

A transcriptomic and metabolomic approach to elucidate the synergistic anti-browning effect of combined L-cysteine and Trehalose treatment on fresh-cut stem lettuce and the mechanisms underlying the maintenance of cell membrane integrity

Ronghua Wu

Shenyang Agricultural University

Zixuan Liu

Shenyang Agricultural University

Chunfan Guo

Shenyang Agricultural University

Jingxuan Ma

Shenyang Agricultural University

Ru Wang

Shenyang Agricultural University

Chunyu Ling

Shenyang Agricultural University

Yan Wang

Shenyang Agricultural University

Mingyue Chen

Shenyang Agricultural University

Zhaoxia Wu

wuzhaoxia@syau.edu.cn

Shenyang Agricultural University

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Abstract

Fresh-cut stem lettuce is highly prone to enzymatic browning and membrane deterioration, limiting its commercial value. This study investigated the synergistic effects and underlying mechanisms of combined L-cysteine (2.5 g/L) and trehalose (1.5 g/L) treatment (L + T) on quality preservation. L + T significantly suppressed browning, with higher lightness and lower browning index than single treatments. It inhibited polyphenol oxidase (PPO) and lipoxygenase (LOX) activities, while activating phenylalanine ammonia-lyase (PAL) and antioxidant enzymes (SOD, CAT, APX), thereby reducing reactive oxygen species and malondialdehyde accumulation. Integrated transcriptomic and metabolomic analyses revealed that L + T coordinately upregulated phenylpropanoid pathway genes (*COMT*, *CAD*) and cysteine/methionine metabolism genes (*cysE*, *metE*), enriching antioxidant phenolics (ferulic acid, sinapyl alcohol) and amino acid precursors. Concurrently, starch/sucrose metabolism was reprogrammed to supply energy, and glycerophospholipid and linoleic acid pathways were modulated to preserve membrane integrity. Notably, trehalose did not activate its own synthesis but boosted energy metabolism, supporting membrane repair. This multi-target network, integrating browning inhibition, oxidative stress alleviation, and membrane homeostasis, provides a mechanistic basis for L + T as a promising natural preservation strategy for fresh-cut produce.

1. Introduction

Freshly cut stem lettuce (*Lactuca sativa* var. *augusta*) is a low-calorie, nutrient-rich vegetable renowned for its diverse phytochemical composition and substantial vitamin and mineral content; it is also recognised for its blood pressure-lowering, gastrointestinal function-improving, and antioxidant properties, as well as its potential chemopreventive effects (Dylag et al., 2023; Shi et al., 2022; Yang et al., 2022). Minor processing steps such as washing, peeling, and cutting into pieces largely retain the fresh, crisp texture, nutritional content, and sensory properties of the fresh produce, consequently enhancing the convenience of both cooking and ready-to-eat consumption for consumers (Dylag et al., 2023; Meng et al., 2026). Accordingly, fresh-cut lettuce stem has emerged as a promising vegetable product with increasing market relevance.

Despite offering benefits, cutting remains vulnerable to causing traumatic stress, which destroys tissue microstructure and subcellular compartments, allowing phenolic compounds to come into contact with polyphenol oxidase (PPO), thus accelerating enzymatic browning. Simultaneously, the mechanical damage triggers oxidative bursts, culminating in accumulated reactive oxygen species (ROS) and membrane lipid peroxidation, which further leads to a decline in membrane selectivity and permeability. Such processes accelerate enzymatic browning, resulting in a softer texture and premature ageing, thereby reducing the product's market value and shelf life (Dylag et al., 2023; Ru et al., 2025). In addition, cuttings induce the metabolism of phenylpropanoids—typically manifested as an elevation in phenylalanine ammonia-lyase (PAL) activity—which enhances the biosynthesis of phenolic compounds, expanding the pool of browning precursors and worsening quality deterioration. Notably, the phenolic precursors, such as ferulic acid, possess highly effective antioxidant properties (Khan et al., 2024; Munir

et al., 2025). With rising concerns regarding toxicological safety, regulatory pressure, and consumer resistance to synthetic preservatives, the development of natural, sustainable, and industry-feasible anti-browning technologies is an urgent priority(Liu et al., 2022a).

L-Cysteine, a GRAS natural sulfur-containing amino acid, acts as an anti-browning agent and antioxidant by inhibiting PPO and PAL activities and enhancing antioxidant defenses. Research has confirmed that L-Cysteine, via mechanisms including downregulation of browning-related enzyme activity and enhancement of antioxidant enzyme activity, effectively inhibits browning in fresh-cut fruit and vegetables such as apples, potatoes, and lotus root, thereby extending their shelf life(Su et al., 2024; Yu et al., 2023; Zhang et al., 2023). Yu et al. (2023) demonstrated its synergistic anti-browning effect with ascorbic acid in fresh-cut stem lettuce coatings. Trehalose, a natural bioprotectant, stabilizes cellular membranes and proteins, enhances stress tolerance, and mitigates quality deterioration in various produce. Studies indicate that its application significantly enhances the shelf life of ingredients ranging from shiitake mushrooms to yellow croaker and cherry tomatoes, whilst minimising quality deterioration(Jiang et al., 2026; Liu et al., 2025; Xue et al., 2024). Despite their individual merits, single applications of either agent yield limited preservation efficacy. L-Cysteine primarily inhibits PPO and activates antioxidant systems, while trehalose stabilizes cell membranes and enhances stress tolerance. We thus hypothesized that their combination could simultaneously suppress enzymatic browning and maintain membrane integrity, achieving a synergistic effect beyond that of either agent alone.

Therefore, this study was designed to test the hypothesis that combined L-cysteine and trehalose (L + T) treatment synergistically inhibits browning and preserves membrane integrity in fresh-cut stem lettuce by coordinating the regulation of phenolic metabolism, antioxidant systems, and membrane lipid homeostasis. To test this hypothesis, we asked the following questions: (i) Does L + T treatment reduce browning index, lipid peroxidation, and ROS accumulation more effectively than single treatments? (ii) How does L + T modulate the activities of PPO, PAL, LOX, and antioxidant enzymes? (iii) Which metabolic pathways and corresponding genes are reprogrammed by L + T? By integrating physiological, transcriptomic, and metabolomic analyses, this work reveals a synergistic network of enzyme modulation, antioxidant reinforcement, and membrane homeostasis, providing a mechanistic basis for natural multi-target preservatives in fresh-cut produce.

2. Materials and Methods

2.1 Plant Materials and Treatments

Stem lettuce used in this study was procured from Dili Fresh Market in Shenyang and had been harvested one day prior to purchase. Uniform, intact heads without mechanical damage were selected, transported immediately to the laboratory, and pre-cooled at 4°C and 80–85% relative humidity for 2 h. The lettuce was then sectioned into cylindrical slices 0.3 cm thick and 5 cm in diameter using a sterile vegetable slicer. Food-grade L-cysteine was obtained from Xinrong Biotechnology Co., Ltd. and trehalose from Dezhou Huiyang Biotechnology Co., Ltd.

2.2 Single-Factor Experiment

Single-factor experiments were performed to screen concentrations, with L-cysteine evaluated from 1.5 to 3.5 g/L in 0.5 g/L increments and trehalose evaluated from 0.5 to 2.5 g/L in 0.5 g/L increments, with an immersion time of 6 min. Immersion durations for the combined solution composed of 2.5 g/L L-cysteine and 1.5 g/L trehalose were evaluated over a range of 2 to 10 min (Fig. S1) (Table S1).

