips while avoiding oversaturation or dripping, and was kept constant for all EO samples to allow standardized comparison.

The olfactory profile of each EO was assessed using the structured evaluation sheet, including 11 descriptors (olfactory intensity, floral, fruity, fresh vegetal, dry vegetal, aromatic herbs and spices, balsamic, animal, chemical, pharmaceutical, and olfactory persistence) and one hedonic parameter (overall pleasantness). Each attribute was rated on a 9-point intensity scale (0 = absence; 9 = maximum intensity).

The EOs final selection was based on the following criteria:

(i) High scores in pleasantness and fruity/fresh descriptors;
(ii) Low intensity of off-notes;
(iii) Overall compatibility with the typical aromatic profile of strawberries.

Based on these criteria, a subset of three EOs (*Illicium verum* from steam-distilled fruits and flowering tops—originated in China; *Citrus sinensis* and *Citrus limon* from cold pressed peels—originated in Italy) was selected for further application.

2.3. Preparation of Chitosan Solutions

The pure chitosan (CHT) solution was prepared using commercial CHT (CAS: 9012-76-4) obtained from crab exoskeletons, according to the protocol reported by Parichanon et al. [11] for kumquat fruits. A 1% (*w*/*v*) CHT concentration was dissolved in a 1% (*v*/*v*) aqueous lactic acid solution and stirred magnetically for 4 h at 25 °C. Subsequently, Tween 80 (0.6% *v*/*v*), vegetal glycerol (0.5% *v*/*v*), and two different concentrations (i.e., 1 and 2% *v*/*v*) of the three selected EOs (i.e., *I. verum*, *C. sinensis*, and *C. limon*) were incorporated into the CHT starting solution to obtain the aromatized coating (CHT + EO). The 1% and 2% EO concentrations were selected as practical formulation levels to compare a moderate and a higher EO enrichment of the CHT coating and to evaluate concentration-dependent effects on volatile profile and sensory acceptability.

The mixture was then stirred magnetically for an additional 20 min at 18 °C, resulting in a viscous, opaque white emulsion.

2.4. Strawberry Fruits Treatment

The strawberry fruits were subjected to eight conditions (summarized in Table 2).

For the treatments, previously rinsed and pat dried with paper towel strawberries were singularly dipped for 20 s in the various CHT coatings by holding them by the leafy cap (calyx). All the fruits were then placed on a plastic mesh grid (hole size 1.2 cm × 1.2 cm) to allow excess coating to drain and let dry for 1 h under a fume hood.

Table 2. Description of the coatings applied on strawberries and their sample codes.

Treatment	Sample Code
Blank control strawberries	Z1
Strawberries with 1% CHT	C
Strawberries + 1% CHT + 1% *Illicium verum* EO	A1
Strawberries + 1% CHT + 2% *Illicium verum* EO	A2
Strawberries + 1% CHT + 1% *Citrus sinensis* EO	AR1
Strawberries + 1% CHT + 2% *Citrus sinensis* EO	AR2
Strawberries + 1% CHT + 1% *Citrus limon* EO	L1
Strawberries + 1% CHT + 2% *Citrus limon* EO	L2

CHT = chitosan; EO = essential oil.

2.5. Headspace Solid Phase Micro-Extraction

The volatile emission of the strawberry fruits coated with CHT added or not with EOs, as well as all the CHT solutions used for the treatments, were analysed by Headspace Solid Phase Micro-Extraction (HS-SPME). For the analysis, samples were placed in glass vials, filling one third of the whole volume. The vials were covered with aluminium foils and left to equilibrate for at least 30 min. During the equilibration period, the volatile molecules emitted by the sample create an equilibrium with the air molecules of the headspace (HS). Once the equilibration time was finished, the headspace was sampled using a special syringe equipped with an adsorbent fibre of divinylbenzen/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) pre-conditioned following the manufacturers' instructions. Sampling times were experimentally determined to obtain an optimal adsorption of the volatiles, and to avoid both under- and over-saturation of the fibre and of the mass spectrometer detector. In detail, for samples C, Z1, A1, A2, AR1, AR2, L1, and L2 the time was 20 s, for CHT 90 s, and for CHT + 1% *Illicium verum* EO, CHT + 1% *Citrus sinensis* EO, and CHT + 1% *Citrus limon* EO 1 s. Once sampling time was finished, the fibre was withdrawn into the needle and transferred to the injection port of the Gas Chromatography-Mass Spectrometry (GC-MS) system.

2.6. Gas Chromatography-Mass Spectrometry Analyses

GC-MS analyses were performed in triplicate (n = 3) with an Agilent 7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP- 5MS capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and an Agilent 5977B single quadrupole mass detector. For the HS-SPME and EOs analyses, the injector temperature was set at 250 °C and 220 °C, respectively, while the transfer line temperature was 240 °C for both analyses. The oven temperature was programmed from 60 to 240 °C at 3 °C min^{-1} ramp, with helium as the carrier gas (flow rate 1 mL min^{-1}) using the splitless injection mode for HS and the split mode (1:25 ratio) for EO analyses. The acquisition parameters were: full scan; scan range: 30–300 m/z; scan time: 1.0 s. The constituents were identified by comparing the retention times with those of the standard samples and their linear retention indices relative to a series of n-hydrocarbons. Experimental mass spectra were matched against commercial [22,23] and a homemade library of mass spectra built up from pure substances and components of known EOs and the literature data. Quantitative comparisons of relative peaks areas (%) were performed between the same chemicals in the different samples.

2.7. Sensory Evaluation of Strawberry Fruits

The sensory evaluation of strawberries treated with CHT combined with the three selected EOs (i.e., *I. verum*, *C. sinensis*, and *C. limon*) at two concentrations (i.e., 1% and 2% *v*/*v*) was performed using Quantitative Descriptive Analysis (QDA), in accordance with ISO 13299:2016 standards.

The analysis was carried out by a trained panel of ten assessors from the DAFE, University of Pisa, to increase analytical robustness. They were all experienced in the sensory evaluation of produce and EOs and were selected and trained according to ISO 8586 guidelines [21]. Sensory sessions were conducted in individual booths in a controlled sensory laboratory compliant with ISO 8589:2010 conditions.

The sensory evaluation sheet used in this phase (Figure S1) was based on a previously validated protocol [24], which was adapted to the specific characteristics of strawberry fruits. In particular, the original scheme was integrated with additional olfactory descriptors defined through the preliminary *consensus panel*, as described in Section 2.2, in order to better capture the aromatic contribution of EOs.

All attributes were rated on a 9-point intensity scale (0 = absence; 9 = maximum intensity). Data were digitally collected using Microsoft Forms and processed using the PanelCheck software v. 1.4.2 to evaluate panel performance (in terms of discrimination ability, repeatability, and agreement) and to perform statistical analysis.

2.8. Antifungal Activity of Selected Essential Oil Against Botrytis cinerea

The three selected EOs (i.e., *I. verum*, *C. sinensis*, and *C. limon*) were tested at a 1% (*v*/*v*) concentration for their ability to inhibit the growth of *Botrytis cinerea*. Their activity was assessed by examining both diffusible and volatile compounds.

For the first assay (diffusible compounds), a central well (1.6 cm diameter) was created in 9 cm Petri dishes containing Potato Dextrose Agar (PDA, 39 g L^{-1}, Difco, Italy), and the agar plug was removed. Each well was filled with 200 µL of a 1% (*v*/*v*) aqueous emulsion of the selected EO (in 1% (*v*/*v*) Tween 80). Plates were kept at 24 °C for 24 h to allow diffusion of the compounds into the medium. Then, each plate was inoculated with three 8 mm agar plugs containing actively growing *B. cinerea* mycelium, positioned along the plate edge at 120° intervals. Control plates contained 200 µL of sterile water added with 1% (*v*/*v*) Tween 80. All treatments, including the control, were performed in triplicate (n = 3). Plates were incubated at 24 °C under a 12 h light/12 h dark photoperiod. The experiment was stopped when the fungal colonies in the control plates reached the edge of the central well. At that point, colony radii in EO-treated plates were measured and subjected to statistical analysis.

The same EOs were also evaluated for their antifungal volatile activity against the pathogen (second experiment). PDA plates were centrally inoculated with an 8 mm agar plug taken from actively growing fungal cultures on PDA. A 9 cm filter paper disc was placed on the lid of each plate and added with 1 mL of an aqueous solution containing 1% (*v*/*v*) Tween 80 and the EO at a final concentration of 1% (*v*/*v*). Control plates were prepared using filter paper discs added with 1 mL of sterile water containing 1% (*v*/*v*) Tween 80. Each treatment, including the control, was carried out in triplicate (n = 3). Plates were sealed with Parafilm® and incubated at 24 °C. Fungal growth was monitored daily by measuring colony diameter in two perpendicular directions until the colonies in the control plates reached the plate edge and then submitted to statistical analysis.

2.9. Protective Effect of Chitosan Added with Selected Essential Oils Against Botrytis cinerea on Strawberry Fruits

Based on the antifungal activity assays, *I. verum* and *C. sinensis* EOs were selected for in vivo tests against *B. cinerea* on strawberry fruits, using 1% (*w*/*v*) CHT as a carrier (see Section 2.3). Strawberry fruits were subjected to four treatments: *B. cinerea* (positive control); 1% CHT [C] + *B. cinerea*; 1% CHT + 1% *I. verum* EO [A1] + *B. cinerea* and; 1% CHT + 1% *C. sinensis* EO [AR1] + *B. cinerea*. Before treatment, fruits were surface sterilized in a solution of NaClO (1% active chlorine) in 50% ethanol for 5 s, rinsed three times (5 s each) with sterile distilled water, and gently pat dried with sterile paper towel. Each fruit was then individually dipped for 20 s in the respective CHT-based coatings, by holding the strawberry by the calyx. After treatment, fruits were placed on a plastic mesh grid (1.2 × 1.2 cm) and left to dry for 1 h under a sterile laminar flow hood to allow excess coating to drain. Subsequently, strawberries were inoculated with *B. cinerea* spores by spraying each fruit twice (total volume 0.173 mL). Spores were collected from one-week-old PDA cultures using sterile Milli-Q water and filtered through sterile Miracloth™. The suspension was adjusted to a final concentration of 1×10^5 spores mL^{-1} in 0.05% (*v*/*v*) Tween 20 to ensure uniform adhesion to the fruit surface. For each treatment, three biological replicates ($n = 3$) were performed, each consisting of ten fruits. All trays were sealed in plastic bags to maintain humidity in order to favor spore germination and fruit infection, and they were incubated at 24 00B0C under a 12 h light/12 h dark photoperiod for three days. At the end of the experiment, Disease Incidence (DI) was determined as the percentage of symptomatic fruits, while Disease Severity Index (DSI) was assessed using the following scale: 0 = no symptoms; 1 = rotted fruit; 2 = visible mycelium (Figure 1).

