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RESEARCH

Metabolomic Insights into Apple Responses to Sepiolite-Based Active Packaging and Modified Atmosphere Storage for Shelf-Life Extension

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Abstract

The development of innovative and sustainable postharvest strategies to preserve fruit quality is a major challenge in the fresh produce chain. In this study, we evaluated a chitosan–cassava starch film enriched with sepiolite, used as an active packaging, alone and combined with modified atmosphere (MA; 1% O_2, 50% CO_2), to extend the shelf life of apples. Quality traits (color, texture, pH) were monitored up to 60 days and integrated with untargeted UHPLC–QTOF metabolomics on peel and pulp. Sepiolite film (SF) and MA both delayed softening and browning at room temperature, compared with the control. Furthermore, the MA + SF combination maintained peel hardness levels comparable to those of low-temperature storage after 60 days, highlighting the potential of this strategy to preserve postharvest apple quality under ambient conditions at levels comparable to refrigerated storage. Metabolomics highlighted a significant time-dependent reprogramming of the apple metabolome, with storage driving extensive changes in membrane lipids and specialized metabolites. SF promoted the accumulation of phenylpropanoids, phytohormones, and vitamins, suggesting improved membrane stability and antioxidant capacity. MA further modulated primary and functional metabolism, notably modulating jasmonate-related compounds, flavonoids, and several B and E vitamins. AMOPLS analysis indicated that atmosphere factor was the main driver of metabolic variance in peel, whereas both atmosphere and sepiolite significantly contributed in pulp. Overall, sepiolite-based active film combined with modified atmospheres represents a promising bio-based approach for ambient postharvest preservation of apples.

Keywords Postharvest · Food packaging · Texture · Polysaccharide film · Food quality · Foodomics

Introduction

Food waste has been a critical global challenge for years, with significant implications for consumers, environmental sustainability, and supply chain dynamics. Current estimates indicate that one-third of all food produced—approximately 1.3 billion tons annually, sufficient to feed 2 billion people—is lost or wasted (Alonso-Salinas et al., 2024). Among all food categories, fruits and vegetables account for 45–55% of global waste, primarily due to their highly perishable nature (Navarro-Martínez et al., 2021). Specifically, climacteric fruits, such as apples, are particularly vulnerable to postharvest losses. Their rapid ripening and senescence are characterized by excessive ethylene biosynthesis and elevated respiration rates, which accelerate firmness loss, nutritional decline, and sensory quality deterioration. Consequently, climacteric fruits generally exhibit a shorter shelf life compared to non-climacteric ones. Apples represent the third most

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widely produced and consumed fruit worldwide, and their large-scale cultivation contributes to several tons of waste (Dhillon et al., 2013). Given the significant contribution of climacteric fruits to global food waste, innovative solutions are needed to maintain fresh fruit quality and extend shelf life. Hence, active packaging has emerged as a promising approach, as it modifies the conditions of packaged food to maintain and improve its quality (Alam et al., 2021; Salgado et al., 2021). Polysaccharides and other biopolymers are considered environmentally sustainable candidates for the preservation of fresh fruits. Among the different polymers employed, chitosan and cassava starch have been widely investigated due to their biodegradability and excellent film-forming ability. Additionally, chitosan exhibits antimicrobial and antifungal properties, and it represents a cost-effective and commercially available natural polysaccharide (Flórez et al., 2022). As complementary materials, cassava starch, commonly known as tapioca, revealed flexibility and transparency and can be easily obtained from renewable sources (Gunathilake & Somendrika, 2024).

An interesting naturally occurring ingredient is sepiolite, a magnesium silicate clay known for its non-toxic, inexpensive, and eco-friendly properties. Owing to its unique fibrous structure, composed of an octahedral magnesium sheet enclosed by two layers of tetrahedral silica sheets ($Mg_8[Si_{12}O_{30}](OH)_4(H_2O)_4.nH_2O$), this filler exhibits a high capacity for ethylene adsorption. This ability is attributed to its polar and active surface, which contains oxygen ions in the tetrahedral sheet, water molecules at the structure edge, SiOH groups, and microporous channels along the fiber axis (Bugnotti et al., 2023; Gaikwad et al., 2020; Sakizci, 2013). Consequently, sepiolite may reduce the ripening rate of climacteric fruits, thereby extending their shelf life and mitigating postharvest losses.

Reducing oxygen levels in the storage environment has therefore been widely applied to extend the shelf life of such fruits. In apples, for example, controlled-atmosphere storage under low-oxygen conditions effectively delays ripening by postponing the climacteric ethylene production burst (Watkins & Nock, 2012). Combining oxygen reduction through modified-atmosphere packaging with the ethylene-adsorbing capacity of sepiolite incorporated into film matrices can represent a promising strategy to maintain apple quality and extend the postharvest life of climacteric fruits. Beyond assessing the phenotypic effects of these treatments, it is of great scientific interest to elucidate the underlying metabolic modulations occurring in both pulp and peel tissues that contribute to the observed extension of shelf life. Metabolomics has emerged as a powerful approach to explore compositional and biochemical changes that occur during fruit postharvest storage (Castro-Cegrí et al., 2025; Yun et al., 2022). In apples, metabolomic profiling provided valuable insights into processes such as enzymatic browning (Hatoum et al., 2016), responsive mechanisms to compression damage (Z. Yang et al., 2023a, 2023b), and ethylene-related regulatory mechanisms mediated by 1-methylcyclopropene (L. Zhang et al., 2023a, 2023b). However, research investigating metabolic modulation induced by modified-atmosphere conditions, sepiolite-based films, and their combined application in smart packaging in apple fruit remains limited. Therefore, the present study employs an untargeted metabolomics approach to characterize the metabolic adjustments associated with extended shelf life in apples subjected to modified atmospheres, sepiolite film incorporation, and their combination. This strategy aims to identify novel metabolic responses that contribute to postharvest fruit quality preservation.

Materials and Methods

Film Preparation and Formulation

Film preparation was done following the method of Pramitasari et al. (2022), with modifications. The films were prepared by first dissolving 1% (w/v) chitosan in 1% acetic acid, which was homogenized at 300 rpm for 24 h. Separately, a 2% (w/v) cassava starch solution was prepared in distilled water and gelatinized on a magnetic hotplate stirrer at 80 °C. The two solutions were then combined at volume ratios of 1:1 (v/v) and 3:1 (v/v), as detailed in Table 1. Glycerol (0.4% v/v, 85%) was added as a plasticizer. Sepiolite (1, 1.5, or 2% w/v) was gradually incorporated into the mixture and stirred at 500–800 rpm at 80 °C until a homogeneous solution was obtained. The resulting films were designated as follows: 1:1 ratio of chitosan-cassava starch, 0% sepiolite (1C0S); 1:1 ratio of chitosan-cassava starch, 1% sepiolite (1C1S); 1:1 ratio of chitosan-cassava starch, 1.5% sepiolite (1C1.5S);

Table 1 Characterization of tested films with a ratio of chitosan-cassava starch 1:1 or 3:1, with sepiolite as additive at 0, 1, 1.5, and 2%

Ratio	Sepiolite (%)	Thickness	Transparency	WVTR
1:1	0	0.05 ± 0.01^{e}	40.5 ± 0.1^{a}	1.85 ± 0.01^{b}
	1	0.25 ± 0.01^{d}	1.6 ± 0.3^{c}	1.70 ± 0.01^{f}
	1.5	0.36 ± 0.02^{b}	0.3 ± 0.6^{d}	1.63 ± 0.01^{g}
	2	0.47 ± 0.02^{a}	0.4 ± 0.5^{d}	1.72 ± 0.01^{e}
3:1	0	0.07 ± 0.01^{e}	28.2 ± 0.1^{b}	1.93 ± 0.01^{a}
	1	0.25 ± 0.02^{d}	2.0 ± 0.3^{c}	1.79 ± 0.01^{c}
	1.5	0.30 ± 0.02^{c}	-1.5 ± 0.1^{e}	1.73 ± 0.01^{d}
	2	0.38 ± 0.02^{b}	-0.1 ± 0.3^{d}	1.63 ± 0.01^{h}

The data presented as the mean ± standard deviation of 5 films. Different letters indicate significant differences according to ANOVA by Duncan's test ($p < 0.05$).

These films were tested for thickness, transparency, and water vapor transmission rate (WVTR).

1:1 ratio of chitosan-cassava starch, 2% sepiolite (1C2S); 3:1 ratio of chitosan-cassava starch, 0% sepiolite (3C0S); 3:1 ratio of chitosan-cassava starch, 1% sepiolite (3C1S); 3:1 ratio of chitosan-cassava starch, 1.5% sepiolite (3C1.5S); 3:1 ratio of chitosan-cassava starch, 2% sepiolite (3C2S).

Film Forming

Packaging films were prepared using the solvent casting method. Aliquots of 175 mL of each film-forming solution were poured into 18 × 13 cm plastic trays and allowed to dry in a fume cupboard for 72–96 h until complete solvent evaporation. The dried films were carefully peeled off and stored in a desiccated container. For treatments containing sepiolite, films were cut into 9 × 13 cm rectangles and placed inside the packaging with apples (Fig. S1).

Film Characterization

Thickness and Transparency

Film thickness was measured using a digital caliper with a 0.01 mm accuracy at 10 different randomly chosen spots on the film (Upadhyay et al., 2022). For the transparency measurements, the light of transmittance was measured at a wavelength of 600 nm using a UV–Vis spectrophotometer (UV-1280, Shimadzu, Canby, USA). Film transparency was calculated according to Pramitasari et al. (2022) using absorbance at 600 nm (A600) and film thickness (D, mm), according to the following equation:

$$Transparency = \frac{(2 - A600)}{D}$$

Transparency values can become negative when absorbance exceeds 2, corresponding to transmittance values below 1%; therefore, these values indicate highly opaque films rather than physically negative light transmission.