2.3 Response Surface Methodology

Following optimization by response surface methodology (Fig. S2) with ANOVA (Table S2-4), four treatments were established: control group CK immersed in distilled water for 4 min, trehalose group T treated with 1.5 g/L trehalose for 4 min, L-cysteine group L treated with 2.5 g/L L-cysteine for 4 min, and combined treatment group L + T treated with 2.5 g/L L-cysteine and 1.5 g/L trehalose for 4 min. After treatment, surface moisture was blotted dry, samples were sealed in polypropylene boxes, and the boxes were stored at 4°C and 80–85% relative humidity for 8 days. Sampling was carried out every 2 days. Quality indices were measured on fresh samples, whereas samples designated for physicochemical analyses were ground in liquid nitrogen and stored at –80°C until analysis. All experiments were conducted in triplicate.

2.4 Quality Evaluation of Fresh-Cut Stem Lettuce

Colorimetric measurements of fresh-cut stem lettuce were performed with a CR-400 colorimeter (Konica Minolta, Tokyo, Japan) following Chu et al. (2023). The L^* , a^* , and b^* values were recorded, and the browning index (BI) was calculated according to the established protocols of Shen et al. (2024). Total bacterial content was evaluated by the method of Ru et al. (2024), Texture (firmness) was assessed using a TA.XTplus texture analyzer (Brookfield, USA) fitted with a P2 probe; measurement parameters were set as follows: penetration depth 3 mm, trigger force 5 N, and results reported in Newtons (N). Weight loss was determined relative to the initial sample mass and expressed as a percentage (%). Total Soluble solids content (TSS) was measured with an Abbe refractometer and reported as mass fraction (%).

2.5 Determination of Total Phenolic Content

Total phenolic content (TPC) was quantified using the Folin-Ciocalteu method as described by Lin et al. (2024). Briefly, 0.5 g of the sample was homogenized with 6.0 mL of 50% ethanol and centrifuged. A 0.3 mL aliquot of the supernatant was mixed with 1.5 mL of 10% Folin-Ciocalteu reagent. After 3 min, 2 mL of 7% sodium carbonate solution was added. The mixture was incubated at 30°C in darkness for 1 h, and absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalents (GAE).

2.6 Determination of Flavonoid Content

2.0 g of sample tissue was weighed, and a small amount of pre-chilled 1% HCl-methanol solution was added. The mixture was ground and homogenized in an ice bath, then transferred to a 20 mL graduated test tube. The mortar was rinsed with 1% HCl-methanol solution, and the rinse solution was transferred

to the test tube, made up to the mark, and mixed thoroughly. The mixture was extracted at 4°C in the dark for 20 minutes, shaken several times, then filtered, and the filtrate was collected for later use. The instrument was adjusted to zero using a 1% HCl-methanol solution as a blank reference. The absorbance of the filtrate was measured at 325 nm, repeated three times, and the results were plotted against a rutin standard curve for calibration. It should be noted that this method measures UV-absorbing compounds at 325 nm and is used for relative comparison among treatments, not absolute quantification of total flavonoids.

2.7 Determination of Total Chlorophyll Content

Total chlorophyll content was determined using a modified method of Zhang et al. (2024). A 1.0g sample was ground with 3.0 mL of acetone for extraction, centrifuged at $10,000 \times g$ for 10 min at 4°C, and the supernatant was diluted to 30 mL with acetone. Absorbance was measured at 663 nm and 645 nm using acetone as the blank. Chlorophyll content was calculated and expressed as mg/kg, with three replicates.

2.8 Determination of Malondialdehyde Content and Ascorbic Acid Content

Malondialdehyde (MDA) content was measured according to Zhao et al. (2025) with minor modifications. A 1.0 g sample was homogenized with 5.0 mL of 100 g L^{-1} trichloroacetic acid (TCA), centrifuged at $10,000 \times g$ for 20 min at 4°C, and the supernatant was collected and stored at low temperature. A 2.0 mL aliquot of the supernatant (2.0 mL of 100 g/L TCA for the blank) was mixed with 2.0 mL of 0.67% thiobarbituric acid (TBA), boiled in a water bath for 20 min, cooled, and centrifuged again. Absorbance was measured at 450 nm, 532 nm, and 600 nm in triplicate. Ascorbic acid content was measured by DCPIP. Briefly, 5.0 g of the sample was homogenized with 20 mL of 1% oxalic acid and centrifuged at $10,000 \times g$ for 10 min at 4°C. An aliquot of the supernatant was titrated with 0.1 mg mL^{-1} DCPIP solution until a pink color persisted for 15 s. Ascorbic acid content was calculated from a standard curve of L-ascorbic acid and expressed as mg/100g fresh weight.

2.9 Determination of antioxidant enzyme activities

APX activity: followed the measurement conducted by Zhao et al. (2021) with a simple adjustment. A 2.0 g sample was homogenized with 2.0 mL extraction buffer and centrifuged. The supernatant was mixed with H_2O_2 and buffer to form a reaction system, and absorbance was measured at 290 nm. Units are given as U/g.

CAT activity: Assayed with minor modifications to the method of Aqeel et al. (2024). A 1.0 g sample was homogenized with 1.0 mL extraction buffer, centrifuged at $12,000 \times g$ for 30 min at 4°C. A 100 μL aliquot of the enzyme extract was mixed with 2.9 mL of 20 mmol/L H_2O_2 , and absorbance was immediately measured at 240 nm. The measurement unit is defined as U/g.

POD activity: Determined as described by Saleh et al. (2026). A 2.0 g sample was homogenized in extraction buffer, centrifuged at $12,000 \times g$ for 30 min at 4°C. A 0.5 mL aliquot of the extract was mixed

with 3.0 mL of 25 mmol L⁻¹ guaiacol solution, followed by the addition of 200 µL of 0.5 mol/L H₂O₂. Absorbance was immediately measured at 470 nm. Values were reported in U/g. SOD activity was assayed using a commercial kit (Solarbio, Cat. No. BC0170) according to the manufacturer's instructions.

2.10 Determination of PAL, LOX, and PPO enzyme activities

PAL activity: Assayed using a modified method of Zhao et al. (2025). A 2.0 g sample was homogenized with 10.0 mL extraction buffer (containing 2 mM EDTA, 4% PVP, and 5 mM β-mercaptoethanol), centrifuged at 12,000 × g for 30 min at 4°C. A 0.5 mL aliquot of the supernatant was mixed with 0.5 mL of 0.02 M phenylalanine and 3.0 mL of borate buffer (pH 8.8), incubated at 37°C for 70 min, and PAL activity was calculated from absorbance at 290 nm. The unit is U/g.

LOX activity: Determined as described by Shi et al. (2016). A 2.0 g sample was homogenized in buffer, and the extract was mixed with sodium linoleate and acetate–sodium acetate buffer. LOX activity was measured by absorbance at 234 nm. Activity was reported in U/g.

PPO activity: According to the method of Li et al. (2024b). A 2.0 g sample was homogenized in 5.0 mL buffer, centrifuged at 12,000 × g for 30 min, and the supernatant was mixed with catechol solution. PPO activity was quantified by measuring absorbance at 420 nm following standard protocols. The unit is expressed as U/g.