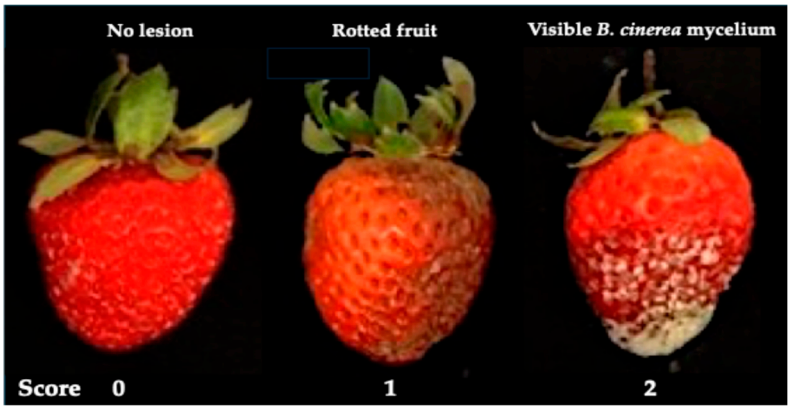


Figure 1. Pathometric scale used to assess *Botrytis cinerea* infection on strawberry. Score: 0 = no symptoms; 1 = rotted fruit; 2 = visible *B. cinerea* mycelium.

The DSI was then calculated using the following formula:

$$DSI_i = \frac{(n_i \times 0) + (n_i \times 1) + (n_i \times 2)}{n_i \times 2} \times 100$$

where n_i is the number of observed fruits, and 2 is the maximum score. This formula was adapted from De Tender and colleagues [25], and data were submitted to statistical analysis.

2.10. Statistical Analysis

Considering the different EOs, the complete chemical composition of the volatile emissions of control strawberries, of strawberry fruits treated with the different CHT coatings, and CHT pure and enriched with EO was subjected to multivariate statistical analysis with the Principal Component Analysis (PCA) and the Hierarchical Cluster Analysis (HCA)

methods. The PCA was performed on covariance data matrices, selecting the two highest PCs obtained by the linear regressions. The HCA was performed using Ward's method, with squared Euclidean distances as a measure of similarity. Such analyses were performed using the JMP Pro 18.0.0 software package (SAS Institute, Cary, NC, USA).

Raw data of the sensory analysis were processed by the open-source PanelCheck software performing a two-way ANOVA analysis and a multivariate statistical analysis with the PCA, by considering panelists, sensory scores, and samples as the main factors. In both analyses, differences with a $p < 0.05$ were considered statistically significant.

Fungal growth values registered from the first antifungal in vitro experiment (diffusible compounds) were subjected to statistical analysis of variance (ANOVA) to determine whether *B. cinerea* growth was affected by the presence of soluble compounds released by the EOs, assuming $p < 0.05$ as significant level. Conversely, for the second experiment (volatile compounds), *B. cinerea* colony diameters were used to construct a growth curve, which was subsequently subjected to analysis of variance of regression (by GraphPad Prism 8) to compare the slope in the presence of water (control) vs. those recorded in the presence of each of the three EOs, assuming $p < 0.05$ as a significant value.

Raw data from in vivo test were submitted to angular transformation using Microsoft Excell, while ANOVA One-Way was run using 'aov' and Tuckey's 'TukeyHSD' in R version 4.5.0, to evaluate the effect of the selected EOs on fruits protection, with $p < 0.05$ assumed as significant level.

2.11. Use of Generative AI-Tools

ChatGPT was used during manuscript preparation to improve the English language and readability of the text. All AI-assisted edits were reviewed by the authors, who take full responsibility for the accuracy and integrity of the manuscript.

3. Results

3.1. Preliminary Selection of Essential Oils

Through the sensory analysis conducted by the *consensus panel* based on the olfactory properties of the 17 EO samples listed in Table 1, six candidate EOs were firstly identified as suitable for potential application in food systems: *Citrus aurantium*, *C. limon*, *C. paradisi*, *C. sinensis*, *I. verum*, and *M. arvensis* (Figure 2a,b).

The final selection was guided by the objective of identifying EOs compatible with the natural aromatic profile of strawberries, characterized by fruity and fresh notes. Among the candidates, citrus EOs (*C. limon*, *C. sinensis*, and *C. paradisi*) were associated with fresh and fruity descriptors, while *I. verum* EO contributed with spicy and aromatic notes and *M. arvensis* EO provided fresh vegetal nuances.

Based on the overall sensory profile and *panel consensus*, three EOs— *I. verum*, *C. sinensis*, and *C. limon*—were ultimately selected for further experiments, according to the criteria described above (see Section 2.2). This selection reflected a balance between aromatic compatibility with strawberry flavor and the potential to modulate the sensory profile through complementary or contrasting notes. This preliminary selection was based on EO aroma alone and did not account for matrix effects occurring after the mixing with CHT and following application to the fruit.

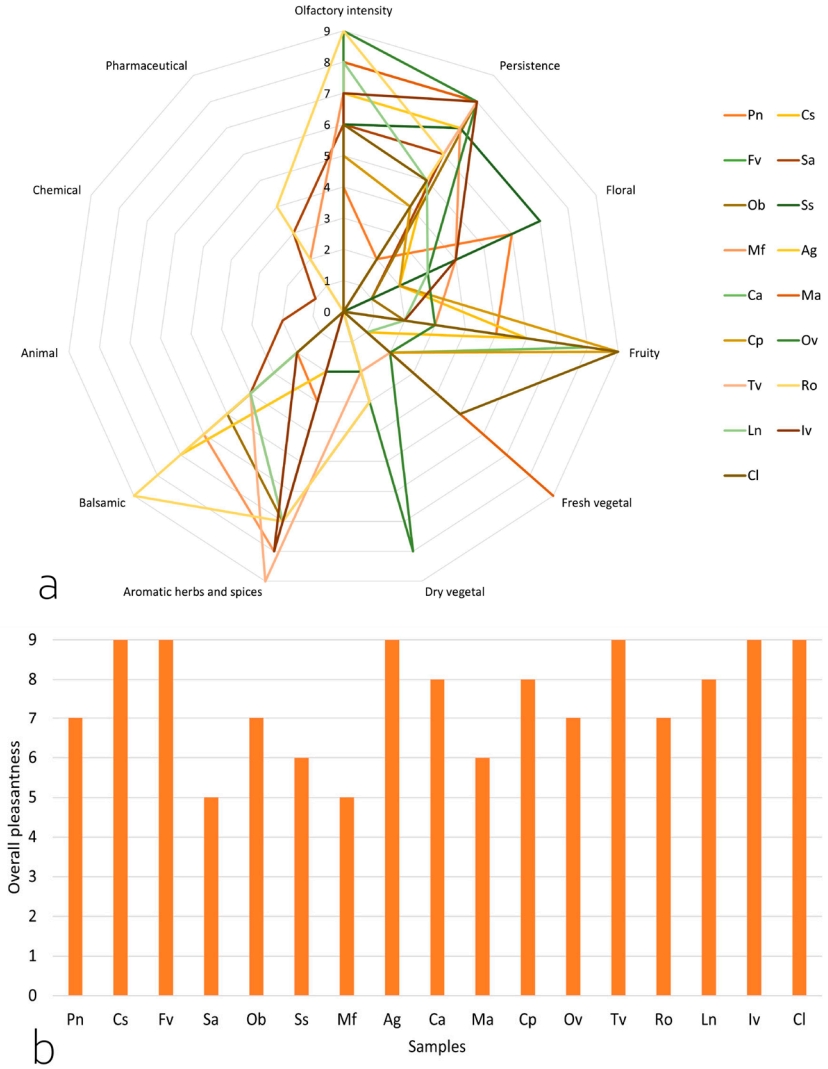


Figure 2. Descriptive sensory attributes (**a**) and olfactory hedonic index score (**b**) of the 17 essential oils listed in Table 1.

3.2. Chemical Composition of the Essential Oils Selected for Strawberry Treatment

The complete chemical composition of the three EOs selected for this study is reported in Table 3.

The EO of *I. verum* was characterized by a clear predominance of compounds belonging to the phenylpropanoid class, which accounted for 97.6% of the entire composition. Within this class, the predominant molecule was *trans*-anethole, representing 91.8%. The *cis* isomer of the same molecule was also detected, although in very low relative amounts (0.2%). Other relevant compounds, such as methyl chavicol and foeniculin, were also identified in amounts below 5%.

The EOs of *C. sinensis* and *C. limon* shared the presence of monoterpene hydrocarbons as the main chemical class, mainly represented by limonene. Specifically, limonene was detected at 97.9% in sweet orange EO and 66.3% in lemon EO. The latter showed a qualitatively more complex composition, with other compounds present in significant percentages, including β-pinene (10.6%), γ-terpinene (8.7%), sabinene (1.6%), and α-pinene (1.5%). These compounds also belong to the monoterpene hydrocarbon class, complemented by oxygenated monoterpenes such as geranial (3.4%), α-terpineol (1.7%), and neral (1.6%). In contrast, *C. sinensis* EO exhibited a less complex chemical composition, with all other detected molecules present in amounts below 1%.

Table 3. Chemical composition of the essential oils of *Illicium verum*, *Citrus sinensis*, and *Citrus limon* expressed as relative abundance (%) ± standard deviation (n = 3).

Compound	l.r.i. [1]	Class	Relative Abundance (%) ± Standard Deviation (n = 3)		
			Illicium verum	*Citrus sinensis*	*Citrus limon*
α-thujene	926	mh	- [2]	-	0.3 ± 0.02
α-pinene	933	mh	-	0.3 ± 0.02	1.5 ± 0.08
Sabinene	973	mh	-	0.2 ± 0.01	1.6 ± 0.26
β-pinene	977	mh	-	-	10.6 ± 0.56
Myrcene	991	mh	-	0.9 ± 0.02	1.1 ± 0.24
Octanal	1003	nt	-	0.2 ± 0.02	-
α-phellandrene	1006	mh	0.1 ± 0.02	-	-
δ-3-carene	1011	mh	-	0.1 ± 0.00	-
p-cymene	1024	mh	-	-	1.2 ± 0.13
Limonene	1029	mh	0.6 ± 0.09	97.9 ± 0.04	66.3 ± 1.9
γ-terpinene	1058	mh	-	-	8.7 ± 0.74
Terpinolene	1088	mh	-	-	0.3 ± 0.05
Linalool	1101	om	0.6 ± 0.04	0.2 ± 0.01	0.2 ± 0.07
α-terpineol	1191	om	-	-	1.7 ± 1.36
methyl chavicol	1201	pp	3.8 ± 0.12	-	-
Decanal	1205	nt	-	0.2 ± 0.01	-
Neral	1241	om	-	-	1.6 ± 1.51
Carvone	1241	om	-	0.1 ± 0.01	-
cis-anethole	1254	pp	0.2 ± 0.00	-	-
p-anisaldehyde	1256	pp	0.4 ± 0.01	-	-
Geranial	1271	om	-	-	3.4 ± 0.24
trans-anethole	1286	pp	91.8 ± 0.34	-	-
neryl acetate	1365	om	-	-	0.6 ± 0.01
geranyl acetate	1385	om	-	-	0.6 ± 0.03
β-caryophyllene	1419	sh	0.4 ± 0.01	-	0.5 ± 0.07
trans-α-bergamotene	1436	sh	0.5 ± 0.01	-	-
β-bisabolene	1509	sh	0.1 ± 0.00	-	-
Foeniculin	1684	pp	1.4 ± 0.04	-	-
Chemical classes			*Illicium verum*	*Citrus sinensis*	*Citrus limon*
Monoterpene hydrocarbons (mh)			0.7 ± 0.11	99.4 ± 0.00	91.6 ± 0.17
Oxygenated monoterpenes (om)			0.6 ± 0.04	0.3 ± 0.01	8.1 ± 0.12
Sesquiterpene hydrocarbons (sh)			1.0 ± 0.02	-	0.5 ± 0.07
Phenylpropanoids (pp)			97.6 ± 0.17	-	-
Non terpene derivatives (nt)			-	0.4 ± 0.01	-
Total identified (%)			99.9 ± 0.01	100.1 ± 0.01	100.2 ± 0.02

[1] LRI: linear retention index on an HP-5MS capillary column used for compound identification. Chemical classes: mh = monoterpene hydrocarbons; om = oxygenated monoterpenes; sh = sesquiterpene hydrocarbons; pp = phenylpropanoids; nt = non-terpene derivatives. [2]: Not detected.