Water Vapor Transmission Rate (WVTR)

The WVTR was calculated using the cup method following Santoso et al. (2023), where each film was first cut into circular pieces with a 4.5 cm diameter. The cut film was placed as a lid for a 50 mL beaker containing 5 g of silica gel, then put in a desiccator filled with distilled water. The container with silica gel and film was weighed periodically every 24 h for 5 days. The result was used to obtain a graph of the mass of the container against time, and the linear equation with the formula y = mx + c was acquired. The WVTR value is calculated following Eq. 2, where m is obtained from the linear equation, A is the film area (m^2), and t is the time (h):

$$WVTR = \frac{m}{A \times t}$$

Fruit Material and Experimental Design

Freshly harvested apples (*Malus domestica*, cultivar Golden Delicious, supplied by Garda frutta, Brescia, Italy) were packaged in a 20 cm × 7 cm × 45 cm packaging made of polyamide-polyethylene (Azienda Cartaria Lombarda, Malagnino, Italy). Five treatments were tested in this study to preserve apples during the postharvest period, consisting of control (C), low temperature (LT), sepiolite film (SF), modified atmosphere (MA), and modified atmosphere + sepiolite (MA + SF), with 5 biological replicates of 5 fruits each per treatment and storage period. Apples were stored in a temperature-controlled chamber at 4 ºC for LT treatment, and the rest at room temperature. The sepiolite film formulation 1C1S was chosen for the application on apple packaging based on the results obtained, and the MA was composed of 1% O_2 and 50% CO_2. All fruits were then stored in permanent darkness in a controlled environment for 6, 18, and 60 days. At each time point, the peel and pulp of fruits were removed entirely, pooled separately, frozen, and powdered in liquid nitrogen, and stored at −80 ºC.

Color and pH Evaluation

A colorimeter (Konica Minolta CM-600d, Osaka, Japan) was used to evaluate the color changes in apples. The color parameters were measured as lightness (L*), red-green (a*), and yellow-blue (b*) values according to the CIE Lab system (Whale & Singh, 2007), reflecting the visual appearance of the apples. The pH was measured in homogenized apple tissue (5.0 g) using a pH meter (HI2202 Hanna Instruments, Eibar, Spain).

Texture Evaluation

The apple texture analysis was performed using a Texture Analyser (TVT 6700, Perten, Sweden). Fruits were measured for peel hardness with a test profile performed with a single-cycle compression. Apples with the peel were cut in half, and a portion 25 mm thick was taken from the equatorial region for the peel hardness. The analysis was performed using a two-millimeter flat-head probe with a test speed of 1.00 mm/s. The maximum force recorded during the experiment corresponds to the force required to penetrate the peel, characterizing its hardness (Zhu et al., 2018). For the pulp compression, peel was removed, and two portions of 25 mm thick pulp were taken, one for the axial direction and the other for the radial direction. The axial direction corresponds to the longitudinal axis of the

fruit from the peduncle to the base, while the radial direction follows the transversal axis and corresponds to that along the cell rows. The analysis was performed using a three-millimeter flat head probe with a test speed of 1.00 mm/s and a compression of 80%. The mechanical responses were characterized by pulp axial and radial firmness (N) and the work of axial and radial compression (Khan & Vincent, 1993).

Sample Extraction and Metabolomics Analysis

For metabolomic analysis, the samples were homogenized (1:10, *w/v*) with methanol/H_2O/formic acid solution (80.0/19.9/0.1; *v/v/v*) and subjected to ultrasound-assisted extraction using a sonication bath for 15 min. Samples were then centrifuged at 5500 × *g* for 10 min at 4 ºC. Then, the supernatant was incubated overnight at −18 ºC to promote protein precipitation, filtered through a 0.22 µm cellulose syringe filter, and collected in vials. In parallel, quality controls (QC) were obtained by pooling 20 µL of each sample into the same vial.

The untargeted metabolomics analysis was done using a Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (HRMS) (Thermo Scientific, Waltham, MA, USA) coupled to a Vanquish ultra-high-pressure liquid chromatography (UHPLC). The chromatographic separation of compounds was conducted on an Agilent Zorbax Eclipse Plus C18 column (50 × 2.1 mm, 1.8 µm) as the stationary phase, which was maintained at 35 ºC. The mobile phase was composed of LC–MS grade water and acetonitrile, following a gradient elution from 6% up to 94% acetonitrile in 35 min. Additionally, 0.1% formic acid was added to both mobile phases as a phase modifier. All the optimized HRMS parameters are reported in a previous work (Becchi et al., 2023). The full scan MS analysis was carried out in a positive ionization mode with a nominal mass resolution of 70,000 at m/z 200. The injection volume was 3 µL, with a 0.2 mL/min flow rate and a mass m/z range of 80–1200. In addition, Data-dependent (Top N = 3) MS/MS mode with full scan mass resolution reduced to 17,500 at m/z 200, with an AGC target value of $1e^5$, maximum IT of 100 ms, and isolation window of 1.0 m/z, respectively. The Top N ions were selected for fragmentation under stepped Normalized Collisional Energy (i.e., 10, 20, 40 eV). The HESI parameters for both MS and MS/MS were as follows: sheath gas flow 40 arb (arbitrary units), auxiliary gas flow 20 arb, spray voltage 3.5 kV, capillary temperature 320 °C. Before data collection, the mass spectrometer was calibrated using Pierce™ positive ion calibration solution (Thermo Fisher Scientific, San Jose CA, USA). To avoid possible bias, the injection sequence was randomized.

Data Processing

The data processing was done according to Castro-Cegrí et al. (2025). Briefly, once the detection of features was completed, the acquired raw data were processed by MS-DIAL software (v. 4.90) and identified in FooDB database (https://foodb.ca/), including peak finding, feature identification by library matching, gap filling, and alignment steps. Firstly, the raw '.d' format files were converted into '.abf' files by the Reifycs Abf Converter. These '.abf' data files were imported into MS-DIAL, searching the features in the retention time range of 1–32 min, *m/z* range 100–1200 for MS-only data and 80–1200 for MS/MS data, setting a minimum of 3,000 counts for peak detection. The FooDB database (https://foodb.ca/) was chosen to identify features with tolerances of 0.01 Da and 0.05 Da for MS and MS/MS, respectively, and with an identification score cut-off > 80%. Feature identification was based on mass accuracy data, isotopic patterns, and spectral matching. For isotope recognition, only the maximum number of isotopes was set to 1, and the maximum charge number was set to 2, as the mass range for metabolite annotation was < 2000 Da. The adducts considered for annotation were selected based on the experimental matrix and the analytical procedure, i.e. $[M+H]^+$, $[M+H_2O]^+$, $[M+H\text{-}H2O]^+$ (neutral loss), $[M+ACN]^+$, $[M+Na]^+$, $[M+K]^+$, and all the possible combinations among them (Risoli et al., 2025; Tsugawa et al., 2015). All features that were not detected in at least 83.33% of replications per group were removed. According to the Metabolomics Standards Initiative (MSI) (Salek et al., 2013), a level 2 of confidence in features' annotation was achieved, conferring the assessment of putatively annotated compounds further involved in multivariate statistical analysis.

Statistical Analysis

The statistical analysis of the results from quality parameters was performed by one-way analysis of variance (ANOVA) using the SPSS 30.0 program (SPSS Inc.). Means were compared with Duncan's least significant differences test, assuming significant differences at $p < 0.05$.

Metabolomics raw data were first subjected to $\log_2$ transformation and normalization at the 75th percentile by the software Mass Profiler Professional 15.1 (Agilent Technologies®), before applying multivariate statistical analysis. Next, unsupervised hierarchical cluster analysis (HCA; Euclidean distance, Ward's linkage method) was performed to provide a metabolome-wide naive clustering as a function of the type of treatment and days of storage. Volcano analysis, by means of fold change analysis (FC), setting cut-off = ±2, combined with one-way ANOVA followed by Tukey's post hoc test ($p < 0.01$) was performed by Mass Profiler Professional 15.1 software to identify the differentially

accumulated metabolites (DAMs) due to each treatment and storage period compared against T0.

Thereafter, ANOVA Multiblock Orthogonal Partial Least Squares (AMOPLS) analysis was performed in R software (version 4.2.3) according to the previous workflow reported by Becchi et al. (2024). To validate the AMOPLS model and evaluate the statistical significance of the factors under investigation, a set of 100 random permutations was executed to avoid overfitting. To assess the overall contribution of a specific metabolite related to each factor, the quadratic Variable Importance in the Projection (VIP^2) metric was also calculated (Boccard & Rudaz, 2016).

Results

Evaluation of Chitosan-Sepiolite Film

Clear differences were observed among the prepared film formulations (Fig. 1). Films containing higher sepiolite concentrations and higher chitosan ratios exhibited more surface defects, including visible damage and holes. In contrast, the formulation with the lowest sepiolite content (1%) and a chitosan:cassava starch ratio of 1:1 (1C1S) produced the most stable film, with a homogeneous dispersion of sepiolite particles (Fig. 1).

Film thickness and transparency were strongly influenced by sepiolite concentration. Increasing sepiolite content led to a progressive increase in thickness (Table 1). Transparency was also markedly reduced by sepiolite, with higher concentrations lowering transparency values by up to 30–40 fold. The chitosan:cassava starch ratio further influenced this parameter: as 1C0S showed 43% higher transparency compared to 3C0S (Table 1). Regarding water vapor transmission rate (WVTR), sepiolite primarily contributed to reducing permeability, while higher chitosan ratios generally increased WVTR. Overall, the 1C1S formulation was the film incorporating sepiolite that displayed the most favorable characteristics, combining stability and homogeneity with balanced thickness, transparency, and WVTR. Consequently, this formulation was selected to evaluate the effect of sepiolite for extending the shelf life of apples stored under normal and MA.