2.11 Determination of O₂⁻ and H₂O₂ Contents

O₂⁻ and H₂O₂ contents: Determined using commercial kits: O₂⁻ (Regen, Cat. No. T01123) and H₂O₂ (Solarbio, Cat. No. BC3590), following the manufacturers' instructions.

2.12 Transcriptomic Analysis

Fresh-cut lettuce stems from the control (CK) and combined L-cysteine and trehalose treatment (L + T) groups were sampled at day 0, 4, and 8 days of storage to prepare sequencing libraries for transcriptome analysis. For each treatment group at each sampling time, three independent libraries were constructed (n = 3). Total RNA was extracted and quality-checked: concentration and purity were determined using a Nanodrop spectrophotometer, and integrity was verified via agarose gel electrophoresis or an Agilent 2100 Bioanalyzer. Qualified RNA (≥ 1 µg) was used for library construction: mRNA enrichment, fragmentation, cDNA synthesis, end repair, adapter ligation, fragment selection, and PCR amplification. Libraries were quality-checked using an Agilent 2100 Bioanalyzer, PicoGreen, and qPCR, normalized, pooled, and sequenced on an Illumina platform in paired-end 150 bp (PE150) mode, and then aligned to the lettuce reference genome (<https://db.cngb.org/lettuce/omics/genome>). Differentially expressed genes (DEGs) were determined using DESeq2 (|log₂FC| ≥ 1.0 and adjusted p < 0.05). Functional enrichment of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was performed.

2.13 Metabolomic Analysis

Fresh-cut lettuce stems from the control (CK) and combined L-cysteine and trehalose treatment (L + T) groups were sampled at day 0, 4, and 8 days of storage and selected for widely targeted metabolomics. Samples were freeze-dried, ground, and 25 mg of powder was extracted with methanol–water (4:1, v/v) containing an internal standard. The mixture was vortexed, homogenized, ultrasonicated in an ice bath (3 repetitions), incubated at -40°C for 1 h, and centrifuged at 12,000 rpm for 15 min at 4°C . The supernatant was filtered through a $0.22\text{ }\mu\text{m}$ membrane filter, and equal volumes of all samples were mixed to prepare quality control (QC) samples. Metabolites were separated using a Vanquish ultra-high-performance liquid chromatograph (UPLC) with a C18 column, with 0.01% aqueous acetic acid and 50% acetonitrile/isopropanol as the mobile phase. Detection was performed using a Stellar mass spectrometer in parallel reaction monitoring (PRM) mode with a heated electrospray ionization (H-ESI) source. Data were processed using Biobud JZGB software and the Biotree PDB v6.0 database. After quality control validation, metabolites were accurately identified, and relative quantification was performed based on peak area. Differentially abundant metabolites (DAMs) were identified by integrating $|\text{FC}| \geq 1$, an adjusted P-value < 0.05 , and variable importance in projection (VIP) > 1 derived from orthogonal partial least squares-discriminant analysis (OPLS-DA). Significant DAMs were functionally characterized by conducting a KEGG pathway enrichment analysis.

2.14 Data Analysis

All data were subjected to analysis of variance (ANOVA) using SPSS 26.0 software. Significant differences were determined via Duncan's multiple range test ($P < 0.05$). Graphs were plotted using Origin 2024 software.

3 Results and Discussion

Mechanical injury incurred during fresh-cut processing triggers enzymatic browning, lipid peroxidation of cellular membranes, and excessive accumulation of reactive oxygen species (ROS), all of which collectively curtail the shelf life of fresh-cut stem lettuce (Zhang et al., 2023). The combined application of natural preservatives represents a viable alternative for preserving postharvest quality in fresh-cut horticultural commodities (Li et al., 2023a). L-Cysteine exhibits both anti-browning and antioxidant activities (Pace et al., 2015; Su et al., 2024), whereas trehalose is known to stabilise membrane architectures and mitigate postharvest stress damage (Ding & Wang, 2018). Nevertheless, each compound used alone provides only limited efficacy in suppressing browning and delaying senescence in fresh-cut stem lettuce. The present study demonstrates that a combined treatment with L-cysteine and trehalose (designated L + T) exerts synergistic effects in inhibiting discolouration and preserving membrane integrity. This section presents experimental observations alongside mechanistic interpretations, aiming to elucidate the regulatory basis underlying the preservative action of L + T in fresh-cut stem lettuce.

3.1 Appearance and Colour Quality

The control (CK) and trehalose-alone (T) groups rapidly developed severe yellow-brown discolouration during storage; the L-cysteine-alone (L) group showed only a mild delay in browning, whereas the L + T group retained a bright green flesh colour throughout the entire storage period (Fig. 1A). Colourimetric data (Fig. 1B–D) revealed that on day 8, the a^* value of the L + T group was -4.8, significantly lower than those of the control (2.0), T (5.9), and L (-0.9) groups. Concurrently, the b^* and L^* values of the L + T group reached 17.2 and 47.1, respectively, markedly exceeding the corresponding values of 12.0 and 37.0 recorded for the control and T groups. The browning index (BI) rose steadily across all groups, with the T group exhibiting the highest BI and the L + T group the lowest throughout the storage course.

Consistent with visual assessments, the control group displayed a progressive decline in L^* value and a notable increase in a^* value and BI during storage. Single-agent treatments (L or T) exerted only modest mitigating effects, whereas the L + T group consistently maintained elevated L , low a , and low BI values, thereby effectively preserving the natural yellow-green hue of the flesh. Enzymatic browning constitutes the principal cause of visual quality deterioration in fresh-cut lettuce under cold storage (Zhang et al., 2023). The combined visual and colourimetric evidence confirms that L-cysteine and trehalose act synergistically to inhibit enzymatic browning, thus effectively sustaining the visual quality of the fresh-cut product. The preservative effect of the combined treatment substantially surpasses that of individual treatments and the control, aligning with previously reported combined preservation strategies using natural anti-browning agents in fresh-cut vegetables (Yu et al., 2023).

3.2 Storage Quality

The physicochemical and visual attributes of fresh-cut stem lettuce underwent pronounced changes during storage (Fig. 2A–C). Total bacterial counts increased continuously over time (Fig. 2A). On day 8, counts reached 8.3 lg CFU/g in the control, 8.6 lg CFU/g in the T group, and 7.3 lg CFU/g in the L + T group, whereas the L group showed a significantly lower value of 7.1 lg CFU/g. Firmness exhibited an overall upward trend throughout storage (Fig. 2B). On day 8, firmness values were 6.8 N and 7.3 N for the control and T groups, respectively, while the L + T group remained at 5.9 N, indicating a retarded progression of tissue hardening. Weight loss increased linearly with storage time (Fig. 2C). On day 8, the weight loss rate reached 27.2% in both the control and T groups, 17.9% in the L group, and only 15.2% in the L + T group.

These data indicate that the combined treatment effectively reduced moisture loss, maintained tissue plumpness, and retarded lignification-related hardening during cold storage. Water loss and microbial proliferation are key accelerators of quality decay in fresh-cut produce (Li et al., 2024a). Trehalose has been shown to enhance tissue water-retention capacity through stabilisation of cell structures (Ding & Wang, 2018), while L-cysteine inhibits microbial growth and tissue senescence (Pace et al., 2015). Their combination thus achieves a synergistic effect in preserving the storage quality of fresh-cut stem lettuce.