3.3. Volatile Emission of Strawberry Fruits Samples

The untreated strawberries (sample Z1) exhibited a total of 14 volatile compounds (Table S1), all classified as non-terpenic esters. The volatile profile was predominantly composed of ethyl acetate (30.9%), ethyl butyrate (22.1%), and ethyl hexanoate (25.9%). The application of the 1% CHT coating, without any added EO, determined noticeable alterations in the volatile profile of the investigated fruit (sample C). Indeed, a significant increase in the relative abundance of certain esters, including methyl butanoate (39.9%), methyl hexanoate (14.8%), 1-hexyl acetate (7.3%), and (*E*)-2-hexenyl acetate (8.4%), was observed, accompanied by a decrease in the three main esters detected in the headspace of the control fruits. Despite these changes, the volatile profile of the CHT-coated strawberry fruits still retained high relative levels of esters (approximately 99%), indicating that the

fruity aroma was largely maintained, though slightly altered in composition. Nevertheless, chemical analysis also evidenced that CHT coatings did not directly contribute to the fruits' volatile composition, since almost none of the volatile molecules identified in the CHT solution were detected in sample C.

The treatment with CHT coating enriched with *I. verum* EO, at both 1% (A1) and 2% (A2), had a pronounced impact on the aroma profile of the strawberry fruits (Table S2). Indeed, samples A1 and A2 exhibited a marked presence of phenylpropanoids, primarily (*E*)-anethole and methyl chavicol. The presence of these compounds in the headspace of the treated strawberries clearly derived from the EO itself, supporting the observed dose-dependent response linked to the increasing concentrations of EO. The analysis also showed a clear increase in terpenic content, including monoterpenes and sesquiterpenes, which were not typically found in untreated strawberries. Consequently, the relative abundance of typical strawberry esters strongly decreased from 100% in Z1 to 47.2% in A1 and 31.1% in A2. Chemical results evidenced significant variations in the volatile emission of the treated strawberries, not only due to the direct contribution of the EO, but also possibly due to the interaction among CHT, the EO, and the fruit metabolism. The changes were both qualitative, since new compounds were identified, and quantitative, as great shifts in relative abundance occurred. The profiles of A1 and A2 seemed quite similar, but sample A2 (treated with CHT + 2% EO) tended to have higher relative concentrations of EO-related volatiles, such as phenylpropanoids, indicating a clear dose–effect relationship.

The complete chemical compositions of the volatile profiles were subjected to multivariate statistical analysis with the HCA and PCA methods.

The dendrogram resulting from HCA (Figure 3) grouped all the strawberry samples, both control and treated, into a common cluster, likely due to the shared presence of non-terpenic esters in their volatile emissions. In detail, A1 and Z1 clustered closely together, suggesting that strawberries treated with 1% *I. verum* EO (A1) maintained a volatile profile more similar than expected to that of untreated fruits (Z1). A2, on the other hand, was clearly separated, indicating that the 2% *I. verum* EO treatment significantly altered the volatile profile, especially compared to Z1 and A1. C sample appeared in a separate branch, suggesting a distinct chemical profile, possibly due to metabolic changes in the fruit volatile emission. Notably, this sample clustered far from the pure CHT solution, indicating that the coating effect on strawberry aroma was indirect and did not involve the transfer of CHT-derived volatiles to the fruit samples. Moreover, the CHT solution enriched with *I. verum* EO clustered independently, forming a distinct group, characterized by high relative concentrations of phenylpropanoids, including methyl chavicol and (*E*)-anethole, compounds also found in samples A1 and A2. This aspect highlighted their EO derivation and a clear dose-dependent effect, where higher EO concentrations cause a greater variation in the strawberry volatile profile.

The PCA was performed on a 60 × 6 covariance data matrix (60 compounds × 6 samples = 360 data): the chosen PC1 and PC2 covered 57.6% and 21.7% of the variance, respectively, for a total explained variance of 79.3%.

The PCA score plot (Figure 4, left) showed a clear separation of the samples. All strawberry samples, whether treated or untreated, were plotted in the lower quadrants (PC2 < 0), while the pure chitosan solution (CHT) and the CHT coating enriched with *I. verum* EO were located in the upper quadrants. This positioning suggested that the high abundance of non-terpenic esters in strawberry samples likely contributed to their projection in the lower half of the plot. Specifically, the Z1 sample (untreated control) was positioned in the bottom right quadrant, indicating a volatile profile strongly dominated by characteristic strawberry esters, such as ethyl acetate, ethyl butyrate, and ethyl hexanoate, which pointed distinctly toward the lower right region in the loading plot (Figure 4, right).

The C sample (1% CHT coating) was also located in the bottom right quadrant, relatively close to Z1 along PC1, but closer to the PC2 axis, suggesting some differences in the relative abundance of major esters compared to the control. Conversely, A1 and A2 were both plotted in the bottom left quadrant. The proximity between A1 and A2 indicated similar volatile compositions, but their clear separation from Z1 confirmed that the EO treatments, particularly in A2, caused significant shifts in the aromatic profile of strawberry fruits. These shifts were mainly attributed to the presence of phenylpropanoids, such as (*E*)-anethole and methyl chavicol, which were positioned in the leftmost region of the loading plot. Nonetheless, the slightly closer positioning of A1 to Z1 suggested that the 1% EO treatment had a milder impact on the overall aroma compared to the 2% treatment.

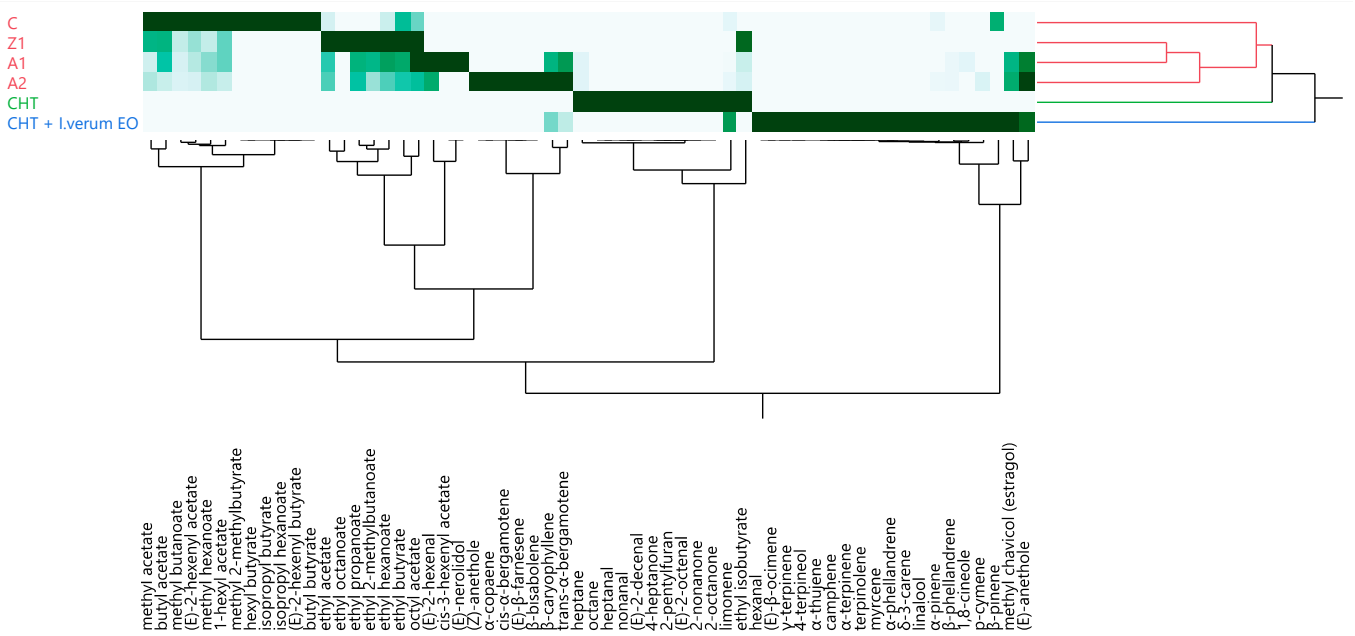


Figure 3. Heatmap of the Hierarchical Cluster Analysis performed on the chemical composition of the volatile emissions of samples related to the treatment with *Illicium verum* essential oil. Sample codes are defined in Table 2.

Strawberry fruits treated with the CHT coating enriched with *C. sinensis* EO, at both 1% (AR1) and 2% (AR2), showed strong differences in the aroma composition if compared to both the control and the CHT-coated samples (Table S3). In particular, AR1 and AR2 were characterized by significantly higher percentages of monoterpene hydrocarbons, mainly represented by limonene, followed by myrcene, both found in the headspace of the CHT enriched by *C. sinensis* EO, whose chemical composition was entirely constituted by this chemical class. Interestingly, similarly to the treatment with CHT enriched with *I. verum* EO, treated strawberries exhibited a dose-dependent variation in their volatile profile. Indeed, the increase in the relative abundance of the detected monoterpene hydrocarbons (55.2% in AR1 and 82.6% in AR2) was in line with the increased concentration of the EO used for the treatment. Analogously, ester content significantly decreased, accounting for 44.9% in AR1 and 17.4% in AR2, thus indicating a strong replacement of natural esters due to EO interaction. Compounds like ethyl butyrate, ethyl hexanoate, and ethyl acetate were still detected, but at much lower levels than in Z1, especially in AR2. This shift suggested that *C. sinensis* EO strongly modifies the aroma composition, partially replacing natural fruit volatiles with EO-derived monoterpenes.