Visual Appearance of Apples Under Different Storage Conditions

Shelf-life evaluation was assessed both visually through fruit appearance (Fig. 2A) and instrumentally by monitoring color changes (Fig. 2B, Table S1). No significant differences were detected after 6 or 18 days of storage. However, a marked deterioration was observed after 60 days in apples stored at room temperature, compared with those maintained at 4 °C. Nevertheless, apples stored under MA conditions retained better visual quality (Fig. 2A), exhibiting reduced browning relative to the control (Fig. 2B). The incorporation of sepiolite film to MA did not result in significant additional differences for the visual appearance of apples.

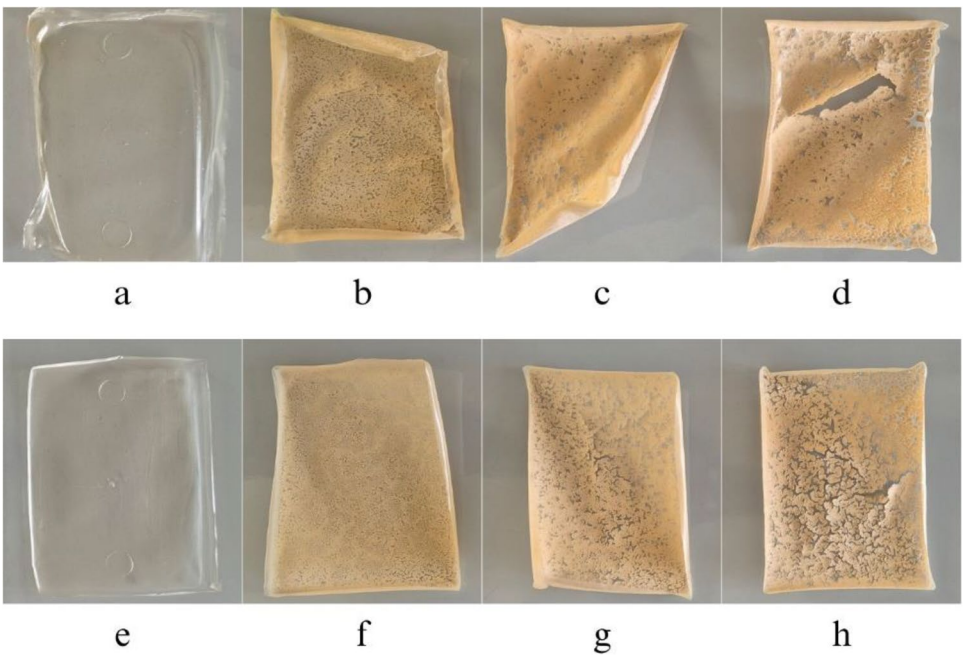


Fig. 1 Film casting result evaluating 1:1 and 3:1 ratios of chitosan and cassava starch (1C and 3C, respectively) and sepiolite (0, 1, 1.5, and 2%). (**a**) 3C0S, (**b**) 3C1S, (**c**) 3C1.5S, (**d**) 3C2S, (**e**) 1C0S, (**f**) 1C1S, (**g**) 1C1.5S, (**h**) 1C2S

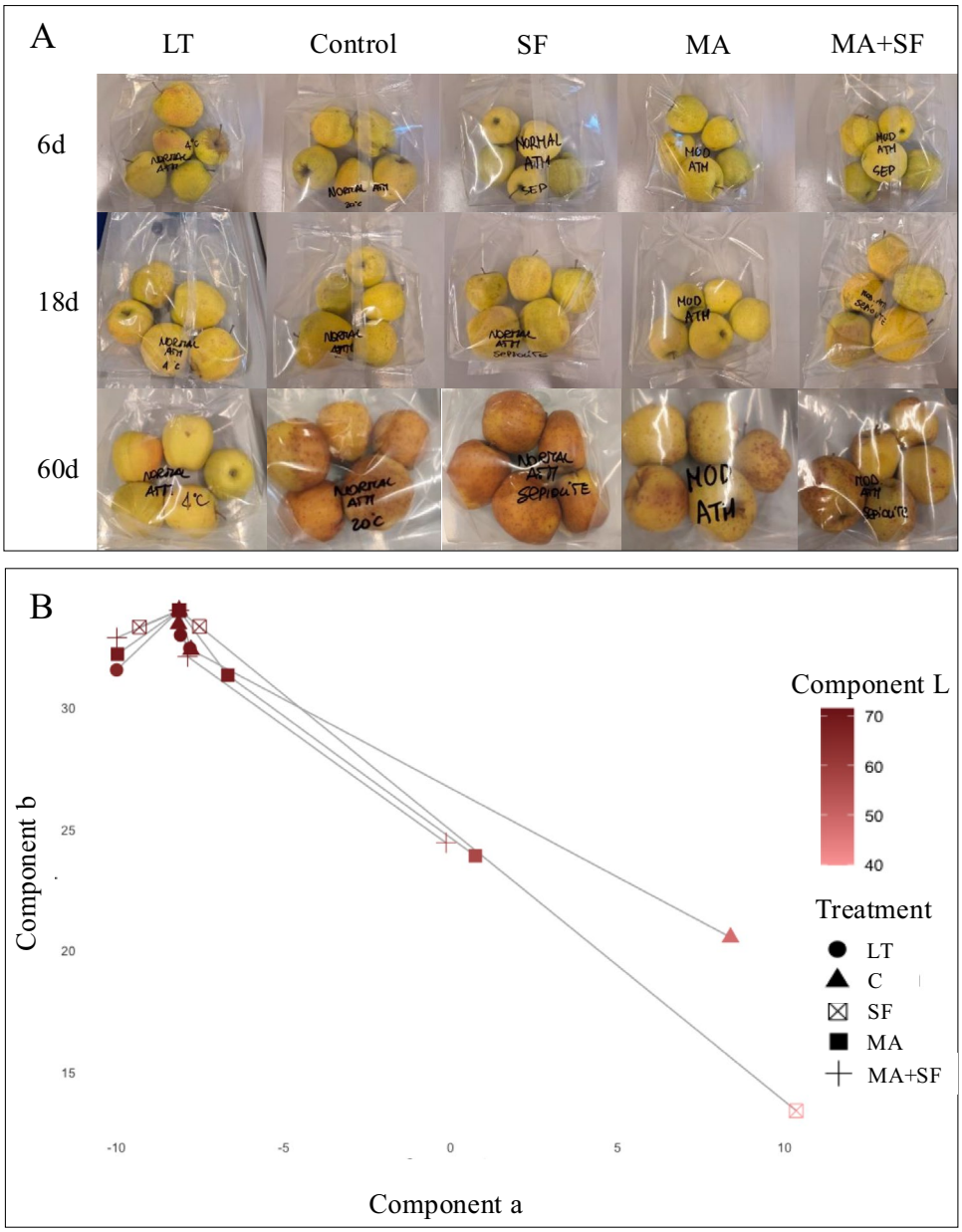


Fig. 2 (**A**) Visualappearanceofapplesstoredunderdifferentpostharvestconditions: lowtemperature (LT), ambient temperature (Control), sepiolite film (SF), modified atmosphere (MA), and the combined treatment (MA + SF). (**B**) Changes in apple color during storage, represented by the evolutionoftheCIELabparametersa, b, and L

Modified Atmosphere and Sepiolite Film Effect on Apple Shelf Life

After 6 days of storage, no significant differences were observed among treatments, with all apples showing an approximate 12% reduction in peel hardness compared to at harvest. However, at 18 days, clear differences emerged; peel hardness was maintained under storage at 4 °C (LT) and with SF treatment, showing values like those at 6 days. Moreover, the MA + SF treatment exhibited higher peel hardness than MA alone. After 60 days, the only treatment at room temperature that maintained peel hardness values comparable to storage at 4 °C was the combination of MA and sepiolite film (MA + SF) (Table 2).

In contrast, pulp axial firmness was more strongly affected by storage duration, with reductions of nearly 40%, 50%, and 80% after 6, 18, and 60 days, respectively. Among treatments, MA alone showed the greatest decline. However, when combined with SF, values remained comparable to those of the control and sepiolite treatments. Storage at 4 °C consistently preserved the highest pulp axial firmness among treatments after 60 days (Table 2). Pulp radial firmness initially increased under room temperature storage after 6 days, with only the SF treatment maintaining a high level after 18 days. Nevertheless, after 60 days, the trend was similar to that observed for pulp axial firmness, with LT showing the highest values and MA the lowest.