Figure 2. Changes in quality attributes of fresh-cut stem as affected by different treatments during 8 days of storage. (A) Total bacterial content; (B) Hardness; (C) Weight loss; (D) Total soluble solids (TSS); (E) Total phenolic content; (F) Flavonoids content; (G) Chlorophyll content; (H) Malondialdehyde (MDA) content; (I) Ascorbic acid content. Data are presented as means $\pm$ standard error ($n = 3$). Different lowercase letters at the same storage time indicate significant differences among treatments ($p < 0.05$).

3.3 Nutritional Quality and Secondary Metabolites

Figures 2D–F depict the temporal evolution of key nutritional and bioactive constituents during storage. Soluble solids content showed an overall increasing trend throughout the storage period (Fig. 2D). On day 8, the value for the L + T group was 2.6%, significantly lower than 3.6% in the control and 4.4% in the T group, suggesting that the combined treatment reduced abnormal nutrient consumption in fresh-cut stem lettuce.

Total phenolics content fluctuated during storage (Fig. 2E). The L-alone group reached a peak of 29.8 $\mu\text{mol/g}$ on day 4; on day 8, total phenolics were 47.5 $\mu\text{mol/g}$ in the L group and 39.9 $\mu\text{mol/g}$ in the L + T group, both markedly higher than 27.9 $\mu\text{mol/g}$ in the control and 32.7 $\mu\text{mol/g}$ in the T group. Flavonoid content increased continuously as storage progressed (Fig. 2F). The T group recorded a peak of 79.7 $\mu\text{mol/g}$ on day 6. On day 8, flavonoid content was 69.8 $\mu\text{mol/g}$ in the control and 74.6 $\mu\text{mol/g}$ in the T group, whereas the L + T group had a value of 40.4 $\mu\text{mol/g}$. Although this flavonoid level was lower than those of the single-treatment groups, the combined treatment alleviated abnormal consumption of phenolic secondary metabolites and maintained functional component stability.

Phenolic secondary metabolites are core bioactive substances that enable fresh-cut produce to counteract postharvest oxidative stress (Li et al., 2023b; Liu et al., 2022a). Further phenolic profiling implied that L + T prioritised metabolic flux towards highly bioactive antioxidant phenolics rather than flavonoids, which contributed to oxidative stress alleviation and browning inhibition, consistent with the metabolic regulatory patterns of natural preservatives on the phenylpropanoid pathway in fresh-cut vegetables (Su et al., 2024).

3.4 Oxidative Stress-Associated Physiological Traits

Figures 2G–I present the physiological metabolism and senescence progression of fresh-cut lettuce during storage. On day 8, chlorophyll content (Fig. 2G) in the control and T groups declined to 0.314 mg/g and 0.283 mg/g, respectively, whereas the L + T group maintained a level of 0.362 mg/g, indicating substantial retardation of chlorophyll degradation and preservation of original colour. Malondialdehyde (MDA, Fig. 2H), a key marker of membrane lipid peroxidation, reached 0.298 $\mu\text{mol/g}$ in the control on day 8, while the L + T group exhibited only 0.167 $\mu\text{mol/g}$. Ascorbic acid content decreased rapidly throughout storage (Fig. 2I). On day 8, ascorbic acid content was merely 0.07 mg/100g in the control, 0.09 mg/100g in the T group, 0.13 mg/100g in the L group, and remained at 0.18 mg/100g in the L + T group.

These physiological markers reflect the status of cell membrane integrity, which serves as the structural foundation for maintaining cellular functions and suppressing browning; cutting-induced oxidative injury

disrupts membrane architecture (Whitaker, 2012). The low MDA level in L + T samples demonstrated that the combined treatment effectively alleviated oxidative damage to cell membranes. Meanwhile, higher chlorophyll and ascorbic acid levels confirmed that L + T markedly preserved photosynthetic pigments and non-enzymatic antioxidant capacity, indirectly verifying the structural integrity of plasma and organellar membranes. Similar results have been reported in fresh-cut strawberries and citrus fruits, where compound preservatives alleviated lipid peroxidation and retained antioxidant substances to delay senescence (He et al., 2019; Lin et al., 2025).

3.5 Antioxidant Enzyme Activities

The dynamic activities of the antioxidant enzymes APX, CAT, POD, and SOD, as well as phenylalanine ammonia-lyase (PAL), a key enzyme in phenylpropanoid metabolism, were measured in fresh-cut stem lettuce during storage (Fig. 3A–E). No significant differences in enzyme activities were detected across all groups at the initial stage. During storage, antioxidant enzyme activities in the control group generally presented a fluctuating pattern of an initial decrease followed by an increase, whereas the combined L + T treatment effectively maintained and activated the antioxidant system.

APX activity (Fig. 3A) dropped substantially in the control group at the early storage period, reaching only 3.5 U/g on day 4, whereas the L + T group kept APX activity at 5.5 U/g at the same stage and remained significantly higher than the control, T, and L groups on day 8. The L + T group maintained consistently high levels of CAT (Fig. 3B) and SOD (Fig. 3D) activities throughout storage; on day 8, CAT activity reached 11.5 U/g and SOD activity was 0.061 U/g, both notably higher than 8.6 U/g and 0.058 U/g measured in the control group. POD activity (Fig. 3C) was effectively regulated to avoid abnormal increases. In addition, PAL activity (Fig. 3E) was remarkably elevated in the L + T group, reaching 0.17 U/g on day 8, thereby activating the phenylpropanoid pathway and synergistically enhancing the overall antioxidant defence capacity of the samples.

Notably, L + T exerted bidirectional regulation: it inhibited browning-causing enzymes while activating PAL to trigger phenolic compound biosynthesis. The activation of antioxidant enzyme systems is a core mechanism by which natural preservatives enhance postharvest stress tolerance in fresh-cut produce (Ma et al., 2025; Yuan et al., 2025). L-Cysteine can directly activate antioxidant enzymes to scavenge ROS, while trehalose stabilises enzyme protein structures to sustain high antioxidant activity (Ali et al., 2019; Ding & Wang, 2018), thus forming a synergistic antioxidant regulatory effect.

3.6 Browning- and Lipid Oxidation-Related Enzyme Activities

The activity changes of lipoxygenase (LOX), a key enzyme mediating lipid oxidation, and polyphenol oxidase (PPO), the principal enzyme associated with enzymatic browning, were measured in fresh-cut lettuce during storage (Fig. 3F–G). These two enzymes are core factors causing quality deterioration in fresh-cut lettuce (Zhang et al., 2023).

LOX activity (Fig. 3F) in the control group rose rapidly in the late storage period, reaching 0.36 U/g on day 6, while the L + T group maintained low LOX activity throughout storage, with a value of only 0.23 U/g on day 8. The combined treatment significantly inhibited lipid peroxidation and reduced structural damage to cell membranes. PPO activity (Fig. 3G) in the control group increased continuously as storage proceeded, reaching 0.21 U/g on day 8, whereas the L + T group recorded merely 0.14 U/g at the same time point.