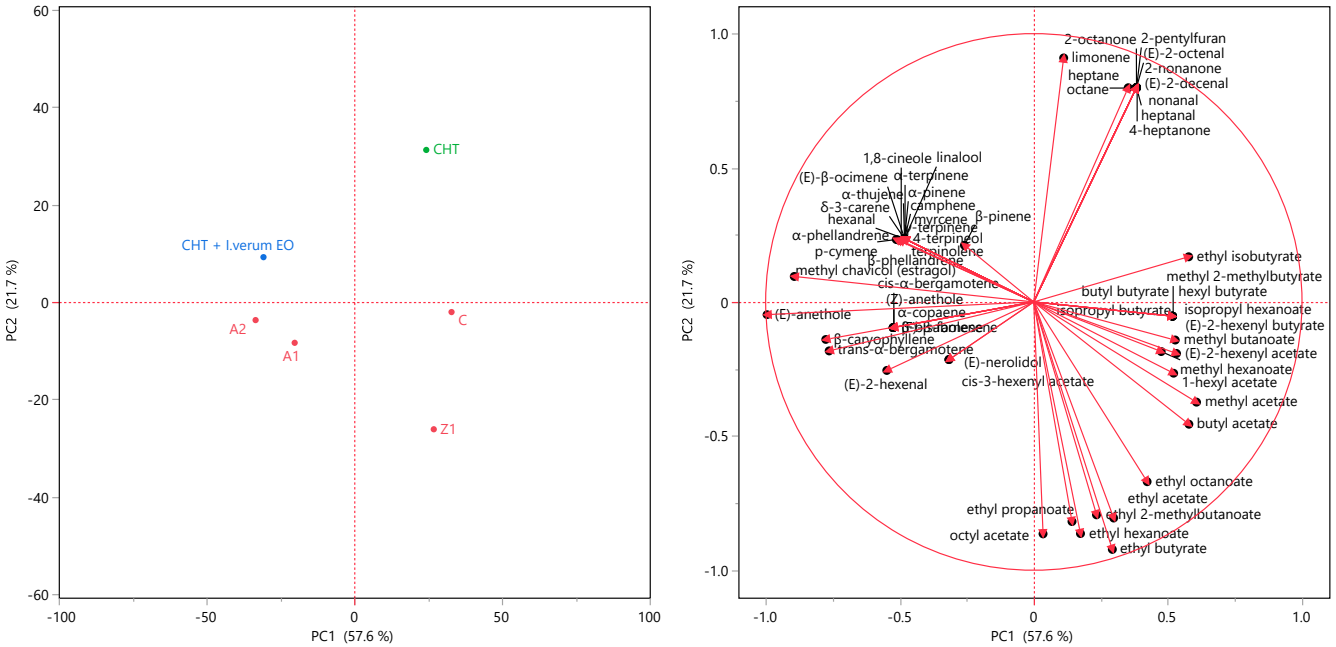


Figure 4. Score (**left**) and loading (**right**) plot of the Principal Component Analysis performed on the chemical composition of the volatile emissions of samples related to the treatment with *Illicum verum* essential oil. Sample codes are defined in Table 2.

The dendrogram resulting from HCA (Figure 5) showed that the untreated strawberry sample clustered close to the sample treated with a CHT coating enriched with 1% *C. sinensis* EO (AR1). This grouping was primarily due to the shared presence of high relative amounts of esters in their volatile profiles, particularly ethyl acetate and ethyl hexanoate. This green cluster also included a subgroup containing AR2 and the headspace of the CHT coating enriched with the EO, both characterized by a strong presence of monoterpene hydrocarbons, especially limonene and myrcene. At a higher level of clustering, the entire green cluster grouped with the CHT solution itself, forming the first major cluster. In contrast, sample C, represented by strawberries treated with the CHT solution only, formed a distinct, separate first-level cluster.

PCA was performed on a 44 × 6 covariance data matrix (44 compounds × 6 samples = 264 data) and the chosen PC1 and PC2 covered 74.5% and 13.6% of the variance, respectively, for a total explained variance of 88.1%. The PCA revealed a certain proximity among AR1, AR2, and the CHT coating enriched with *C. sinensis* EO, all of which were plotted in the left quadrants of the score plot (Figure 6, left). This grouping was likely due to the significant presence of monoterpene hydrocarbons in their volatile emissions, whose vectors pointed in the same direction in the loading plot (Figure 6, right). Although AR1 was grouped with the control sample in the dendrogram, the PCA revealed a noticeable degree of dissimilarity between the two, as they were positioned quite far apart along PC1, which accounted for the majority of the explained variance. Conversely, sample C, despite being clustered far from the others in the HCA dendrogram, shared a similar PC1 value, suggesting that their volatile profiles exhibited certain similarities. However, the samples were still separated along PC2, indicating differences in other aspects of their volatile composition, mainly determined by the quantitative differences in the relative content of their major esters.

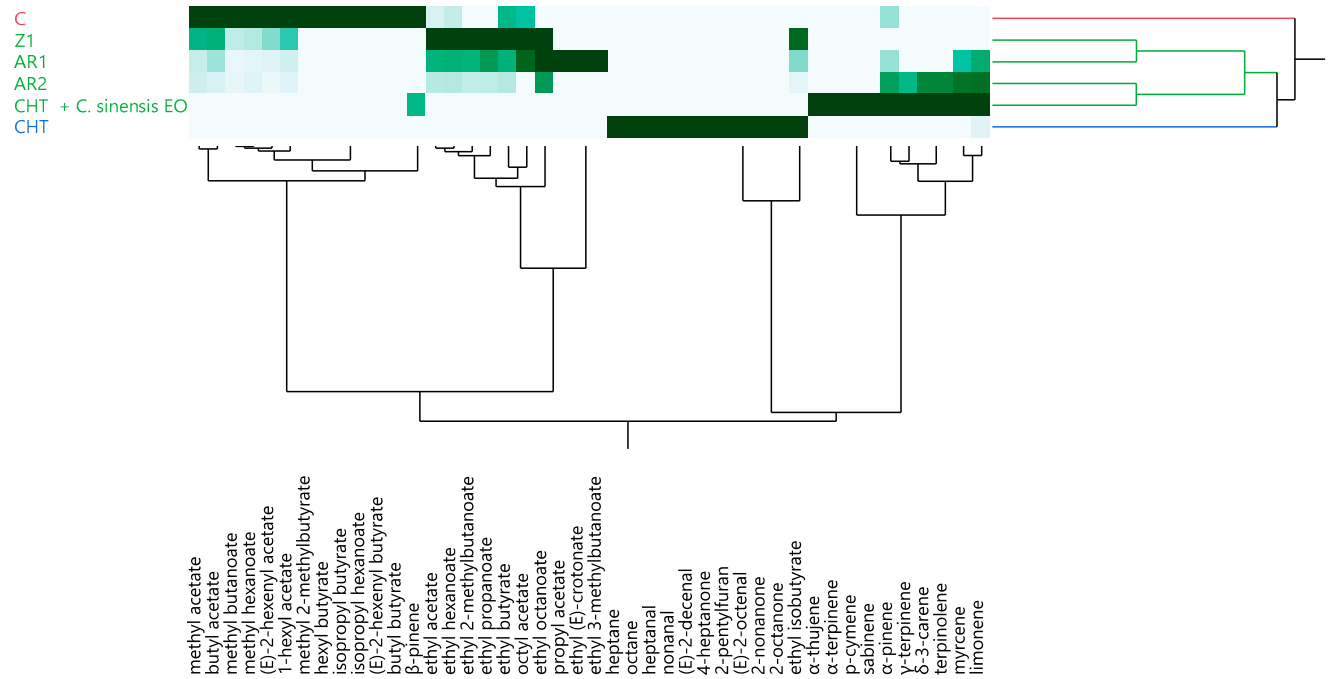


Figure 5. Heatmap of the Hierarchical Cluster Analysis performed on the chemical composition of the volatile emissions of samples related to the treatment with *Citrus sinensis* essential oil. Sample codes are defined in Table 2.

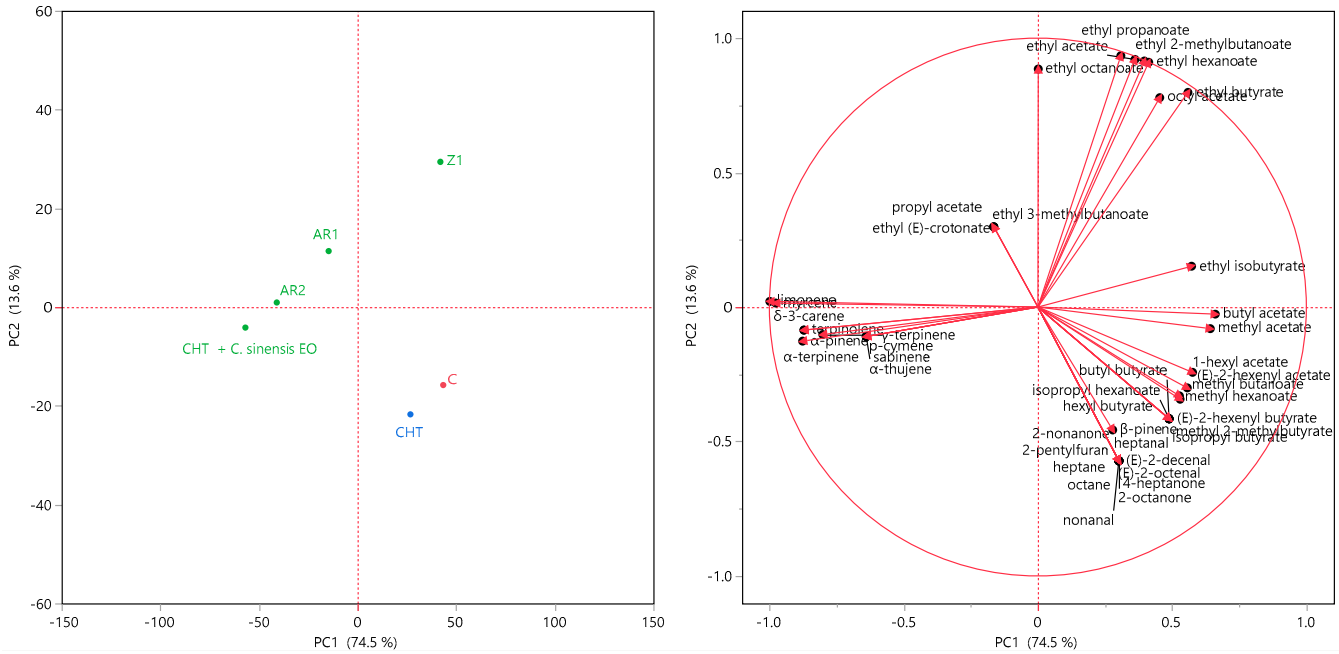


Figure 6. Score (**left**) and loading (**right**) plot of the Principal Component Analysis performed on the chemical composition of the volatile emissions of samples related to the treatment with *Citrus sinensis* essential oil. Sample codes are defined in Table 2.

Samples L1 and L2, treated with CHT coatings enriched with 1% and 2% *C. limon* EO, respectively, were characterized by the presence of esters commonly found in the control strawberries, such as ethyl hexanoate and ethyl butyrate, albeit in significantly lower relative amounts (Table S4). In contrast, these samples exhibited markedly higher levels of monoterpene hydrocarbons, particularly limonene (51.6% in L1 and 53.5% in L2), β-pinene (7.7% in L1 and 9.1% in L2), and γ-terpinene (6.7% in L1 and 8.3% in L2). The presence of

these compounds in the treated strawberries could be directly attributed to the EO, as the CHT coating enriched with *C. limon* volatile extract showed a chemical profile dominated by monoterpene hydrocarbons (99.4%), with the same major components. Notably, a dose-dependent effect was observed also in this case, with increased EO concentration leading to higher levels of these volatiles. Such differences in chemical profiles clearly reflected the impact of EO enrichment and underscored the modulatory role of CHT-based coatings combined with *C. limon* EO on the volatile composition and aroma profile of strawberries.