Table 2 The effect of storage at 4 °C and at room temperature, using packaging with normal and modified atmospheres and sepilite film, was evaluated on the quality parameters of apple fruit

Days of storing	Treatment	Peel Hardness	Pulp axial firmness	Pulp radial firmness	pH
0	At harvest	8.1 ± 0.4^{A}	8.0 ± 0.4^{A}	4.9 ± 0.1^{B}	3.6 ± 0.1^{C}
6	LT	7.0 ± 0.4^{aB}	4.4 ± 0.1^{bcB}	5.2 ± 0.2^{bAB}	3.6 ± 0.1^{aC}
	C	6.9 ± 0.5^{aB}	4.9 ± 0.1^{abB}	6.0 ± 0.3^{aA}	3.7 ± 0.1^{bBC}
	SF	6.9 ± 0.4^{aB}	4.9 ± 0.1^{aB}	6.1 ± 0.3^{aA}	3.4 ± 0.1^{bD}
	MA	6.8 ± 0.3^{aB}	4.2 ± 0.2^{cB}	5.6 ± 0.2^{abA}	3.5 ± 0.1^{abC}
	MA + SF	7.0 ± 0.4^{aB}	4.5 ± 0.2^{abcB}	6.1 ± 0.3^{aA}	3.6 ± 0.1^{aC}
18	LT	6.8 ± 0.4^{aBC}	4.7 ± 0.1^{aB}	5.7 ± 0.2^{abAB}	3.5 ± 0.1^{bC}
	C	5.4 ± 0.3^{bC}	4.7 ± 0.2^{aB}	5.4 ± 0.2^{bB}	3.8 ± 0.1^{aBC}
	SF	6.8 ± 0.4^{aB}	4.8 ± 0.1^{aB}	6.2 ± 0.3^{aA}	3.5 ± 0.1^{bC}
	MA	5.2 ± 0.3^{bC}	4.0 ± 0.1^{bB}	5.0 ± 0.2^{bB}	3.8 ± 0.1^{aB}
	MA + SF	6.1 ± 0.3^{aC}	4.6 ± 0.1^{aB}	5.3 ± 0.2^{bB}	3.7 ± 0.1^{aB}
60	LT	6.1 ± 0.4^{aC}	3.8 ± 0.1^{aC}	4.4 ± 0.2^{aC}	3.5 ± 0.1^{bC}
	C	4.6 ± 0.2^{cC}	1.5 ± 0.2^{bC}	1.6 ± 0.2^{bC}	4.1 ± 0.1^{aA}
	SF	5.1 ± 0.2^{bcC}	1.5 ± 0.1^{bC}	1.5 ± 0.2^{bC}	4.0 ± 0.1^{aA}
	MA	5.1 ± 0.3^{bcC}	0.9 ± 0.1^{cC}	0.9 ± 0.1^{cC}	4.0 ± 0.1^{aA}
	MA + SF	5.8 ± 0.3^{abC}	1.8 ± 0.3^{bC}	1.7 ± 0.2^{bC}	4.1 ± 0.1^{aA}

The data presented as the mean ± standard deviation of 25 fruits. Treatments evaluated were storing at low temperatures (LT), and at room temperature control (C), with sepiolite film (SF), under modified atmospheres (MA), and combination of modified atmospheres and sepiolite (MA + SF). Lower-case letters indicate significant differences between treatments for each day of storage, while upper-case letters indicate significant differences among days of storing with the same treatment, according to ANOVA by Duncan's test ($p < 0.05$).

Peel hardness, pulp axial firmness, pulp radial firmness, and pH were measured after 0, 6, 18, and 60 days of storage.

Regarding pH, considered an index of fruit maturation, apples stored with SF showed a slight decrease after 6 days and maintained lower values after 18 days, similar to storage at 4 °C. In contrast, the other treatments showed an increase, pointing to an earlier maturation. After 60 days, only LT maintained a stable pH, while all other treatments exhibited an increase of approximately 15% (Table 2).

Multivariate Statistics on the Metabolomic Profile in Peel and Pulp

MA and sepiolite film were evaluated, individually and in combination, to assess their effects on the metabolome of apple peel and pulp stored at room temperature, in comparison with cold storage. For this purpose, an untargeted metabolomic analysis was performed on samples collected at T0 (at harvest), T6, T18, and T60. This high-throughput analysis detected 2,905 chemical entities in the analyzed extracts, of which 1,908 compounds were putatively annotated against the FooDB database. Comprehensive information for each feature, including metabolite name, retention time (Rt, min), mass (m/z), molecular formula, ontology, MS and MS/MS spectra, annotation, and total score, is provided in Table S2.

To investigate the influence of these factors on the metabolic profile of apple peel and pulp, an unsupervised hierarchical cluster analysis (HCA) was performed for each tissue (Fig. S2 and S3, respectively). Both tissues showed a similar clustering trend. Storage time emerged as the main factor shaping the metabolome, with samples clustered mainly by time: 0, 6, and 18 days grouped, while 60 days formed a distinct cluster. At T6 and T60, samples were further separated according to storage temperature. Interestingly, at T18, storage at 4 ºC clustered together with the SF treatment, indicating that at this stage the film exerted an effect on the metabolome comparable to cold storage. Finally, the MA condition consistently generated distinct subclusters across all storage periods (Fig. S2 and S3). These results suggest that postharvest storage at room temperature caused significant metabolic alterations in apple peel and pulp, and that these effects were partially mitigated by sepiolite treatment and MA storage.

Pathway Analysis Provides the Functional Signature of Treated Apple Peel and Pulp

Metabolite accumulation by class and subclass was conducted using the differentially accumulated metabolites (DAMs) identified by Volcano analysis ($p < 0.05$ and FC

cut-off = ±2) to further investigate treatment effects in comparison with fruit at harvest (T0). To assess the modulation of biosynthetic metabolism, a total of 1,497 and 1,206 compounds from peel and pulp, respectively, met both criteria and were subjected to pathway analysis. The complete lists of DAMs, including logFC values and chemical ontology annotations, are reported in Tables S3 (peel) and S4 (pulp). This analysis enabled us to characterize the metabolic fingerprint induced by active packaging at room temperature and to compare it with the metabolic modulation observed during storage at 4 °C (Fig. 3).

In terms of general biosynthetic activity, a modest up-accumulation of phenylpropanoid compounds was observed in both peel and pulp at T6, whereas a pronounced down-accumulation of lipids, primarily phospholipids [phosphatidylcholine (PCs), phosphatidylethanolamine (PEs), and phosphatidylglycerol (PGs)], and terpenoid compounds was detected at the same time point. Notably, sepiolite treatment exhibited a distinct modulation pattern, strongly enhancing the accumulation of phenylpropanoids, primary metabolites, and functional metabolites, while minimizing the down-accumulation of lipids and terpenoids. These effects were particularly pronounced in pulp tissue. Within the diverse group of functional and primary metabolites, sepiolite treatment in pulp induced a strong up-accumulation of the phytohormones Gibberellin A84, and (R)−2,3-Dihydro-3,5-dihydroxy-2-oxo-3-indoleacetic acid 5-glucoside (Log FC = 17.24, both). A marked up-accumulation of

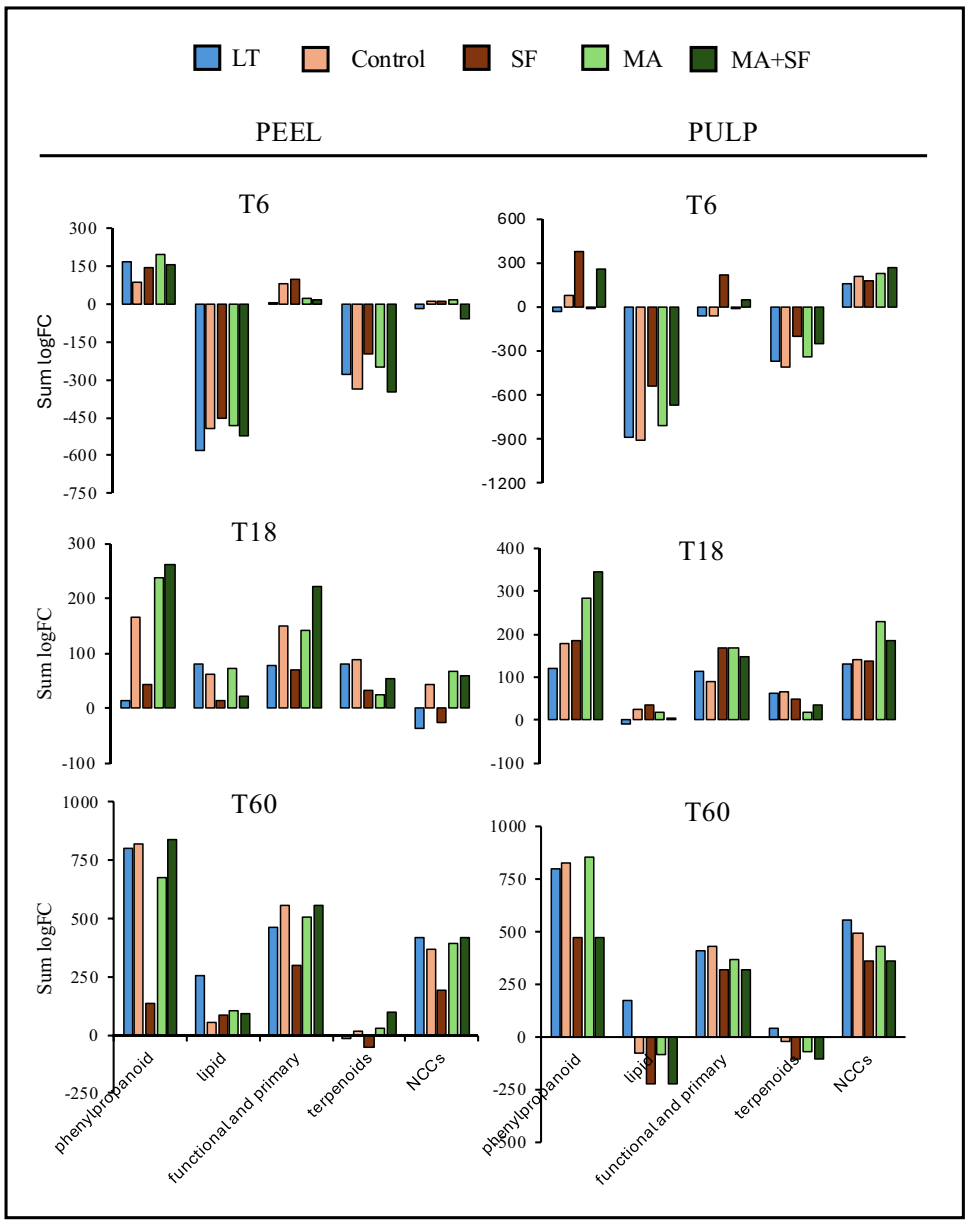


Fig. 3 Pathway analysis on the metabolic profile of apple peel and pulp samples from the treatments evaluated after 6, 18, and 60 days of storage compared against T0. Results are expressed as the sum of the logarithm of fold change (logFC) values of each category. Only the compounds showing a differential abundance (FC > 2 or FC < −2) and statistically significant differences according to one-way through one-way ANOVA and Bonferroni FWER post hoc test ($p < 0.01$) with respect to fresh harvested T0 samples were considered

vitamins was also observed, particularly folic acid derivatives [5,10-Methenyltetrahydrofolic acid; Tetrahydrofolic acid; 10-Formyltetrahydrofolic acid], along with riboflavin derivatives [Riboflavin 5'-(dihydrogen phosphate) and Riboflavin 2',3',4',5'-tetrabutanoate] (Table S4). At 18 days, the influence of treatment factors shifted, and a general metabolic up-accumulation was observed across all treatments. However, in this case, sepiolite no longer promoted higher accumulation and even showed reduced modulation in peel (Fig. 3). At this stage, the most important differences were attributed to MA packaging and its combination with sepiolite, which enhanced the accumulation of phenylpropanoids as well as functional and primary metabolites. After 18 days, no down-accumulation of phospholipids could be observed, unlike previously noted at T6. By T60, peel and pulp exhibited similar trends, with sepiolite treatment showing comparatively lower modulation of the overall metabolism.