These results indicate that the combined treatment effectively retarded the enzymatic oxidation of phenolic substrates and reduced the accumulation of browning products, which was consistent with the variation trend of the browning index reflected in the appearance observations. By restraining PPO activity, L + T blocked the conversion of phenolic compounds into brown pigments at the source of enzymatic browning. LOX inhibition further reduced membrane lipid oxidative damage, which is consistent with the anti-browning and membrane-protective mechanism of compound natural preservatives reported in fresh-cut potatoes and litchi (Ali et al., 2019; Li et al., 2024a).

3.7 Reactive Oxygen Species Accumulation

The changes in superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) contents during storage directly reflected the oxidative stress status of the tissues (Fig. 3H–I). ROS levels in the control group increased markedly as storage progressed. O_2^- content (Fig. 3H) reached 4.4 $\mu\text{mol/kg}$ on day 8, and H_2O_2 content (Fig. 3I) rose sharply at the early stage, attaining 10.9 mmol/kg on day 2. The combined L + T treatment effectively maintained ROS homeostasis: O_2^- content in the L + T group was only 4.2 $\mu\text{mol/kg}$ on day 8, while H_2O_2 content was 4.8 mmol/kg on day 2 and remained at 5.09 mmol/kg on day 8, both values being significantly lower than the 9.98 mmol/kg recorded for the control group on day 8.

Elevated ROS bursts induced by mechanical cutting are key inducers of membrane damage and browning senescence in fresh-cut produce (Whitaker, 2012; Yuan et al., 2025). By alleviating ROS-induced oxidative damage to cell membranes and organelles, the combined treatment delayed the progression of tissue senescence. The restrained ROS burst could be attributed to the enhanced enzymatic and non-enzymatic antioxidant systems under L + T treatment, similar to the ROS-scavenging mechanism of amino acid-based preservatives in postharvest fruit preservation (Lin et al., 2025; Ma et al., 2025).

3.8 Transcriptomic Profiling

Transcriptomic profiling revealed marked transcriptional reprogramming in fresh-cut stem lettuce induced by cold storage and combined L-cysteine and trehalose (L + T) treatment (Fig. 4). Principal component analysis (PCA) was performed to evaluate global transcriptional divergence and sample reproducibility (Fig. 4A). PC1 and PC2 accounted for 62.3% and 17.5% of the total transcriptional variance, respectively. A Pearson correlation heatmap was employed to assess both the reproducibility of the transcriptome sequencing and the transcriptional similarity among different sample groups (Fig. 4B). In this heatmap, the colour gradient represents Pearson correlation coefficients, with deeper red indicating higher inter-sample correlation. Biological replicates within the same treatment group

exhibited extremely high correlation coefficients and clustered tightly together, confirming excellent sequencing reproducibility and data reliability.

A Venn diagram illustrated the shared and unique differentially expressed genes (DEGs) across the four comparison groups (CK0d vs CK4d, CK4d vs LT4d, CK8d vs LT8d, and LT4d vs LT8d), revealing common and stage-specific transcriptional responses to cold storage stress and combined L + T treatment (Fig. 4C). A total of 327 core DEGs were shared among all four comparisons, suggesting that these conserved genes participate in fundamental regulatory processes throughout postharvest storage. Volcano plots were then generated to quantitatively display the global DEG patterns for each pairwise comparison (Fig. 4D–E).

GO and KEGG enrichment analyses, presented as bubble plots, demonstrated that DEGs identified in the CK4d vs LT4d comparison were predominantly enriched in GO terms associated with catalytic activity, oxidoreductase activity, membrane-related functions, abiotic stress responses, photosynthesis, and ion binding; the corresponding KEGG pathways were mainly concentrated in phenylpropanoid biosynthesis, plant hormone signal transduction, the MAPK signalling pathway, cysteine and methionine metabolism, sulfur and nitrogen metabolism, and terpenoid biosynthesis (Fig. 4F, G). In the CK8d vs LT8d comparison, DEGs were primarily enriched in GO terms such as catalytic activity, nucleotide binding, carbohydrate binding, phosphorylation, and protein kinase activity, whereas the overrepresented KEGG pathways were involved in plant hormone signal transduction, phenylpropanoid biosynthesis, starch and sucrose metabolism, glycolysis, the TCA cycle, glycerolipid metabolism, and various amino acid metabolic pathways (Fig. 4H, I). Based on these enrichment results, we ultimately selected phenylpropanoid biosynthesis, starch and sucrose metabolism, cysteine and methionine metabolism, glycerophospholipid metabolism, and linoleic acid/ α -linolenic acid metabolism as the primary pathways for further investigation. These pathway enrichment results are consistent with the transcriptomic regulatory characteristics of fresh-cut vegetables in response to natural preservative treatment (Liu et al., 2022b; Su et al., 2024)

3.9 Metabolomic Profiling

Metabolomic profiling revealed pronounced metabolic reprogramming in fresh-cut stem lettuce during cold storage and in response to combined L-cysteine-trehalose (L + T) treatment. Principal component analysis (PCA; Fig. 5A) showed that PC1 and PC2 explained 46.0% and 16.1% of the total variance, respectively. The distinct clustering and clear spatial separation among CK0d, CK4d, LT4d, CK8d, and LT8d samples indicate that both storage-induced stress and L + T treatment elicited marked shifts in metabolite abundance patterns. The Venn diagram (Fig. 5B) further delineated shared and unique differentially accumulated metabolites (DAMs) across four pairwise comparisons, suggesting the coexistence of conserved metabolic adjustments and stage-specific responses. Volcano plots (Fig. 5C–D) quantified DAM changes: CK4d vs LT4d contained 677 upregulated and 418 downregulated DAMs, whereas CK8d vs LT8d contained 590 upregulated and 477 downregulated DAMs.

KEGG pathway enrichment analysis (Fig. 5E–F) indicated that these DAMs were mainly enriched in amino acid, carbohydrate, and lipid metabolism, including linoleic acid metabolism and glycerophospholipid metabolism, thereby providing metabolic-level evidence supporting stress tolerance and quality preservation in fresh-cut stem lettuce under L + T treatment. Integrated transcriptome and metabolome analysis has been widely used to reveal postharvest preservation mechanisms in fresh-cut produce, and the metabolic pathways identified in this study are consistent with the key regulatory pathways of anti-browning and senescence delay in fresh-cut lily and lettuce (Li et al., 2023a; Liu et al., 2022b).

3.10 Phenylpropanoid Biosynthesis and Correlation Analysis

The phenylpropanoid biosynthesis pathway (Fig. 6A–B) elucidates how combined L-cysteine-trehalose (L + T) treatment modulates secondary metabolism in fresh-cut stem lettuce. In this figure, blue boxes indicate core intermediate metabolites, the gene heatmap depicts the expression patterns of genes encoding key enzymes (COMT, CAD, and POD), and the bar plots present the dynamic accumulation of representative phenolic metabolites. Using phenylalanine and tyrosine as the principal precursors, the pathway produces a series of phenolic intermediates and monolignols, which are subsequently polymerised into distinct lignin types via COMT-, CAD-, and POD-mediated reactions. Notably, L + T significantly upregulated these biosynthetic genes and increased the levels of phenylalanine, sinapyl alcohol, coniferyl aldehyde, and ferulate.