Figure 7 shows the dendrogram and heatmap of volatile compounds across the different strawberry samples relative to the treatment with *C. limon* EO. Three major clusters could be distinguished: one comprising the CHT-coated sample (C) and the untreated strawberries (Z1), one consisting of CHT by itself, and the other one including the samples treated with CHT enriched with *C. limon* EO (L1, and L2), along with the CHT coating enriched with the EO. Z1 and C formed a tight subgroup, primarily due to their high relative abundance of esters such as ethyl acetate, methyl butanoate, and ethyl hexanoate, although great differences in their relative contents were highlighted. In contrast, the second major cluster included the EO-treated samples, which were clearly distinguished for their elevated levels of monoterpene hydrocarbons, including limonene, β-pinene, and γ-terpinene, compounds derived from *C. limon* EO. Indeed, L1 and L2 were clustered close to the *C. limon* EO-CHT, with which they shared the high monoterpene content. The heatmap further illustrates two distinctions, with ester-rich profiles dominating the upper groups of the dendrogram, and terpene-rich profiles characterizing the EO-treated samples, as well as the EO-enriched CHT. This clustering supported the hypothesis that CHT coatings enriched with *C. limon* EO significantly alter the aromatic fingerprint of strawberries, shifting the volatile profile from ester-dominated to terpene-dominated.

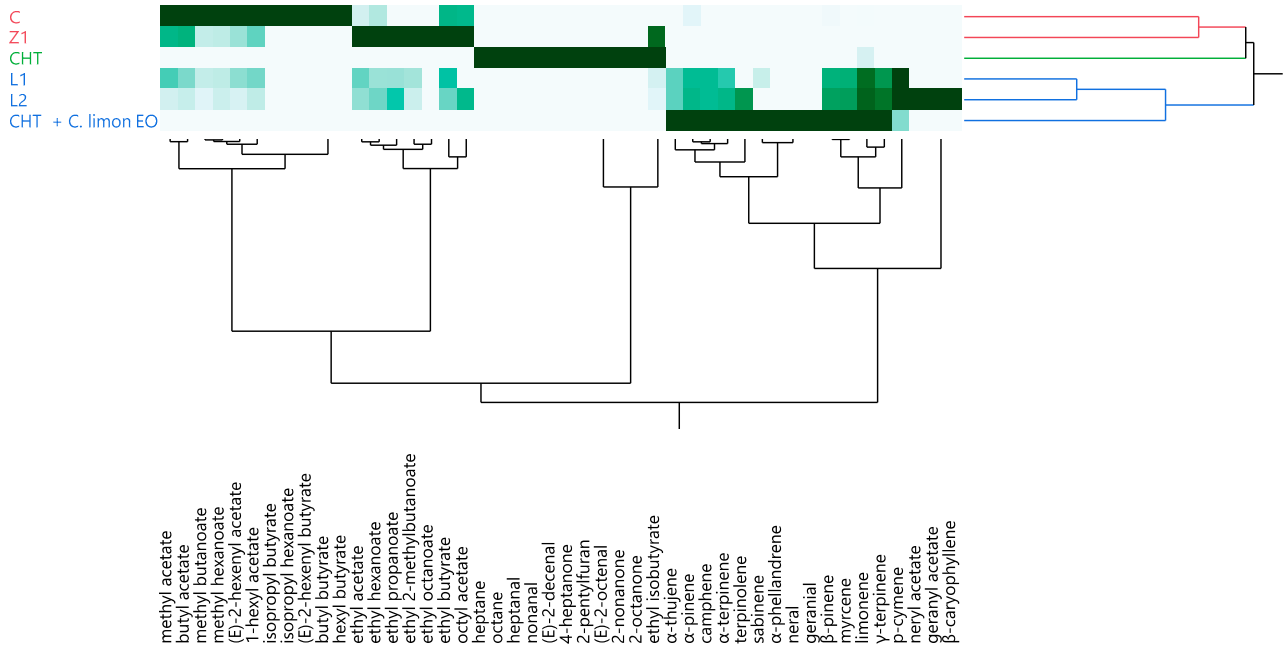


Figure 7. Heatmap of the Hierarchical Cluster Analysis performed on the chemical composition of the volatile emissions of samples related to the treatment with *Citrus limon* essential oil. Sample codes are defined in Table 2.

In this case, PCA was performed on a 46 × 6 covariance data matrix (46 compounds × 6 samples = 276 data), and the chosen PC1 and PC2 covered 62.7% and 18.9% of the variance, respectively, for a total explained variance of 81.6%. The PCA was in line with the HCA outcomes. In fact, in the score plot (Figure 8, left), strawberry samples treated

with CHT enriched with *C. limon* EO (L1 and L2) were grouped closely together in the right-left quadrant, along with the headspace of the EO enriched CHT coating, indicating a high degree of similarity in their volatile composition. This proximity could be attributed to the strong influence of monoterpene hydrocarbons, such as limonene, β-pinene, and γ-terpinene, whose vectors pointed in the rightmost direction of the loading plot (Figure 8, right). Conversely, sample Z1, characterized by high levels of esters, particularly ethyl acetate, ethyl butyrate, and ethyl hexanoate, was plotted in the upper left quadrant, clearly separated from the EO-treated samples along PC1, which explained 62.7% of the total variance. Interestingly, sample Z1, despite clustering closely with the CHT-coated sample (C) in the dendrogram, was located far apart along PC2 in the PCA score plot, suggesting a great chemical dissimilarity between the two samples, as well.

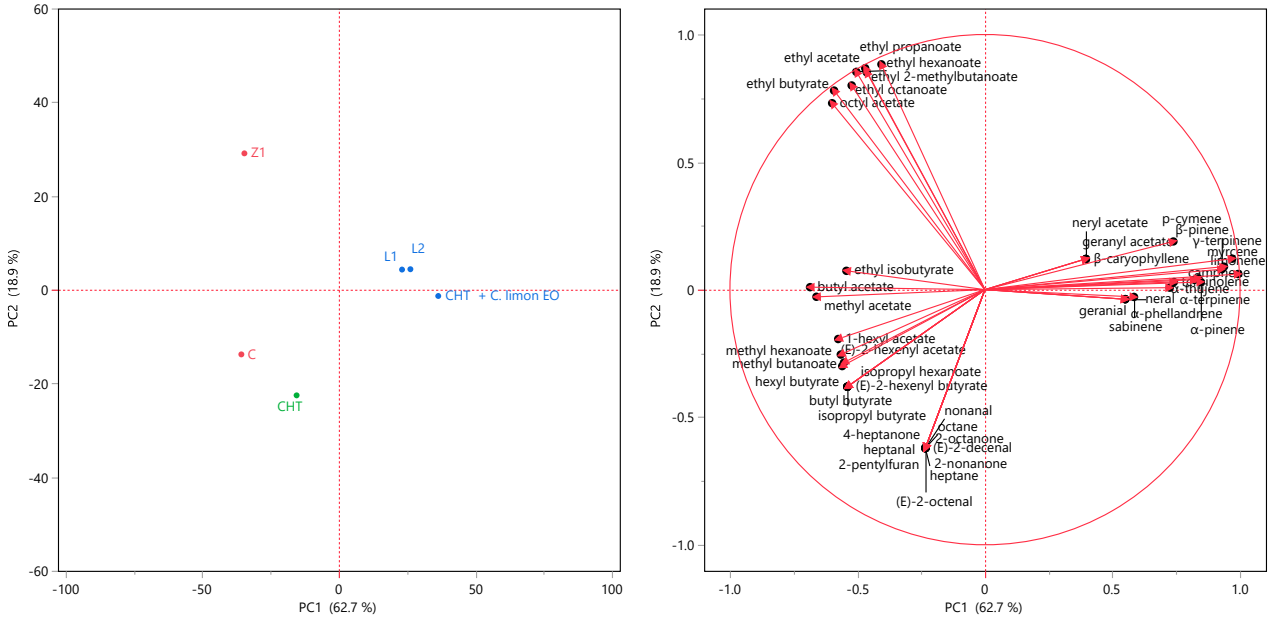


Figure 8. Score (**left**) and loading (**right**) plot of the Principal Component Analysis performed on the chemical composition of the volatile emissions of samples related to the treatment with *Citrus limon* essential oil. Sample codes are defined in Table 2.

3.4. Sensory Profile of Treated Strawberry Fruits

Following the sensory-driven selection of EOs (Section 3.1), the impact of CHT coatings enriched with *I. verum*, *C. sinensis*, or *C. limon* EO on the sensory profile of strawberries was evaluated through QDA. Statistical analysis revealed that the application of CHT + EOs induced significant variations in both quantitative and hedonic sensory attributes. Radar plots were used to visualize quantitative and hedonic descriptors, with statistically significant differences indicated by asterisks (Figure 9).

Among the quantitative attributes, 11 parameters showed significant differences among treatments. In particular, "olfactory intensity", "consistency", "bitterness", "harmony", "typical fruity", "floral", "fruity", and "spicy" attributes were highly significant ($p < 0.001$), while "spherical perception" and "vegetal" attributes were significant at $p < 0.01$ and "biological/biochemical" at $p < 0.05$. Conversely, "acidic", "sweet", and "persistence" attributes did not show significant differences among samples.

Among the tested coatings, strawberries treated with CHT + *C. sinensis* EO at 1% (AR1) exhibited the best preservation of key sensory attributes typical of fresh strawberries, particularly in terms of consistency, aroma, and overall flavor profile. In contrast, treatment with CHT + *I. verum* EO at 2% (A2) showed the lowest ability to preserve the natural sensory

profile, as indicated by increased spiciness, bitterness, and olfactory intensity, coupled with reduced consistency, typical fruity character, and spherical perception.

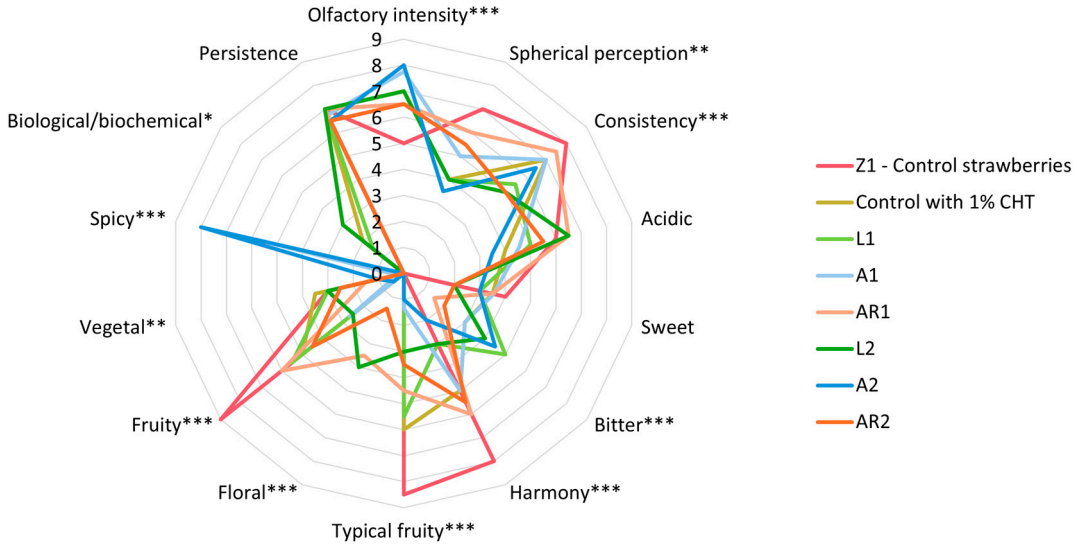


Figure 9. Quantitative descriptors from strawberries coated with chitosan and essential oils scored by intensity with level of significance (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). Sample codes are defined in Table 2.