AMOPLS Models Based on Atmosphere and Sepiolite Factors

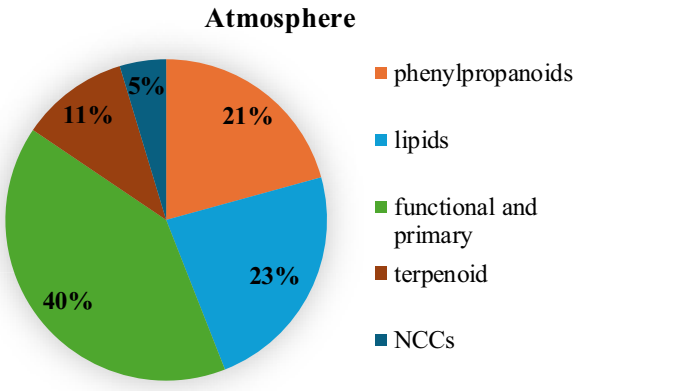
To further explore the metabolic modulation associated with the factors sepiolite film, MA, and their interaction after 60 days at room temperature (T60), two independent AMOPLS analyses were conducted for each tissue. This multivariate approach allowed quantification of the relative contribution of each experimental factor to the overall metabolomic variance and facilitated the identification of metabolites most strongly associated with these effects (VIP^2 markers). The resulting AMOPLS score plots (Fig. S4) demonstrated clear clustering patterns, with distinct separations corresponding to the effects of atmosphere, sepiolite-containing coating, and their interaction (atmosphere × sepiolite), indicating a consistent impact of these factors on the apple metabolome. For simplicity, the term "sepiolite factor" is used throughout the manuscript to refer to the active coating formulation containing sepiolite within the chitosan–cassava starch matrix, rather than to the isolated effect of sepiolite itself. The associated statistical parameters are summarized in Fig. 4, including the relative sum of squares (RSS), residual structure ratio (RSR), their corresponding p-values, and the contributions to predictive and orthogonal components (tp and to, respectively).

In peel, atmosphere was identified as the only statistically significant discriminant factor (RSR p-value = 0.01), whereas sepiolite and the combined interaction showed no significant influences (RSR p-value > 0.05). Although MA + SF resulted in higher peel hardness than MA alone at day 60, this phenotypic difference was not associated with a statistically significant contribution of the sepiolite factor to the overall peel metabolomic variance in the AMOPLS model. This indicates that the effect of sepiolite on peel firmness was not reflected in broad metabolomic discrimination patterns. In contrast, both atmosphere and sepiolite factors were statistically significant in pulp (RSR p-value = 0.01), underscoring a tissue-specific response. These results indicate that peel metabolism is predominantly modulated by storage atmosphere, while pulp metabolism responds to both atmosphere and sepiolite factors. Such divergence highlights the differential susceptibility of tissue compartments to postharvest conditions. Collectively, these findings support the conclusion that sepiolite film and MA modulate fruit maturation and decay in distinct, tissue-dependent ways, with implications for targeted postharvest management strategies. To identify the metabolites with the greatest influence within each factor investigated, a VIP^2 analysis was then conducted. Specifically, the first 200 VIP^2 markers associated with having the higher VIP^2 scores were incorporated into chart diagrams to highlight the most representative chemical classes for each group separation (Fig. 4). The lists of VIP^2 markers are further provided in Table S5. In peel, functional and primary metabolites represented the largest proportion of discriminatory variables under the atmosphere factor (40%), followed by lipids (23%) and phenylpropanoids (21%). In pulp, the same classes contributed most to atmosphere-related discrimination, although the distribution was more homogeneous, with functional and primary metabolites accounting for 33%, lipids for 25%, and phenylpropanoids for 24%. In contrast, for the sepiolite factor in pulp, lipids were the predominant class (30%), followed by functional and primary metabolites (28%) and phenylpropanoids (21%).

Assessing some of the most relevant VIP^2 markers associated with the atmosphere factor in peel, the phosphatidic acid PA(16:0e/18:0) exhibited the highest discriminatory power (VIP^2 value = 10.5), found together with PA(O-20:4(5Z,8Z,11Z,14Z)/2:0) (VIP^2 value = 4.4). Within the functional and primary metabolite class, notable contributors included the amino acid N(6)-Methyllysine (VIP^2 value = 8.4), the jasmonate-related phytohormones 6-Epi-7-isocucurbic acid glucoside (VIP^2 value = 6.6) and methyl epijasmonate (VIP^2 value = 4.9), the cytokinin (E)-Ribosylzeatin (VIP^2 value = 4.9), and the lipid derivative Isobutyryl-L-carnitine (VIP^2 value = 6.4). In pulp, the atmosphere factor highlighted several key metabolites. Among amino acids, L-tyrosine showed a strong discriminatory contribution (VIP^2 value = 8.1). Vitamins such as riboflavin, gamma-tocotrienol, and pyridoxine were also identified as relevant markers (VIP^2 value = 6.5, 5.8, and 4.8, respectively). Compared with peel, pulp exhibited a higher representation of both flavonoids and the relative glucoside derivatives, including 8-demethylthymonin (VIP^2 value = 10.5), isoquercitrin (VIP^2 = 6.1), quercetin 3-rutinoside (VIP^2 value = 5.9), and quercitrin (VIP^2 value = 5.0). Metabolomic analysis of the apple pulp identified several compounds with high discriminant power associated with the sepiolite factor. The lipid fraction was

PEEL

Factor	RSS	RSR	RSS p-value	RSR p-value	R2Y p-value	PermNb	Tp1	Tp2	Tp3	To1
Atmosphere	0.09	1.098	0.01	0.01	0.01	100	0.972	0.01	0.034	0.24
Sepiolite	0.061	1.05	1	1	0.01	100	0.009	0.011	0.893	0.251
Atmosphere x Sepiolite	0.077	1.08	0.69	0.08	0.01	100	0.009	0.967		0.244
residuals	0.772	1	NA	NA	NA	NA	0.01	0.011	0.038	0.264



PULP

Factor	RSS	RSR	RSS p-value	RSR p-value	R2Y p-value	PermNb	Tp1	Tp2	Tp3	To1
Atmosphere	0.071	1.063	0.01	0.01	0.01	100	0.039	0.959	0.013	0.245
Sepiolite	0.074	1.07	0.01	0.02	0.02	100	0.88	0.013	0.013	0.244
Atmosphere x Sepiolite	0.055	1.041	1	0.8	0.03	100	0.04	0.014	0.96	0.25
residuals	0.8	1	NA	NA	NA	NA	0.041	0.014	0.014	0.261

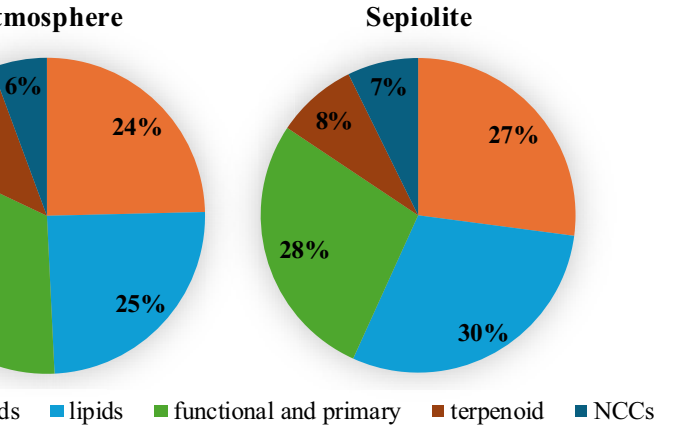


Fig. 4 Results from AMOPLS analysis done and chart diagrams illustrating the metabolic classes of VIP2 markers identified as discriminant for each factor that showed a significant effect in peel and pulp

the most significantly affected metabolite class, with the saturated fatty acids (SFAs) 9,10-Epoxy-18-hydroxy-octadecanoic acid and Nonadecanoic acid (VIP^2 value = 6.3 and 5.6, respectively) being highly relevant. Similarly, the fatty acyl glycerol 1-(5-hydroxydodecanoate) and erythro-7,9-tetratriacontanediol also showed strong discriminative capacities, with VIP2 values of 6.2 and 5.8, respectively. Furthermore, other compound classes exhibited strong discrimination, including the vitamin Pyridoxamine 5'-phosphate (VIP^2 value = 5.9) and the amino acid L-Histidine (VIP^2 value = 5.1). The observed changes in these metabolic markers suggest a mechanism by which sepiolite and atmosphere modulate visual appearance and texture parameters, enhancing the apple postharvest quality and shelf life. The specific mechanisms linking these metabolites to the resulting phenotypic changes will be further discussed.