Within the phenylpropanoid pathway, L + T promoted the synthesis of upstream phenolic precursors and drove substantial accumulation of downstream antioxidant phenolics. These compounds can directly scavenge ROS and inhibit PPO activity, thus interrupting browning at both the enzymatic and oxidative stress nodes (Li et al., 2023a).

The Pearson correlation heatmap (Fig. 6C) quantifies pairwise linear relationships among colour parameters, browning-related enzymes, oxidative stress markers, antioxidant indices, and nutritional traits during storage, providing correlative evidence for mechanisms of quality deterioration and potential targets of L + T treatment. Positive and negative correlations are indicated in red and blue, respectively, with colour intensity and circle size representing correlation magnitude; significant correlations are denoted by asterisks ($p < 0.05$). The browning index (BI) is negatively correlated with L^* and positively correlated with a^* and b^* values as well as PPO and PAL activities, supporting enzymatic browning as a primary driver of discolouration. BI is also positively correlated with MDA content and LOX activity, but negatively correlated with antioxidant enzymes (SOD, CAT, POD and APX), ascorbic acid, total phenolics, and flavonoids. These associations suggest that oxidative stress-induced membrane damage compromises cellular compartmentalisation and thereby accelerates browning (Zhang et al., 2023).

Consistently, LOX activity correlates positively with both MDA and BI, whereas antioxidant indices show positive intercorrelations. Collectively, the correlation network supports an integrated "enzymatic browning–oxidative stress–membrane damage" framework. L + T established a positive regulatory chain

of 'PAL activation → increase in total phenolics and decrease in flavonoids → enrichment of highly active antioxidant phenolics → alleviation of oxidative stress → inhibition of browning', thus effectively blocking the browning cascade, which reveals the multi-target synergistic anti-browning mechanism of L + T composite treatment.

3.11 Starch and Sucrose Metabolism and Cysteine and Methionine Metabolism

As shown in Fig. 7, four core metabolic pathways operate coordinately to confer membrane protection and maintain postharvest quality in fresh-cut stem lettuce under L-cysteine-trehalose (L + T) treatment. In the starch and sucrose metabolism pathway (Fig. 7A), L + T upregulated energy-provision-related genes (INV, TPS, bglX, and glgC), thereby increasing the accumulation of sucrose, UDP-glucose, and glucose-6-phosphate, thus ensuring sufficient substrates to support energy-dependent membrane repair. Notably, exogenous trehalose did not activate its own synthesis or degradation pathway in lettuce tissue; trehalose content exhibited no obvious fluctuation. Instead, L + T moderately down-regulated catabolic genes while activating energy-synthesis-related genes to accumulate high-energy metabolites, providing continuous energetic support for membrane repair and structural maintenance. Energy metabolism reprogramming is essential for maintaining postharvest cellular homeostasis of fresh-cut produce (Ma et al., 2025).

In the cysteine and methionine metabolism pathway (Fig. 7B), L + T induced key biosynthetic genes (including *cysE* and *metE*), leading to elevated levels of L-cysteine, homoserine, serine, and 2-keto-4-methylthiobutyric acid, and consequently enlarging the precursor pool for antioxidant-related biosynthesis. The elevated metabolite pool furnished precursors for non-enzymatic antioxidant (ascorbic acid) synthesis and supplied substrates for the synthesis and maintenance of antioxidant enzyme activity, including SOD, CAT, and APX. Amino acid metabolism regulation is a critical mechanism for L-cysteine to exert antioxidant and anti-browning effects (Lin et al., 2025; Su et al., 2024).

The phenylpropanoid pathway and cysteine-methionine pathway acted synergistically: they jointly enhanced phenolic antioxidant production and ROS-scavenging capacity, forming a closed regulatory loop of 'antioxidant phenolics enrichment – efficient ROS elimination – sustained browning inhibition'. This combined regulatory effect was superior to modulating only a single pathway.

3.12 Glycerophospholipid and Linoleic Acid/ α -Linolenic Acid Metabolism

Within the glycerophospholipid metabolism pathway (Fig. 7C), L + T treatment modulated the expression of GPAT, PLD1_2, LCAT3, and GDE1, thereby promoting the biosynthesis of phosphorylethanolamine and serine and facilitating the remodelling of membrane lipid architecture (Yu et al., 2021). In addition, in the linoleic acid and α -linolenic acid metabolism pathways (Fig. 7D), L + T mitigated lipid peroxidation by adjusting peroxidation-related genes (LOX1_5 and LOX2S), which was accompanied by a reduction in the

accumulation of cytotoxic peroxidation products. Concurrently, L + T increased the levels of protective metabolites (e.g., traumatic acid and coronaric acid), thereby enhancing tolerance to membrane oxidative stress. Lipid metabolism homeostasis is the key to maintaining cell membrane integrity and delaying postharvest senescence of horticultural products (He et al., 2019; Whitaker, 2012).

Collectively, these coordinated multi-pathway regulatory responses, together with energy supply from starch-sucrose metabolism, constructed a "structural repair – oxidative inhibition – energy guarantee" membrane-protection network, contributing to maintaining membrane integrity and delaying quality deterioration of fresh-cut stem lettuce during cold storage.

3.13 Integrated Mechanisms Underlying Fresh-Cut Stem Lettuce Preservation with L-Cysteine and Trehalose

Combined immersion treatment with L-cysteine (2.5 g/L) and trehalose (1.5 g/L) preserves fresh-cut stem lettuce via synergistic regulation of multiple metabolic pathways. On the one hand, the treatment markedly down-regulated the activities of browning-related enzymes, polyphenol oxidase (PPO) and lipoxygenase (LOX), and suppressed accumulation of malondialdehyde (MDA), a marker of membrane lipid peroxidation (Zhang et al., 2023). Moreover, modulation of the phenylpropanoid pathway reduced oxidative consumption of phenolic substrates, while glycerophospholipid metabolism maintained the structural integrity of cellular membranes (Yu et al., 2021). On the other hand, the combined treatment activated the antioxidant system centred on superoxide dismutase (SOD), catalase (CAT), and the ascorbate-APX (AsA-APX) cycle, thereby enhancing scavenging of reactive oxygen species such as superoxide anion and hydrogen peroxide and alleviating oxidative stress-induced damage (Ali et al., 2019; Yuan et al., 2025).

Furthermore, balanced energy supply was sustained through regulation of starch and sucrose metabolism, and modulation of cysteine and methionine metabolism together with linoleic acid and α -linolenic acid pathways, strengthening stress-defence capacity. Collectively, these multi-target synergistic effects – including browning inhibition, membrane stabilisation, reactive oxygen species elimination, and maintenance of metabolic homeostasis – retard senescence and delay the deterioration of quality in fresh-cut stem lettuce.