Interestingly, the treatment with CHT + *C. limon* EO at 1% (L1) showed relatively high bitterness values and a reduced consistency compared to other 1% EO treatments. The pure CHT sample (C) was the closest to the natural strawberry profile in terms of flavor, although it exhibited lower spherical perception and slightly negative scores in the "biological/biochemical" descriptor, similarly to samples containing *C. limon* EO.

Hedonic attributes (Figure 10) showed significant differences across all descriptors. In particular, "fineness" was significant at $p < 0.05$, while all other attributes ("frankness", "fineness retro-olfactory", "frankness retro-olfactory", "aromatic richness", "olfactory pleasantness", "gustatory pleasantness", "retro-olfactory pleasantness", and "hedonic level") were highly significant ($p < 0.001$). The AR1 sample achieved the highest scores for hedonic perception, including olfactory, gustatory, and retro-olfactory pleasantness, outperforming even the pure CHT control. Moreover, quality-related attributes such as "fineness", "frankness", "harmony", and "aromatic richness" were significantly enhanced with this coating.

Conversely, increasing EO concentration to 2% generally resulted in a deterioration of sensory quality, with increased bitterness and reduced consistency, as also observed for AR2 (Figure 9). Despite this, AR2 remained the second-best treatment in terms of overall hedonic acceptability (Figure 11).

To further explore relationships among sensory descriptors and samples, PCA was performed (Figure 12). The first two principal components explained 82.3% of the total variance (PC1 = 70.4%, PC2 = 11.9%). PC1 clearly separated samples with positive attributes (left side), associated with descriptors such as "harmony", "consistency", "spherical perception", and hedonic parameters, from those characterized by more intense and less typical attributes (right side), including "spicy", "bitter", "olfactory intensity", and "biological/biochemical".

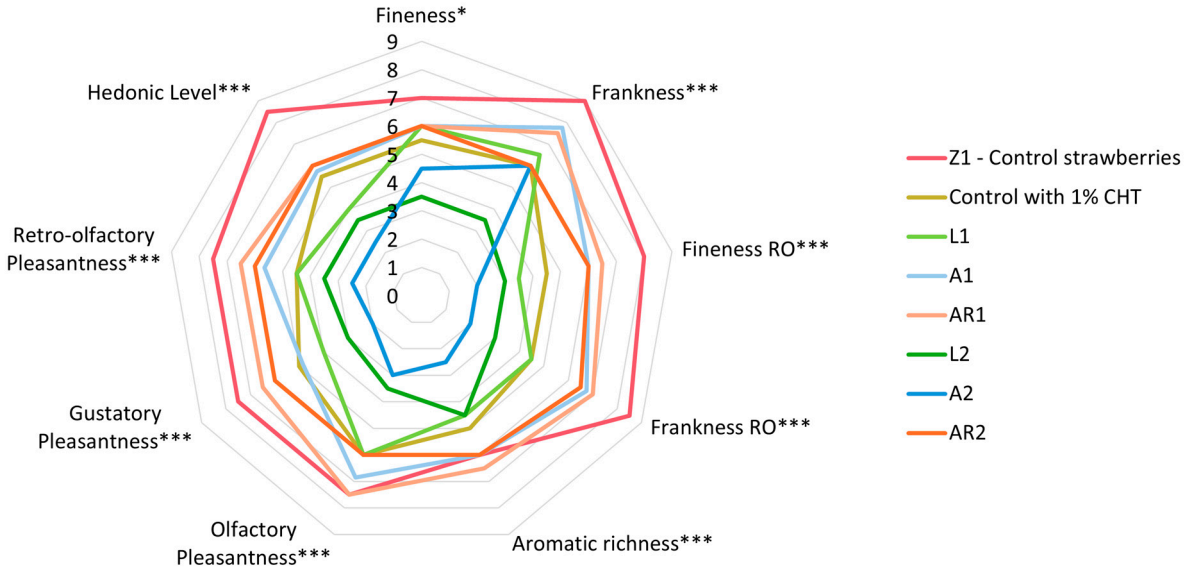


Figure 10. Hedonic descriptors from strawberries coated with chitosan and essential oils scored by intensity with level of significance (* = $p < 0.05$; *** = $p < 0.001$). Sample codes are defined in Table 2.

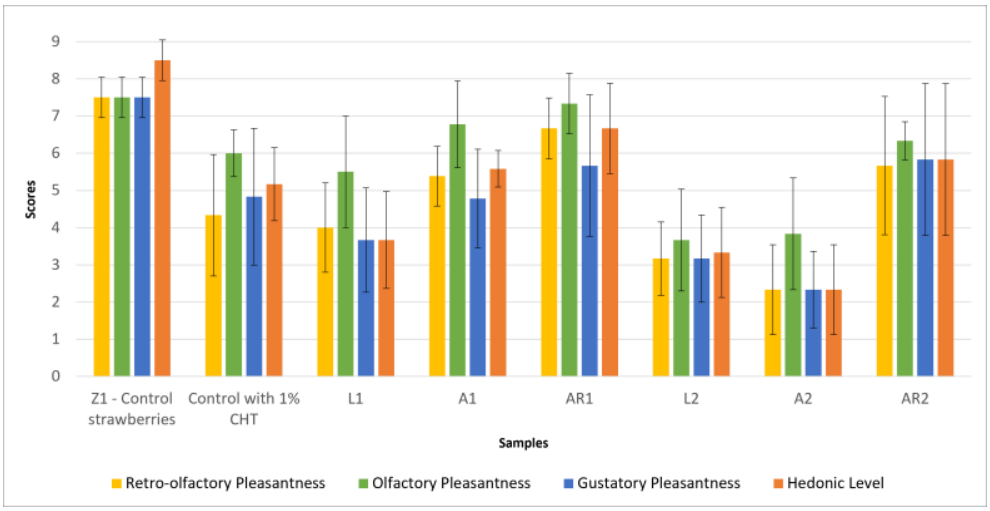


Figure 11. Overall pleasantness of strawberries coated with chitosan and essential oils. Sample codes are defined in Table 2.

The untreated control (Z1) was positioned in the negative region of PC1, closely associated with desirable sensory attributes, confirming its role as the reference profile. The pure CHT-treated sample (C) shifted toward the positive side of PC2, indicating the emergence of slight off-notes such as vegetal and biochemical nuances.

Samples treated with coatings containing *I. verum* EO showed a clear concentration-dependent shift: A1 exhibited moderate deviation from the control, while A2 was strongly associated with "spicy" and "olfactory intensity", indicating a pronounced alteration of the natural strawberry profile.

AR1 was located close to the control sample, confirming its ability to preserve a balanced sensory profile characterized by fruity, sweet, and harmonious attributes. AR2 showed a similar but slightly more intense profile, with increased persistence and acidity.

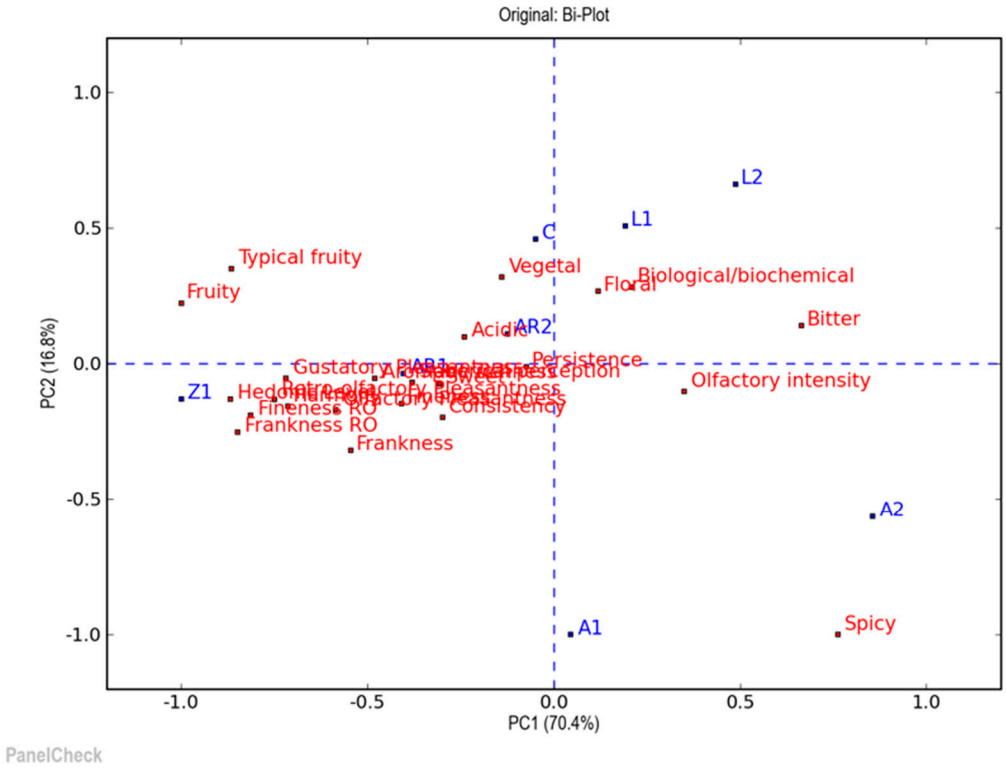


Figure 12. Principal Component Analysis performed on sensory attributes (quantitative and hedonic) of strawberries coated with chitosan and essential oils. Sample codes are defined in Table 2.

Samples treated with coating including *C. limon* EO (L1 and L2) were positioned in regions associated with floral, vegetal, and bitter descriptors, indicating a progressive deviation from the typical strawberry profile, particularly at the higher concentration (L2), which exhibited the most pronounced off-notes and sensory imbalance.

Overall, sensory results indicate that the incorporation of EOs into CHT coatings significantly modulates strawberry sensory properties in a concentration-dependent manner. Among the tested formulations, CHT + *C. sinensis* EO at 1% provided the best balance between preservation of the natural strawberry profile and enhancement of sensory quality.

Such results confirm that the preliminary sensory screening was effective in identifying EOs suitable for food application, with *C. sinensis* at 1% emerging as the best compromise between preservation of the natural strawberry profile and sensory enhancement.

3.5. Antifungal Activity of Selected Essential Oils Against Botrytis cinerea

When the three selected EOs (i.e., *I. verum*, *C. sinensis*, and *C. limon*) were tested for their ability to control *B. cinerea* though their soluble compounds, no one was capable of significantly reducing pathogen's mycelial growth, with the values (expressed in cm) not statistically different from each other or from the control (water).

Conversely, when growth curves, obtained in control plates (water) and in the presence of volatile compounds released by the three tested EOs, where submitted to anaylsis of variance of regression, a significant *p*slope value (<0.0001) was obtained for *I. verum* and *C. sinensis* EOs (Table 4). No significant reduction, compared to control, was observed in the presence of *C. limon* EO (*p*slope = 0.309).