Discussion

As expected, storage at ambient temperature markedly accelerated physiological senescence, resulting in pronounced changes in external appearance and a significant increase in fruit softening compared with low-temperature storage. The application of MA conditions effectively delayed color changes, a response also reported in pomegranates (Selcuk & Erkan, 2014). Although elevated CO2 concentrations may potentially induce fermentative metabolism or off-flavor development in apples, no evident symptoms of abnormal tissue breakdown or visual CO2 injury were observed under the evaluated conditions. Nevertheless, sensory evaluation and the assessment of fermentation-related metabolites were beyond the scope of the present study. Future work should additionally evaluate different MA gas compositions in combination with the active coating system to optimize preservation efficiency and commercial applicability. Incorporating sepiolite film into the packaging further enhanced firmness retention in both peel and pulp relative to the control, with the effect being more pronounced when combined with MA storage. Although the SF condition alone did not markedly improve the external visual appearance of apples under ambient storage, important effects were still observed at the texture and metabolomic levels. This apparent discrepancy suggests that visual deterioration and internal metabolic responses may involve partially distinct physiological processes. In this context, the stronger effect of MA on external quality traits such as browning may be associated with the predominant influence of the atmosphere factor on peel metabolism, whereas sepiolite-related effects appeared more evident in pulp metabolism and firmness retention. Overall, the complementary effects associated with sepiolite incorporation and MA conditions are consistent with delayed postharvest senescence and reduced quality deterioration previously reported in fruits and vegetables (Álvarez-Hernández et al., 2020; Dwibedi et al., 2024). To elucidate the mechanisms underlying these responses, the metabolic mechanisms responsible for these phenotypic responses will be further discussed.

Differential Metabolic Class Induction by Storage Condition During the Postharvest Period in Apple

In recent years, metabolomic approaches have been applied to contribute to the enhancement of nutritional quality and postharvest management in crops, such as banana, strawberry, and apple (Kim et al., 2023; Yang et al., 2023a, 2023b; Yin et al., 2022). In this context, the present study investigates the metabolomic responses underlying differences in apple shelf life as influenced by MA storage, active packaging incorporating a sepiolite film, and their combined application. Because a film matrix treatment without sepiolite was not included during fruit storage, the specific contribution of sepiolite cannot be completely isolated from that of the chitosan–cassava starch matrix. Therefore, the physiological and metabolomic responses observed in the present study should be interpreted as the combined effect of the bio-based film formulation incorporating sepiolite and the modified atmosphere conditions. Storage duration emerged as a key factor driving metabolic reprogramming, with a clear separation of T60 samples into a distinct cluster, indicative of time-dependent shifts in biochemical pathways. Such trends are in agreement with previous metabolomic studies in zucchini fruit, which also reported strong storage-period–dependent clustering patterns (Castro-Cegrí et al., 2024b, 2025).

From a biochemical perspective, the observed down-accumulation of phospholipids in apple fruit can be attributed to a process commonly triggered under stress conditions and associated with membrane destabilization and accelerated senescence (Paliyath et al., 2009). The mitigation of this decline by sepiolite coating suggests an enhanced remodeling capacity of membrane lipids, thereby contributing to chloroplast and mitochondrial stability during storage (Kobayashi et al., 2015). In fact, preserving phospholipid components was noted as a protective mechanism against decay of peach fruit during postharvest storage, especially by phosphatidylcholine (PCs) and phosphatidylethanolamine (PEs) (Song et al., 2022). On the other side, this treatment promoted an accumulation of phytohormones, as gibberellins, indicating that sepiolite may stimulate signaling cascades involved in cell wall remodeling, antioxidant defense, and stress tolerance. Such responses are consistent with the role of GA in delaying ripening-related softening by regulating cell wall–modifying enzymes (Jiang et al., 2025; H. Yang et al., 2023a, 2023b). Similarly, the accumulation of vitamins, particularly those with antioxidant properties, may support defense against oxidative stress and prevent lipid peroxidation, as in grapes treated with folic acid (Pei et al., 2023). Together, these metabolomic shifts reveal a coordinated defense mechanism, integrating membrane lipid preservation with hormone-mediated firmness regulation and antioxidant protection. Such complementary pathways highlight the potential of active packaging with sepiolite to extend fruit quality during postharvest storage by targeting both structural integrity and stress resilience at the metabolic level.

Key Metabolic Markers Associated with Modified Atmosphere and Sepiolite Factors

The AMOPLS analysis of apple peel and pulp revealed the compounds most differentially affected by the factors evaluated, influencing the fruit metabolism during postharvest.

Overall, the combined MA + SF condition appeared to involve complementary tissue-specific metabolic responses that may explain the improved firmness retention observed after prolonged storage. The AMOPLS analysis indicated that the atmosphere factor was the predominant source of metabolic modulation in peel tissue, suggesting a major contribution to external tissue responses associated with visual quality and oxidative metabolism. In contrast, both atmosphere and sepiolite factors significantly contributed to the metabolic modulation observed in pulp tissue, particularly affecting lipid-related and functional metabolites associated with membrane stability and postharvest adaptation. These coordinated responses between peel and pulp tissues may collectively contribute to delaying senescence and maintaining fruit texture during storage.

Atmosphere emerged as the only factor that significantly altered the metabolic profile of apple peel at the end of the storage period. Phosphatidic acids PA(16:0e/18:0) and PA(O-20:4(5Z,8Z,11Z,14Z)/2:0) were identified as key metabolites discriminating the atmosphere effect. Phosphatidic acid (PA) is a well-known stress-associated lipid that accumulates in plants under adverse conditions (Tan et al., 2018; Wu et al., 2022). It acts as a central signaling molecule in hormone-regulated pathways, modulating the expression of genes encoding enzymes involved in phytohormone biosynthesis and signal transduction, including those of auxin, abscisic acid, and jasmonic acid (Kolesnikov et al., 2022). Notably, carbon dioxide treatments have been shown to regulate PA signaling, thereby improving postharvest quality in paprika (Park et al., 2023), and similar roles have been reported in banana (Zhou et al., 2022). Thus, this PA modulation could be linked to the results observed in apples under MA, causing an important effect on functional and primary compounds. Jasmonate derivatives such as 6-Epi-7-isocucurbic acid glucoside (jasmonoid glucoside) and methyl epijasmonate are emerging as major discriminant markers. This aligns with previous findings in peach, where MA extended shelf life through jasmonate induction (Ai et al., 2024). The modulation of jasmonates observed in apple pulp may also explain the concurrent increase in flavonoids and flavonoid glucosides, consistent with the established role of methyl jasmonate in activating the phenylpropanoid pathway and enhancing the accumulation of phenolic and flavonoid compounds, thereby improving fruit quality and storability (Tao et al., 2022; Wang et al., 2021; Zhang et al., 2024).

In addition, atmosphere factor was associated with changes in the vitamin-related metabolite profile of apple pulp, with riboflavin and γ-tocotrienol identified among the relevant discriminatory metabolites. Enhancing the nutritional value of fresh produce through the enrichment of bioactive compounds with antioxidant properties remains a key goal in food research. Tocopherols are isoprenoid compounds that serve as important natural antioxidants in fruits (Charoensiri et al., 2009) and have previously been associated with improved stress tolerance and postharvest performance (Gull et al., 2023; Rey et al., 2021). Riboflavin, in turn, exerts dual functionality: it is essential for human health, contributing to iron absorption, tryptophan metabolism, and mitochondrial activity (Thakur et al., 2017), while also enhancing plant antioxidant capacity. Specifically, riboflavin activates the phenylpropanoid pathway, leading to increased phenolic accumulation and improved storability, as shown in zucchini, litchi, and strawberry (Castro-Cegrí et al., 2024a; Peng et al., 2026; X. Zhang et al., 2023a, 2023b). Pyridoxine-related metabolites were also identified among the compounds modulated atmosphere and sepiolite factors. Previous studies have linked vitamin B6 metabolism with improved postharvest quality maintenance and cell wall stability in fruit tissues (Ali et al., 2024). The sepiolite coating factor was additionally associated with the modulation of lipid-related metabolites, particularly several saturated fatty acids (SFAs) and fatty acyl derivatives identified as relevant VIP_2 markers. Therefore, these findings suggest that sepiolite film incorporation substantially influenced membrane lipid metabolism during storage. Accordingly, the modulation of these lipid-related compounds may reflect membrane remodeling processes associated with postharvest adaptation and quality maintenance, a phenomenon already reported in crops such as banana and cucumber (L. Zhang et al., 2022a, 2022b; X. Zhang et al., 2022a, 2022b).

Conclusions

Developing effective and safe strategies to extend the postharvest shelf life of fruits is becoming increasingly essential. This study evaluated a sepiolite-enriched chitosan–cassava starch film, used as active packaging, in combination with MA storage, as a strategy to extend the shelf life of apples stored at room temperature. From a technological perspective, storage at ambient temperature markedly accelerated senescence, as shown by peel browning, loss of firmness and pH increase. Under these more challenging conditions, both sepiolite film (SF) and MA mitigated quality loss. Notably, the combination MA + SF maintained peel hardness at values comparable to cold-stored fruit after 60 days, highlighting the potential of this strategy to partially reproduce the preservation effect of refrigeration under ambient conditions.

Metabolomic analysis revealed a strong time-dependent metabolic reprogramming in both peel and pulp tissues. Across treatments, storage induced marked changes in membrane lipid profiles and specialized metabolites. Early during storage (T6), SF promoted the accumulation of phenylpropanoids and functional metabolites, especially vitamins and phytohormones, suggesting enhanced antioxidant

capacity. Similarly, MA elicited the accumulation of flavonoids. AMOPLS analysis demonstrated that the atmosphere factor was the predominant source of variation in peel tissue, whereas both atmosphere and sepiolite factors significantly contributed to metabolic modulation in pulp tissue. PA emerged as key discriminant lipids associated with atmosphere factor, supporting a role for phospholipid-derived signalling in the response to altered gas composition. In parallel, vitamin-related metabolites including riboflavin, tocopherols, and pyridoxine were identified among the compounds modulated by the atmosphere and sepiolite factors, suggesting a potential influence of these postharvest conditions on the functional profile of apples.