4. Conclusion

This study demonstrates that combined treatment with L-cysteine and trehalose (L + T) synergistically inhibits browning in fresh-cut stem lettuce and maintains the integrity of its cell membranes. From a physiological perspective, the L + T treatment delays colour deterioration by balancing phenolic metabolism through the inhibition of PPO and activation of PAL, thereby providing a rich supply of antioxidant phenolic compounds and strengthening both enzymatic and non-enzymatic antioxidant systems. This reduces lipid peroxidation and the accumulation of ROS, thereby protecting cellular compartmentalisation and blocking browning at its source. At the molecular level, integrated transcriptomic and metabolomic analyses indicate that the phenylpropanoid pathway, together with the

cysteine and methionine metabolic pathways, mutually drive the synthesis of antioxidants and the scavenging of ROS. The starch and sucrose pathways provide energy for membrane repair while simultaneously stimulating the synthesis of downstream compounds without activating trehalose metabolism; meanwhile, the glycerophospholipid and unsaturated fatty acid pathways ensure membrane remodelling. Consequently, L + T establishes an enzyme regulation, antioxidant enhancement, and membrane homeostasis preservation network that extends shelf life, while also providing theoretical and practical guidance for the development of natural preservatives in fresh-cut produce.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

Ronghua Wu: Conceptualization; investigation; writing—original draft; methodology; validation; visualization; writing—review and editing; software; formal analysis; data curation; resources. Zixuan Liu: Investigation; methodology; validation; visualization; writing—review and editing; formal analysis. Chunfan Guo: Writing—review and editing; formal analysis. Jingxuan Ma: Writing—review and editing; formal analysis. Ru Wang: Writing—review and editing. Chunyu Ling: software; formal analysis. Yan Wang: formal analysis; data curation. Mingyue Chen: methodology; validation. Zhaoxia Wu: Funding acquisition; project administration; conceptualization; writing—review and editing; resources.

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Data Availability

The data will be available upon reasonable request.

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Figures

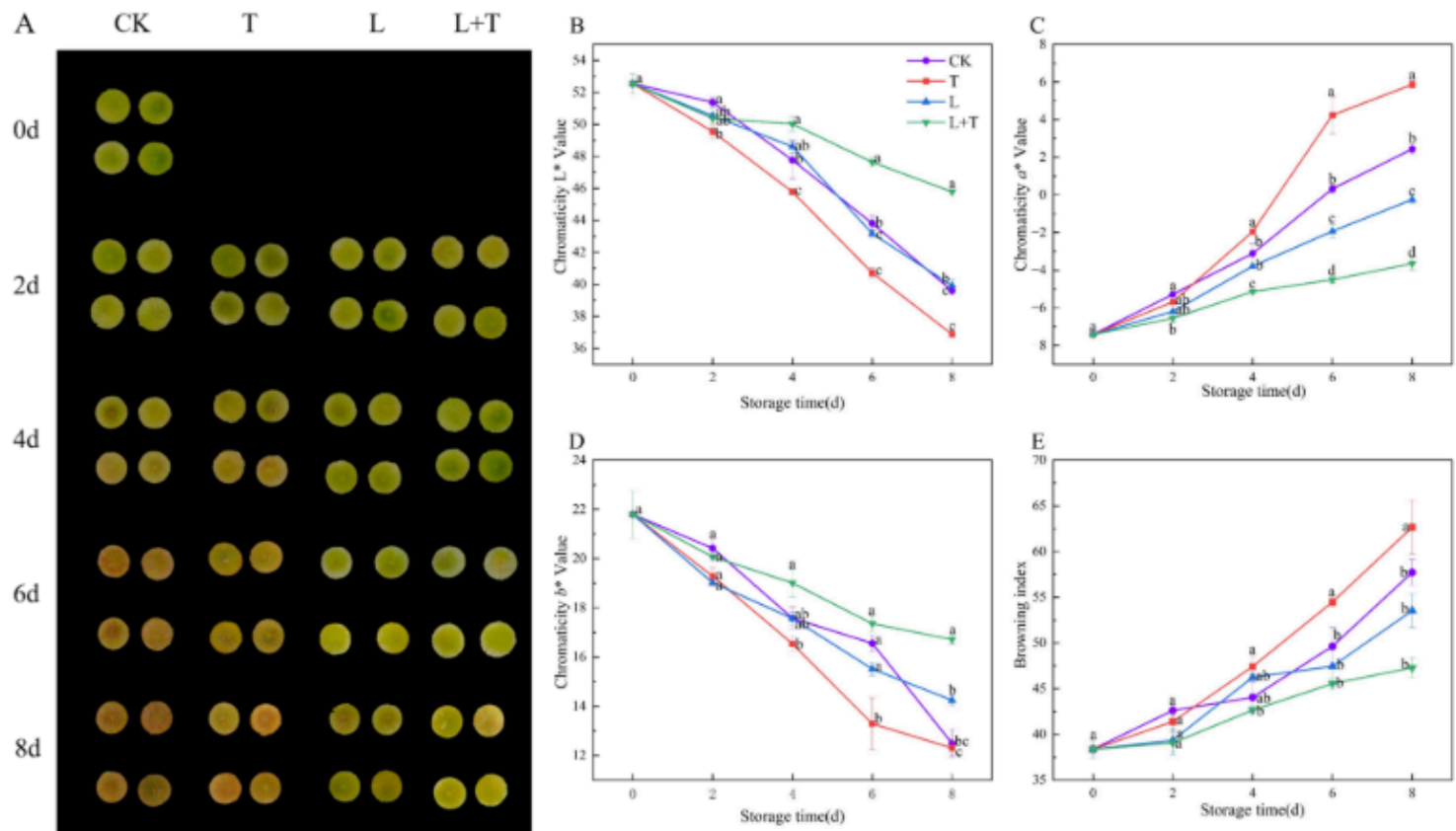


Figure 1

Changes in visual appearance and color parameters of fresh-cut stem lettuce as affected by different treatments during 8 days of storage. (A) Visual appearance of fresh-cut stem lettuce slices; (B) Chromaticity a^* value; (C) Chromaticity b^* value; (D) Chromaticity L^* value; (E) Browning index. CK, untreated control; T, trehalose treatment; L, L-cysteine treatment; L+T, combined L-cysteine and trehalose treatment. Data are presented as means $\pm$ standard error ($n = 3$). Different lowercase letters at the same storage time indicate significant differences among treatments ($p < 0.05$).