Table 4. Effect of volatile compounds released by the selected essential oils on *Botrytis cinerea* growth.

Treatment	Slope (mm h^{-1})	R^2	*p*	*p*slope
Control (water)	0.12	0.93	<0.0001	-
Illicium verum EO	0.03	0.72	<0.0001	<0.0001
Citrus sinensis EO	0.07	0.93	<0.0001	<0.0001
Citrus limon EO	0.11	0.82	<0.0001	0.309

Analysis of variance of regression of *B. cinerea* growth on PDA (at 24 °C) in the presence of volatile compounds released by the three EOs. Slope: fungal growth expressed in mm h^{-1}; R^2 and *p*: significance of the regression; *p*slope: significance of the difference in fungal growth with water (control) vs. each EO ($p < 0.05$).

3.6. Protective Effect of Chitosan Added with the Selected Essential Oils Against Botrytis cinerea on Strawberry Fruits

When the protective effect of CHT—applied alone or added with one of the two selected EOs (i.e., *I. verum* and *C. sinensis*)—was evaluated through visual assessment of strawberry fruits, no significant differences were observed between untreated fruits and those treated with 1% CHT (C) for either the DI or the DSI (Table 5). In contrast, the addition of the two EOs (A1 and AR1) to the coating resulted in a significant reduction in both parameters, with approximately half as many symptomatic fruits observed in the presence of *I. verum* and *C. sinensis* EOs. Interestingly, no significant differences were detected between the two EOs.

Table 5. Effect expressed as Disease Incidence (DI) and Disease Severity Index (DSI) of pure chitosan 1% and added with 1% of the selected essential oils on *Botrytis cinerea* infection of strawberry fruits.

Treatment	DI	DSI
B. cinerea	100.0 ± 0.0 A	80.0 ± 17.8 A
1% CHT [C] + *Botrytis cinerea*	96.7 ± 4.7 A	86.7 ± 2.4 A
1% CHT + 1% *Illicium verum* EO [A1] + *Botrytis cinerea*	46.7 ± 16.9 B	43.3 ± 19.3 B
1% CHT + 1% *Citrus sinensis* EO [AR1] + *Botrytis cinerea*	50.0 ± 0.0 B	40.0 ± 0.0 B

Values correspond to the mean ± Standard Deviation ($n = 3$), with each biological replicate consisting of 10 fruits. DI: Disease Incidence expressed as % of symptomatic fruits; DIS: Disease Severity Index calculated according to a formula adaptation from Tender et al. [25] (see Section 2.9). Within the same column, at the different letter correspond values significantly different ($p < 0.05$).

4. Discussion

The growing interest in bio-based preservation strategies reflects a broader transition toward reducing synthetic inputs in the food system while maintaining product quality and safety [26]. In this context, CHT-based coatings enriched with EOs have emerged as promising options, combining barrier properties with antimicrobial activity [11]. However, their practical implementation depends not only on their effectiveness against postharvest pathogens but also on their impact on sensory quality [14], which remains a key determinant of consumer acceptance.

The present study demonstrates that CHT coatings are not chemically inert systems but can actively modulate the volatile profile of strawberry fruits. Although the ester-dominated signature typical of fresh strawberries was largely preserved, significant shifts in the relative abundance of key compounds were observed. Since these esters are mainly associated with fruit metabolism [27], rather than external contamination, the results suggest that CHT may influence biochemical pathways involved in aroma biosynthesis. This interpretation is consistent with recent findings showing that CHT coatings can affect volatile metabolism [28] and delay ripening-related processes in fresh fruit [29,30].

The incorporation of EOs into the CHT matrix led to more pronounced qualitative and quantitative changes in the volatile emissions of treated fruits. In all cases, the chemical fingerprint of the EO was clearly reflected in the headspace composition of coated straw-

berries. The predominance of phenylpropanoids in *I. verum* EO-containing samples and of monoterpene hydrocarbons in citrus EO—including treatments confirms that EO-derived compounds are effectively transferred to the fruit surface through the coating matrix. This outcome supports the role of CHT as a carrier system capable of modulating the release of volatile bioactives.

A key result of this work is the consistent dose-dependent effect observed across all EO-including treatments. Increasing EO concentration resulted in a higher contribution of EO-derived volatiles and a concomitant reduction in native strawberry esters. This highlights a critical trade-off between functional efficacy and preservation of the natural aroma profile. At higher concentrations, the dominance of exogenous compounds may lead to a loss of product identity, which is undesirable from a sensory standpoint. This effect was particularly evident for *I. verum* EO, where the strong aromatic character of anethole significantly altered the overall profile of the fruit, in agreement with previous studies describing the impact of EO components on aroma modulation [12].

The integration of chemical and sensory data further strengthens this interpretation. The sensory results indicate that not all EOs are equally suitable for application in strawberry coatings, and that their effectiveness depends on both composition and concentration. Among the tested formulations, *C. sinensis* EO at 1% emerged as the most balanced option, maintaining a sensory profile close to that of untreated fruit while improving hedonic perception. This conclusion is consistent with recent studies demonstrating that moderate EO concentrations can enhance shelf-life while preserving sensory acceptability in strawberries [31,32]. Conversely, higher EO concentrations and certain EO types resulted in increased bitterness, spiciness, and sensory imbalance, confirming that excessive volatile enrichment can negatively affect consumer perception.

From a functional perspective, the antifungal assays highlighted the importance of volatile-mediated activity. While no significant inhibition was observed for diffusible compounds, the vapor phase of *I. verum* and *C. sinensis* EOs significantly reduced the growth of *B. cinerea*. This finding aligns with previous reports showing that EO volatiles can exert strong antifungal effects through membrane disruption and metabolic interference [33,34]. The lack of significant activity observed for *C. limon* EO further emphasizes that antifungal efficacy depends on EO composition rather than on the mere presence of volatile compounds. Differences in activity among citrus EOs have already been reported and are generally attributed to variations in minor components and synergistic interactions [35].

Importantly, the in vivo experiments confirmed that the combination of CHT with selected EOs significantly reduced both disease incidence and severity on strawberry fruits. This demonstrates that the incorporation of EOs into a polymeric matrix enhances their effectiveness under realistic conditions, likely due to sustained release and improved interaction with the fruit surface. Similar results have been reported for CHT-based coatings enriched with EOs, showing improved control of *B. cinerea* and extended shelf-life of strawberries [36].

5. Conclusions

Taken together, these results indicate that the same volatile compounds responsible for antifungal activity may also induce sensory modifications, highlighting a functional–sensory trade-off that must be carefully managed when designing active coatings. In this context, the integration of sensory-driven selection with chemical and microbiological analyses represents a key strength of the present study, allowing the identification of formulations that are both effective and acceptable. Under the present experimental conditions, 2% EO treatments did not appear advisable as first-choice formulations for fresh strawberry coatings because of their impact on sensory quality and aromatic profile.

From an application standpoint, the findings suggest that CHT coatings enriched with selected EOs can be tailored to achieve specific goals, including shelf-life extension, reduction in postharvest losses, and controlled modulation of fruit aroma. This approach is particularly relevant for low-input or "zero residue" supply chains, where the use of synthetic preservatives is limited. Moreover, the dipping-based application carried out in this study is compatible with existing postharvest handling practices and could be adapted for industrial-scale implementation.

Nevertheless, some limitations of the study should be acknowledged. The experiments were conducted under controlled laboratory conditions and on a single strawberry cultivar, which may not fully capture the variability encountered under commercial supply chain conditions. Factors such as storage temperature fluctuations, packaging systems, and cultivar-specific responses could influence both coating performance and sensory outcomes. Moreover, a specific shelf-life assessment under commercial or simulated storage conditions was not performed for each EO coating, and future studies should address this aspect to better evaluate the practical applicability of the proposed formulations. In addition, only three EOs were selected for detailed analysis, and only two concentration levels were tested, which does not allow the identification of optimal formulations within a finer concentration range.

Despite the relatively short duration of the in vivo experiments, it is worth emphasizing that the trials were conducted at 24 °C, a temperature optimal for the germination of *B. cinerea* spores and subsequent strawberry infection. Under these highly conducive conditions, the coatings based on EOs combined with CHT still exhibited a significant protective action on the fruits, highlighting their effectiveness even in a strongly disease-promoting environment. The antifungal assay performed on strawberry fruits should be considered a preliminary proof-of-concept evaluation aimed at exploring the potential practical application of the selected essential oils, whereas the primary focus of the present study was the characterization of their volatile composition and sensory properties. Furthermore, sensory evaluation was carried out by a trained panel, which ensures analytical reliability but may not fully reflect consumer perception under real market conditions. Finally, although clear relationships between coating composition, volatile profile, and antifungal activity were observed, the underlying physiological mechanisms involved in the modulation of fruit metabolism were not specifically investigated and warrant further study.

Overall, this study integrates sensory-driven selection with instrumental and biological validation, providing a multidisciplinary framework for optimizing bioactive coatings, and contributes to a better understanding of how bio-based coatings can be strategically designed to modulate postharvest fruit quality. The results highlight the importance of balancing antimicrobial efficacy with sensory preservation and provide a framework for the development of sustainable coating systems tailored to specific commodities and market requirements.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy16121202/s1, Figure S1: Sensory wheel from chitosan–essential oil coated strawberries; Table S1: Chemical composition of the volatile emissions of CHT, untreated strawberries (Z1), and chitosan-coated strawberries (C); Table S2: Chemical composition of the volatile emissions of samples related to the treatment with *Illicium verum* essential oil; Table S3: Chemical composition of the volatile emissions of samples related to the treatment with *Citrus sinensis* essential oil; Table S4: Chemical composition of the volatile emissions of samples related to the treatment with *Citrus limon* essential oil.

Author Contributions: Conceptualization, B.C., F.V., and S.S.; methodology, F.V. and S.S.; software, P.D.-G.; validation, B.C., F.V. and S.S.; formal analysis, A.P., C.S., D.M., P.D.-G. and Y.P.; investigation, A.P., D.M., P.F. and Y.P.; data curation, A.P., C.S., D.M., P.D.-G. and Y.P.; writing—original draft prepa-

ration, F.V., P.D.-G., S.S. and Y.P.; writing—review and editing, B.C., F.V., P.F. and S.S.; supervision, B.C., F.V. and S.S.; project administration, B.C.; funding acquisition, B.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was granted by the PRIMA program, project FoWRSaP. The PRIMA program is supported by the European Union and the Italian Ministero dell'Università e della Ricerca.

Institutional Review Board Statement: The research obtained the approval of the bioethical committee of the University of Pisa (protocol n. 0088081/2020).