The combined MA + SF strategy therefore represents a promising bio-based alternative to extend apple shelf life under ambient conditions, potentially reducing reliance on continuous refrigeration. Nonetheless, further studies under pilot- and industrial-scale conditions, including sensory evaluation and commercial storage validation, will be necessary to assess scalability and practical applicability. Future research could additionally incorporate complementary ionization modes and targeted metabolomic approaches to achieve a more comprehensive characterization of postharvest metabolic responses. In addition, the absence of a film matrix treatment without sepiolite should be considered a limitation of the present study and addressed in future research to better discriminate the individual contribution of each component.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11947-026-04453-4.

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Data Availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

References

Ai, S., Xu, S., Wu, C., Grierson, D., Chen, K., & Xu, C. (2024). Novel insights into modified atmosphere mediated cold tolerance in peach fruit during postharvest storage. *Postharvest Biology and Technology, 218*, Article 113187. https://doi.org/10.1016/j.postharvbio.2024.113187

Alam, A. U., Rathi, P., Beshai, H., Sarabha, G. K., & Deen, M. J. (2021). Fruit quality monitoring with smart packaging. *Sensors, 21*, Article 1509. https://doi.org/10.3390/s21041509

Ali, M., Zhao, X., Hussain, S., Li, X., Li, F., & Xinhua, Z. (2024). Physiological and transcriptomic analysis revealed the quality regulatory mechanism of pyridoxine (vitamin B6) in fresh-cut apple. *Postharvest Biology and Technology, 217*, Article 113098. https://doi.org/10.1016/j.postharvbio.2024.113098

Alonso-Salinas, R., López-Miranda, S., Pérez-López, A. J., & Acosta-Motos, J. R. (2024). Strategies to delay ethylene-mediated ripening in climacteric fruits: Implications for shelf life extension and postharvest quality. *Horticulturae, 10*, Article 840. https://doi.org/10.3390/horticulturae10080840

Álvarez-Hernández, M. H., Martínez-Hernández, G. B., Avalos-Belmontes, F., Miranda-Molina, F. D., & Artés-Hernández, F. (2020). Postharvest quality retention of apricots by using a novel sepiolite–loaded potassium permanganate ethylene scavenger. *Postharvest Biology and Technology, 160*, Article 111061. https://doi.org/10.1016/j.postharvbio.2019.111061

Becchi, P. P., Rocchetti, G., Vezzulli, F., Lambri, M., & Lucini, L. (2023). The integrated metabolomics and sensory analyses unravel the peculiarities of mountain grassland-based cheese production: The case of Parmigiano Reggiano PDO. *Food Chemistry, 428*, Article 136803. https://doi.org/10.1016/j.foodchem.2023.136803

Becchi, P. P., Rocchetti, G., García-Pérez, P., Michelini, S., Pizzamiglio, V., & Lucini, L. (2024). Untargeted metabolomics and machine learning unveil quality and authenticity interactions in grated Parmigiano Reggiano PDO cheese. *Food Chemistry, 447*, Article 138938. https://doi.org/10.1016/j.foodchem.2024.138938

Boccard, J., & Rudaz, S. (2016). Exploring Omics data from designed experiments using analysis of variance multiblock Orthogonal Partial Least Squares. *Analytica Chimica Acta, 920*, 18–28. https://doi.org/10.1016/j.aca.2016.03.042

Bugnotti, D., DalleVacche, S., Esposito, L. H., Callone, E., Orsini, S. F., Ceccato, R., D'Arienzo, M., Bongiovanni, R., Dirè, S., & Vitale, A. (2023). Structure of starch–sepiolite bio-nanocomposites: Effect of processing and matrix-filler interactions. *Polymers, 15*, 1207. https://doi.org/10.3390/polym15051207

Castro-Cegrí, A., García, A., Garrido, D., & Palma, F. (2024a). Riboflavin improves postharvest cold tolerance in zucchini fruit inducing non-enzymatic antioxidant response and phenolic metabolism. *Plant Physiology and Biochemistry, 217*, Article 109270. https://doi.org/10.1016/j.plaphy.2024.109270

Castro-Cegrí, A., García-Pérez, P., Jamilena, M., Garrido, D., Palma, F., & Lucini, L. (2024b). Exogenous abscisic acid mitigates chilling injury in zucchini during cold storage by eliciting a time-dependent shaping of specialized metabolites. *Postharvest Biology and Technology, 212*, Article 112864. https://doi.org/10.1016/j.postharvbio.2024.112864

Castro-Cegrí, A., Garcia-Perez, P., Garrido, D., Palma, F., & Lucini, L. (2025). The riboflavin-mediated reprogramming of specialized metabolites enhances postharvest cold tolerance and functional traits of zucchini fruits. *Food Chemistry, 485*, Article 144543. https://doi.org/10.1016/j.foodchem.2025.144543

Charoensiri, R., Kongkachuichai, R., Suknicom, S., & Sungpuag, P. (2009). Beta-carotene, lycopene, and alpha-tocopherol contents of selected Thai fruits. *Food Chemistry, 113*, 202–207. https://doi.org/10.1016/j.foodchem.2008.07.074

Dhillon, G. S., Kaur, S., & Brar, S. K. (2013). Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. *Renewable and Sustainable Energy Reviews, 27*, 789–805. https://doi.org/10.1016/j.rser.2013.06.046

Dwibedi, V., Kaur, G., George, N., Rana, P., Ge, Y., & Sun, T. (2024). Research progress in the preservation and packaging of fruits and vegetables: From traditional methods to innovative technologies. *Food Packaging and Shelf Life, 46*, Article 101385. https://doi.org/10.1016/j.fpsl.2024.101385

Flórez, M., Guerra-Rodríguez, E., Cazón, P., & Vázquez, M. (2022). Chitosan for food packaging: Recent advances in active and intelligent films. *Food Hydrocoll., 124*, Article 107328. https://doi.org/10.1016/j.foodhyd.2021.107328

Gaikwad, K. K., Singh, S., & Negi, Y. S. (2020). Ethylene scavengers for active packaging of fresh food produce. *Environmental Chemistry Letters, 18*, 269–284. https://doi.org/10.1007/s10311-019-00938-1

Gull, A., Masoodi, F. A., Masoodi, L., Gani, A., & Muzaffar, S. (2023). Effect of sodium alginate coatings enriched with α-tocopherol on quality of fresh walnut kernels. *Food Chem. Adv., 2*, Article 100169. https://doi.org/10.1016/j.focha.2022.100169

Gunathilake, I. A. D. S. R., & Somendrika, M. A. D. (2024). Development of a biodegradable packaging with antimicrobial properties from cassava starch by incorporating *Ocimum tenuiflorum* extract. *Food Chemistry Advances, 4*, Article 100658. https://doi.org/10.1016/j.focha.2024.100658

Hatoum, D., Hertog, M. L. A. T. M., Geeraerd, A. H., & Nicolai, B. M. (2016). Effect of browning related pre- and postharvest factors on the 'Braeburn' apple metabolome during CA storage. *Postharvest Biology and Technology, 111*, 106–116. https://doi.org/10.1016/j.postharvbio.2015.08.004

Jiang, K., Zhang, X., Li, T., Liu, J., Liu, M., & Han, S. (2025). Gibberellin and shikimic acid enhance ascorbic acid accumulation and ROS scavenging ability to delay the senescence of postharvest jujube fruit. *Postharvest Biology and Technology, 222*, Article 113340. https://doi.org/10.1016/j.postharvbio.2024.113340

Khan, A. A., & Vincent, J. F. V. (1993). Anisotropy in the fracture properties of apple flesh as investigated by crack-opening tests. *Journal of Materials Science, 28*, 45–51. https://doi.org/10.1007/BF00349031

Kim, D.-S., Park, K.-J., Choi, J. H., Lim, J.-H., & Kim, H.-J. (2023). Metabolomic analysis of strawberries at different maturities according to postharvest storage period. *Scientia Horticulturae, 321*, Article 112283. https://doi.org/10.1016/j.scienta.2023.112283

Kobayashi, K., Fujii, S., Sato, M., Toyooka, K., & Wada, H. (2015). Specific role of phosphatidylglycerol and functional overlaps with other thylakoid lipids in Arabidopsis chloroplast biogenesis. *Plant Cell Reports, 34*, 631–642. https://doi.org/10.1007/s00299-014-1719-z

Kolesnikov, Y., Kretynin, S., Bukhonska, Y., Pokotylo, I., Ruelland, E., Martinec, J., & Kravets, V. (2022). Phosphatidic acid in plant hormonal signaling: From target proteins to membrane conformations. *International Journal of Molecular Sciences, 23*, 3227.

Navarro-Martínez, A., López-Gómez, A., & Martínez-Hernández, G. B. (2021). Potential of essential oils from active packaging to highly reduce ethylene biosynthesis in broccoli and apples. *ACS Food Science & Technology, 1*, 1050–1058. https://doi.org/10.1021/acsfoodscitech.1c00071

Paliyath, G., Murr, D. P., Handa, A. K., & Lurie, S. (2009). *Postharvest biology and technology of fruits, vegetables, and flowers*. John Wiley & Sons.

Park, M.-H., Ku, K.-M., Do, K.-R., Eum, H. L., Cho, J. H., Park, P. H., & Malka, S. K. (2023). Carbon dioxide treatment modulates phosphatidic acid signaling and stress response to improve chilling tolerance and postharvest quality in paprika. *Frontiers in Plant Science, 14*. https://doi.org/10.3389/fpls.2023.1287997

Pei, M.-S., Liu, H.-N., Yu, Y.-H., Wei, T.-L., & Guo, D.-L. (2023). Folic acid delays postharvest quality deterioration of table grape by regulating cell wall metabolism-associated hub WRKY31 transcription factor. *Postharvest Biology and Technology, 197*, Article 112207. https://doi.org/10.1016/j.postharvbio.2022.112207

Peng, J., Li, T., Liu, M., Zhang, J., Duan, X., Li, Y., Liang, H., Garcia-Caparros, P., Jiang, Y., & Duan, X. (2026). Application of riboflavin delays the browning of harvested litchi during storage at room temperature. *Food Control, 180*, Article 111646. https://doi.org/10.1016/j.foodcont.2025.111646

Pramitasari, R., Gunawicahya, L. N., & Anugrah, D. S. B. (2022). Development of an indicator film based on cassava starch–chitosan incorporated with red dragon fruit peel anthocyanin extract. *Polymers, 14*, Article 4142. https://doi.org/10.3390/polym14194142

Rey, F., Rodrigo, M. J., & Zacarias, L. (2021). Accumulation of tocopherols and transcriptional regulation of their biosynthesis during cold storage of mandarin fruit. *Postharvest Biology and Technology, 180*, Article 111594. https://doi.org/10.1016/j.postharvbio.2021.111594

Risoli, S., García-Pérez, P., Quaratiello, G., Cotrozzi, L., Sarrocco, S., Pellegrini, E., Nali, C., Lorenzini, G., & Lucini, L. (2025). Metabolomic insights on the response of winter wheat cultivars to *Fusarium* head blight infection and inoculation with a biocontrol strain in open field. *Plant Stress, 16*, Article 100807. https://doi.org/10.1016/j.stress.2025.100807

Sakizci, M. (2013). Effect of salt modification and acid activation on ethylene adsorption properties of sepiolite. *Adsorption, 19*, 1083–1091. https://doi.org/10.1007/s10450-013-9531-6

Salek, R. M., Steinbeck, C., Viant, M. R., Goodacre, R., & Dunn, W. B. (2013). The role of reporting standards for metabolite annotation and identification in metabolomic studies. *GigaScience*. https://doi.org/10.1186/2047-217X-2-13

Salgado, P. R., Di Giorgio, L., Musso, Y. S., & Mauri, A. N. (2021). Recent developments in smart food packaging focused on biobased and biodegradable polymers. *Frontiers in Sustainable Food Systems, 5*. https://doi.org/10.3389/fsufs.2021.630393

Santoso, V. R., Pramitasari, R., & Anugrah, D. S. B. (2023). Development of indicator film based on cassava starch-chitosan incorporated with red dragon fruit peel anthocyanins-gambier catechins to detect banana ripeness. *Polymers, 15*, 3609. https://doi.org/10.3390/polym15173609

Selcuk, N., & Erkan, M. (2014). Changes in antioxidant activity and postharvest quality of sweet pomegranates cv. Hicrannar under modified atmosphere packaging. *Postharvest Biology and Technology, 92*, 29–36. https://doi.org/10.1016/j.postharvbio.2014.01.007

Song, C., Wang, K., Xiao, X., Liu, Q., Yang, M., Li, X., Feng, Y., Li, S., Shi, L., Chen, W., & Yang, Z. (2022). Membrane lipid metabolism influences chilling injury during cold storage of peach fruit. *Food Research International, 157*, Article 111249. https://doi.org/10.1016/j.foodres.2022.111249

Tan, W.-J., Yang, Y.-C., Zhou, Y., Huang, L.-P., Xu, L., Chen, Q.-F., Yu, L.-J., & Xiao, S. (2018). Diacylglycerol acyltransferase and diacylglycerol kinase modulate triacylglycerol and phosphatidic acid production in the plant response to freezing stress. *Plant Physiology, 177*, 1303–1318. https://doi.org/10.1104/pp.18.00402

Tao, X., Wu, Q., Li, J., Huang, S., Cai, L., Mao, L., Luo, Z., Li, L., & Ying, T. (2022). Exogenous methyl jasmonate regulates phenolic compounds biosynthesis during postharvest tomato ripening. *Postharvest Biology and Technology, 184*, Article 111760. https://doi.org/10.1016/j.postharvbio.2021.111760

Thakur, K., Tomar, S. K., Singh, A. K., Mandal, S., & Arora, S. (2017). Riboflavin and health: A review of recent human research. *Critical Reviews in Food Science and Nutrition, 57*(17), 3650–3660.

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., Kanazawa, M., VanderGheynst, J., Fiehn, O., & Arita, M. (2015). MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nature Methods, 12*, 523–526. https://doi.org/10.1038/nmeth.3393

Upadhyay, A., Kumar, P., Kardam, S. K., & Gaikwad, K. K. (2022). Ethylene scavenging film based on corn starch-gum acacia impregnated with sepiolite clay and its effect on quality of fresh broccoli florets. *Food Bioscience, 46*, Article 101556. https://doi.org/10.1016/j.fbio.2022.101556

Wang, B., Wu, C., Wang, G., He, J., & Zhu, S. (2021). Transcriptomic analysis reveals a role of phenylpropanoid pathway in the enhancement of chilling tolerance by pre-storage cold acclimation in cucumber fruit. *Scientia Horticulturae, 288*, Article 110282. https://doi.org/10.1016/j.scienta.2021.110282

Watkins, C. B., & Nock, J. F. (2012). Rapid 1-methylcyclopropene (1-MCP) treatment and delayed controlled atmosphere storage of apples. *Postharvest Biology and Technology, 69*, 24–31. https://doi.org/10.1016/j.postharvbio.2012.02.010

Whale, S. K., & Singh, Z. (2007). Endogenous ethylene and color development in the skin of 'Pink Lady' apple. *Journal of the American Society for Horticultural Science, 132*, 20–28. https://doi.org/10.21273/JASHS.132.1.20

Wu, J., Nadeem, M., Galagedara, L., Thomas, R., & Cheema, M. (2022). Recent insights into cell responses to cold stress in plants: Signaling, defence, and potential functions of phosphatidic acid. *Environmental and Experimental Botany, 203*, Article 105068. https://doi.org/10.1016/j.envexpbot.2022.105068

Yang, H., Li, J., Li, X., Wu, R., Zhang, X., Fan, X., Li, G., Gong, H., Yin, X., & Zhang, A. (2023a). The mechanism of gibberellins treatment suppressing kiwifruit postharvest ripening processes by transcriptome analysis. *Postharvest Biology and Technology, 198*, Article 112223. https://doi.org/10.1016/j.postharvbio.2022.112223

Yang, Z., Lin, M., Yang, X., Zhu, C., Wu, D., & Chen, K. (2023b). Mechanisms of the response of apple fruit to postharvest compression damage analyzed by integrated transcriptome and metabolome. *Food Chem. X, 20*, Article 100972. https://doi.org/10.1016/j.fochx.2023.100972

Yin, Z., Dong, T., Huang, W., Du, M., Chen, D., Fernie, A. R., Yi, G., & Yan, S. (2022). Spatially resolved metabolomics reveals variety-specific metabolic changes in banana pulp during postharvest senescence. *Food Chemistry X, 15*, Article 100371. https://doi.org/10.1016/j.fochx.2022.100371

Yun, Z., Gao, H., & Jiang, Y. (2022). Insights into metabolomics in quality attributes of postharvest fruit. *Current Opinion in Food Science, 45*, Article 100836. https://doi.org/10.1016/j.cofs.2022.100836

Zhang, L., Cao, X., Wang, Z., Zhang, Z., Li, J., Wang, Q., & Xu, X. (2022a). Brassinolide alleviated chilling injury of banana fruit by regulating unsaturated fatty acids and phenolic compounds. *Scientia Horticulturae, 297*, Article 110922. https://doi.org/10.1016/j.scienta.2022.110922

Zhang, X., Liu, T., Zhu, S., Wang, D., Sun, S., & Xin, L. (2022b). Short-term hypobaric treatment alleviates chilling injury by regulating membrane fatty acids metabolism in peach fruit. *Journal of Food Biochemistry, 46*, Article e14113. https://doi.org/10.1111/jfbc.14113

Zhang, L., Zhang, P., Gao, M., Zhao, Y., Zhang, C., & Zhu, H. (2023a). Integrative metabolomics and transcriptomics analyses reveal pivotal regulatory mechanisms of 1-methylcyclopropene in maintaining postharvest storage quality of 'Fuji' apples. *Food Quality and Safety, 7*, Article fyac063. https://doi.org/10.1093/fqsafe/fyac063

Zhang, X., Wang, M., Gan, C., Ren, Y., Zhao, X., & Yuan, Z. (2023b). Riboflavin application delays senescence and relieves decay in harvested strawberries during cold storage by improving antioxidant system. *LWT, 182*, Article 114810. https://doi.org/10.1016/j.lwt.2023.114810

Zhang, K., Zhang, J., Zheng, T., Gu, W., Zhang, Y., Li, W., Zhou, P., Fang, Y., & Chen, K. (2024). Preharvest application of MeJA enhancing the quality of postharvest grape berries via regulating terpenes biosynthesis and phenylpropanoid metabolisms. *Food Chemistry, 438*, Article 137958. https://doi.org/10.1016/j.foodchem.2023.137958

Zhou, Z., Yang, Y., Shan, W., Zhang, H., Wei, W., Kuang, J., Chen, J., & Lu, W. (2022). Ethylene attenuates chilling injury of banana fruit via the MabHLH060/183 module in controlling phosphatidic acid formation genes. *Postharvest Biology and Technology, 183*, Article 111724. https://doi.org/10.1016/j.postharvbio.2021.111724

Zhu, D., Liang, J., Liu, H., Cao, X., Ge, Y., & Li, J. (2018). Sweet cherry softening accompanied with moisture migration and loss during low-temperature storage. *Journal of the Science of Food and Agriculture, 98*, 3651–3658. https://doi.org/10.1002/jsfa.8843