Informed Consent Statement: The research was conducted according to the ethical guidelines, and informed consent was obtained from all participants.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Acknowledgments: The graphical abstract was designed using resources from Flaticon.com (accessed on 16 May 2026). The authors acknowledge the use of ChatGPT 5.5 (OpenAI, San Francisco, CA, USA) for language editing and readability improvement during manuscript preparation.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CHT	Chitosan
DVB/CAR/PDMS	Divinylbenzen/carboxen/polydimethylsiloxane
EO	Essential Oil
FDA	Food and Drug Administration
GC-MS	Gas Chromatography-Mass Spectrometry
GRAS	Generally Recognized As Safe
HCA	Hierarchical Cluster Analysis
HS-SPME	Headspace Solid Phase Micro-Extraction
PCA	Principal Component Analysis
QDA	Quantitative Descriptive Analysis

References

1. Angelino, D.; Godos, J.; Ghelfi, F.; Tieri, M.; Titta, L.; Lafranconi, A.; Marventano, S.; Alonzo, E.; Gambera, A.; Sciacca, S.; et al. Fruit and vegetable consumption and health outcomes: An umbrella review of observational studies. *Int. J. Food Sci. Nutr.* **2019**, *70*, 652–667. [CrossRef] [PubMed]
2. Plazzotta, S.; Manzocco, L.; Nicoli, M.C. Fruit and vegetable waste management and the challenge of fresh-cut salad. *Trends Food Sci. Technol.* **2017**, *63*, 51–59. [CrossRef]
3. FAO Food and Agriculture Organization of the United Nations. Global Food Losses and Food Waste—Extent, Causes and Prevention. 2011. Available online: https://www.fao.org/sustainable-food-value-chains/library/details/en/c/266053/ (accessed on 9 April 2026).
4. Ambuko, J.L.; Masakhwe, S.M.; Amwoka, E.; Mujuka, E.; Fabi, C. Food loss and waste data gaps in fruit and vegetable value chains: A review of the literature. *Front. Hortic.* **2025**, *4*, 1529040. [CrossRef]
5. da Silva, T.P.; Castro, B.P.S.; Cordeiro, N.G.; de Souza Andrade, A.C.; Souza Lopes, A.C.; Vieira de Lima Costa, B. Waste of fruits and vegetables in retail: Cause, destination and reduction strategies. *J. Mater. Cycles Waste Manag.* **2025**, *27*, 1626–1636. [CrossRef]
6. Guo, J.; Xu, Y.; Liang, S.; Zhou, Z.; Zhang, C.; Li, K.; Peng, X.; Qin, S.; Xing, K. Antifungal activity of volatile compounds from *Bacillus tequilensis* XK29 against *Botrytis cinerea* causing gray mold on cherry tomatoes. *Postharvest Biol. Technol.* **2023**, *198*, 112239. [CrossRef]
7. Carocho, M.; Barreiro, M.F.; Morales, P.; Ferreira, I.C.F.R. Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 377–399. [CrossRef] [PubMed]
8. Nichifor, B.; Zait, L.; Timiras, L. Drivers, barriers, and innovations in sustainable food consumption: A systematic literature review. *Sustainability* **2025**, *17*, 2233. [CrossRef]

9. Girotto, F.; Alibardi, L.; Cossu, R. Food waste generation and industrial uses: A review. *Waste Manag.* **2015**, *45*, 32–41. [CrossRef] [PubMed]
10. Díaz-Guerrero, P.; Panzani, S.; Sanmartin, C.; Muntoni, C.; Taglieri, I.; Venturi, F. "Pepper": Different spices, one name—Analysis of sensory and biological aspects. *Molecules* **2025**, *30*, 1891. [CrossRef] [PubMed]
11. Parichanon, P.; Farina, P.; Vicente, I.; Cesarini, M.; Hotaj, E.; Sarrocco, S.; Pellegrini, E.; Conti, B. Chitosan/mandarin essential oil-based films on citrus fruits for the control of the medfly attack and to prevent the occurrence of grey and blue mould in post-harvest. *J. Stored Prod. Res.* **2024**, *108*, 102380. [CrossRef]
12. Perricone, M.; Arace, E.; Corbo, M.R.; Sinigaglia, M.; Bevilacqua, A. Bioactivity of essential oils: A review on their interaction with food components. *Front. Microbiol.* **2015**, *6*, 76. [CrossRef] [PubMed]
13. Maurya, A.; Prasad, J.; Das, S.; Dwivedy, A.K. Essential oils and their application in food safety. *Front. Sustain. Food Syst.* **2021**, *5*, 653420. [CrossRef]
14. Sharma, S.; Barkauskaite, S.; Jaiswal, A.K.; Jaiswal, S. Essential oils as additives in active food packaging. *Food Chem.* **2021**, *343*, 128403. [CrossRef] [PubMed]
15. Parichanon, P.; Santin, M.; Khemakhem, M.; Falher, T.; Petrucci, A.; Mencarini, D.; Sarrocco, S.; Conti, B.; Castagna, A. Mandarin essential oil-flavoured bio-based active packaging for quality preservation and fungal control in fresh produce. *Postharvest Biol. Technol.* **2026**, *231*, 113906. [CrossRef]
16. Jugreet, B.S.; Suroowan, S.; Rengasamy, R.R.K.; Mahomoodally, M.F. Chemistry, bioactivities, mode of action and industrial applications of essential oils. *Trends Food Sci. Technol.* **2020**, *101*, 89–105. [CrossRef]
17. Derbassi, N.b.; Pedrosa, M.B.; Heleno, S.; Carocho, M.; Ferreira, I.C.F.R.; Barros, L. Plant volatiles: Using scented molecules as food additives. *Trends Food Sci. Technol.* **2022**, *122*, 97–103. [CrossRef]
18. Mihut, A.; Mihut, C.; Gal, T.; Dascalu, I.; Iordanescu, O. Analysis of the importance of strawberry (*Fragaria* × *ananassa*) cultivation in the European Union and Romania: Economic, technological and pedoclimatic implications. *J. Hortic. For. Biotechnol.* **2024**, *29*, 414–418. [CrossRef]
19. Duarte-Molina, F.; Gómez, P.L.; Agueda Castro, M.; Alzamora, S.M. Storage quality of strawberry fruit treated by pulsed light: Fungal decay, water loss and mechanical properties. *Innov. Food Sci. Emerg. Technol.* **2016**, *34*, 267–274. [CrossRef]
20. U.S. Food and Drug Administration. The Substances Added to Food Inventory Replaces What Was Previously Known as Everything Added to Foods in the United States (EAFUS). 2026. Available online: https://hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances (accessed on 9 April 2026).
21. Tonacci, A.; Billeci, L.; Di Mambro, I.; Marangoni, R.; Sanmartin, C.; Venturi, F. Wearable sensors for assessing the role of olfactory training on the autonomic response to olfactory stimulation. *Sensors* **2021**, *21*, 770. [CrossRef] [PubMed]
22. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allured Pub. Corp: Carol Stream, IL, USA, 2007.
23. NIST. *NIST/EPA/NIH Mass Spectral Library*; Stein, S.E., Ed.; National Institute of Standards and Technology, Mass Spectrometry Data Center: Gaithersburg, MD, USA, 2014.
24. Bedini, S.; Cosci, F.; Tani, C.; Pierattini, E.C.; Venturi, F.; Lucchi, A.; Ioriatti, C.; Ascrizzi, R.; Flamini, G.; Ferroni, G.; et al. Essential oils as post-harvest crop protectants against the fruit fly *Drosophila suzukii*: Bioactivity and organoleptic profile. *Insects* **2020**, *11*, 508. [CrossRef] [PubMed]
25. De Tender, C.; Vandecasteele, B.; Verstraeten, B.; Ommeslag, S.; De Meyer, T.; De Visscher, J.; Dawyndt, P.; Clement, L.; Kyndt, T.; Debode, J. Chitin in strawberry cultivation: Foliar growth and defense response promotion, but reduced fruit yield and disease resistance by nutrient imbalances. *Mol. Plant-Microbe Interact.* **2021**, *34*, 227–239. [CrossRef] [PubMed]
26. Carpena, M.; Nuñez-Estevez, B.; Soria-Lopez, A.; Garcia-Oliveira, P.; Prieto, M.A. Essential oils and their application on active packaging systems: A Review. *Resources* **2021**, *10*, 7. [CrossRef]
27. Xiao, Z.; Hu, Y.; Niu, Y.; Zhang, J.; Yang, B. Five representative esters and aldehydes from fruits can enhance sweet perception. *LWT* **2024**, *194*, 115804. [CrossRef]
28. Perdones, Á.; Escriche, I.; Chiralt, A.; Vargas, M. Effect of chitosan–lemon essential oil coatings on volatile profile of strawberries during storage. *Food Chem.* **2016**, *197*, 979–986. [CrossRef] [PubMed]
29. Priyadarshi, R.; El-Araby, A.; Rhim, J.W. Chitosan-based sustainable packaging and coating technologies for strawberry preservation: A review. *Int. J. Biol. Macromol.* **2024**, *278*, 134859. [CrossRef] [PubMed]
30. Cao, M.; Qiao, C.; Han, L.; Zhuang, M.; Wang, S.; Pang, R.; Guo, L.; Yang, M.; Gui, M. Volatile profile of postharvest hardy kiwifruits treated with chitosan-silica nanocomposite coatings. *Food Res. Int.* **2025**, *205*, 115981. [CrossRef] [PubMed]
31. Carhuavilca-Paredes, Y.; Chirinos-Molina, B.; Inga, M.; Castro-Mandujano, O.N.; Bautista-Cruz, N.; Romero y Otiniano, P.; Chauca-Cerrutti, A.; Bravo-Araníbar, N. Enhancing strawberry preservation: A novel edible coating combining chitosan, mango seed starch (*Mangifera indica*), and orange peel essential oil (*Citrus sinensis*). *CyTA J. Food* **2025**, *23*, 2532496. [CrossRef]
32. Hassani, B.; Ebrahimi, F.; Najafi, A.; Ziaolhagh, S. Investigation of the effect of aloe vera gel coating combined with free and encapsulated savory essential oil on the shelf life of strawberries. *Appl. Food Res.* **2025**, *5*, 101269. [CrossRef]

33. Aouadi, G.; Grami, L.K.; Taibi, F.; Bouhlal, R.; Elkahoui, S.; Zaagueri, T.; Jallouli, S.; Chaanbi, M.; Rabeh Hajlaoui, M.; Mediouni Ben Jemâa, J. Assessment of the efficiency of *Mentha pulegium* essential oil to suppress contamination of stored fruits by *Botrytis cinerea*. *J. Plant Dis. Prot.* **2022**, *129*, 881–893. [CrossRef]
34. Tančinová, D.; Mašková, Z.; Mendelová, A.; Foltinová, D.; Barboráková, Z.; Medo, J. Antifungal activities of essential oils in vapor phase against *Botrytis cinerea* and their potential to control postharvest strawberry gray mold. *Foods* **2022**, *11*, 2945. [CrossRef] [PubMed]
35. de Albuquerque Sousa, T.C.; da Cunha, W.M.; Gomes Rosas, A.L.; Quevedo Oppelt, C.; Ávila Gandra, E.; Valmor Rombaldi, C.; Dillenburg Meinhart, A. Essential oils as natural sources for the control of *Botrytis cinerea*: Chemical composition and antifungal effect. *Food Biosci.* **2024**, *62*, 105516. [CrossRef]
36. Wang, D.; Yang, H.; Lu, X.; Wu, Y.; Blasi, F. The inhibitory effect of chitosan based films, incorporated with essential oil of *Perilla frutescens* leaves, against *Botrytis cinerea* during the storage of strawberries. *Processes* **2022**, *10*, 706. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.