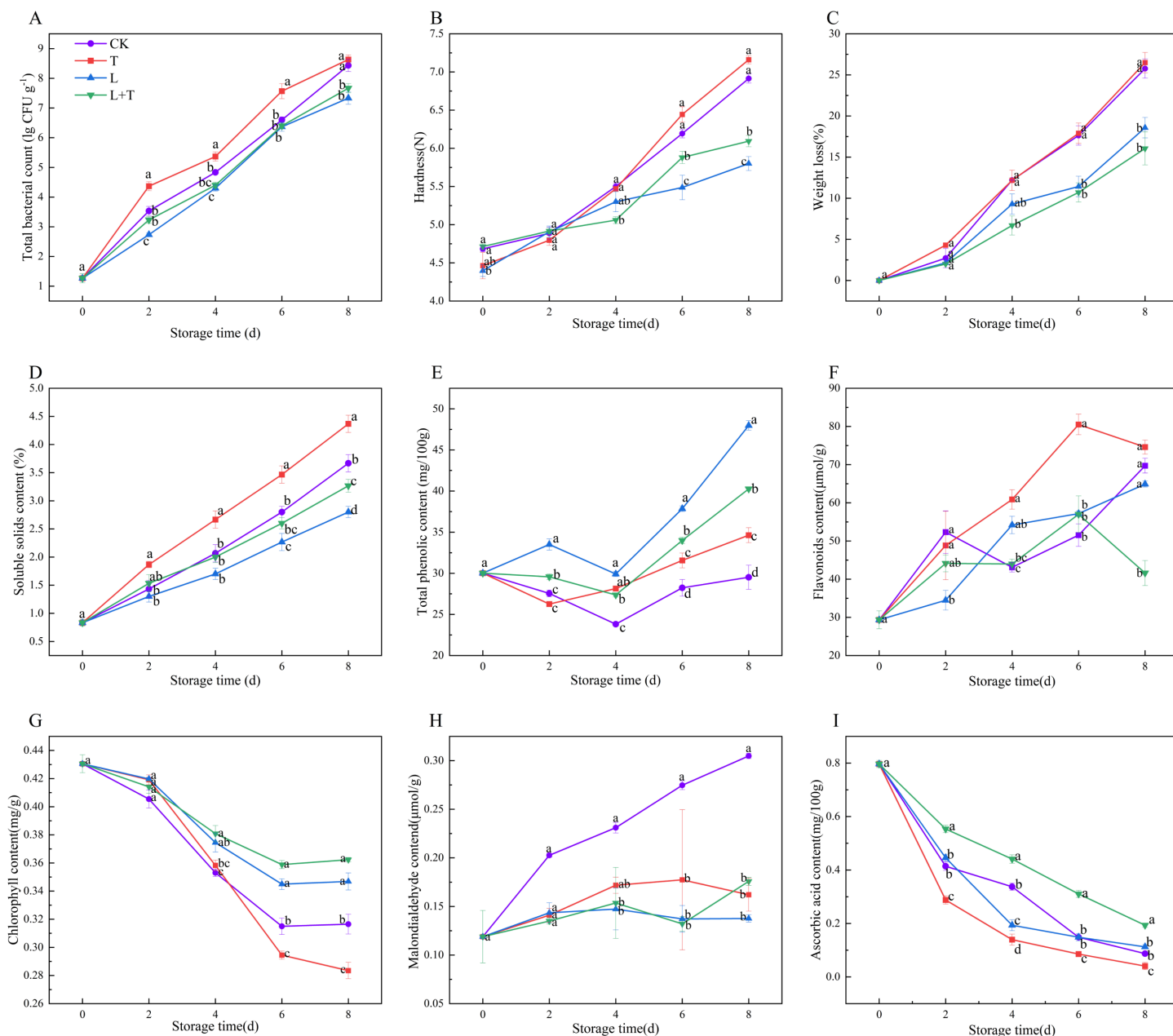


Figure 2

Changes in quality attributes of fresh-cut stem as affected by different treatments during 8 days of storage. (A) Total bacterial content; (B) Hardness; (C) Weight loss; (D) Total soluble solids (TSS); (E) Total phenolic content; (F) Flavonoids content; (G) Chlorophyll content; (H) Malondialdehyde (MDA) content; (I) Ascorbic acid content. Data are presented as means $\pm$ standard error ($n = 3$). Different lowercase letters at the same storage time indicate significant differences among treatments ($p < 0.05$).

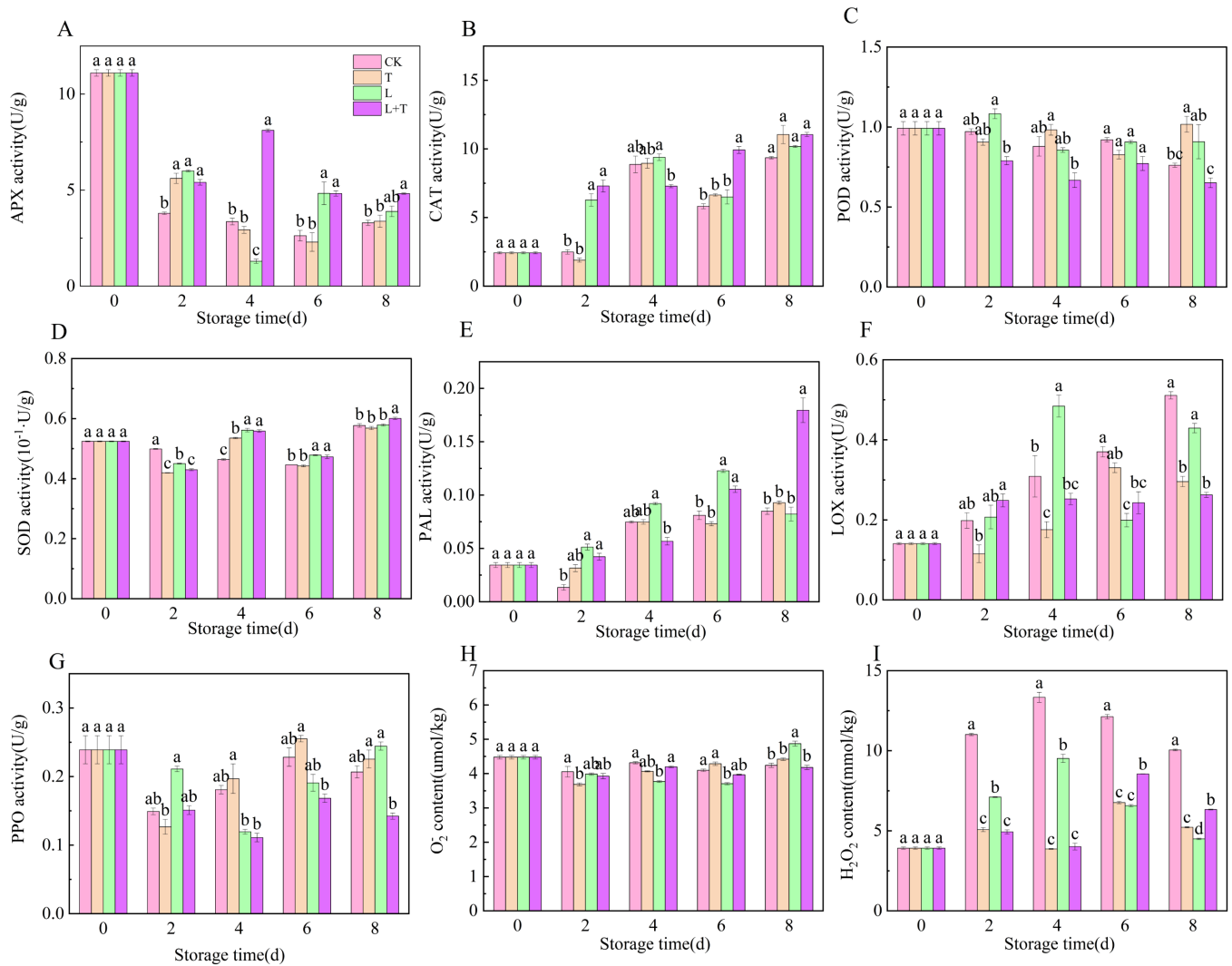


Figure 3

Changes in antioxidant enzyme activities, browning-related enzyme activities and reactive oxygen species levels in fresh-cut stem lettuce as affected by different treatments during 8 days of storage. (A) APX activity; (B) CAT activity; (C) POD activity; (D) SOD activity; (E) PAL activity; (F) LOX activity; (G) PPO activity; (H) O₂⁻ content; (I) H₂O₂ content. Data are presented as means ± standard error (n = 3). Different lowercase letters at the same storage time indicate significant differences among treatments (p < 0.05).

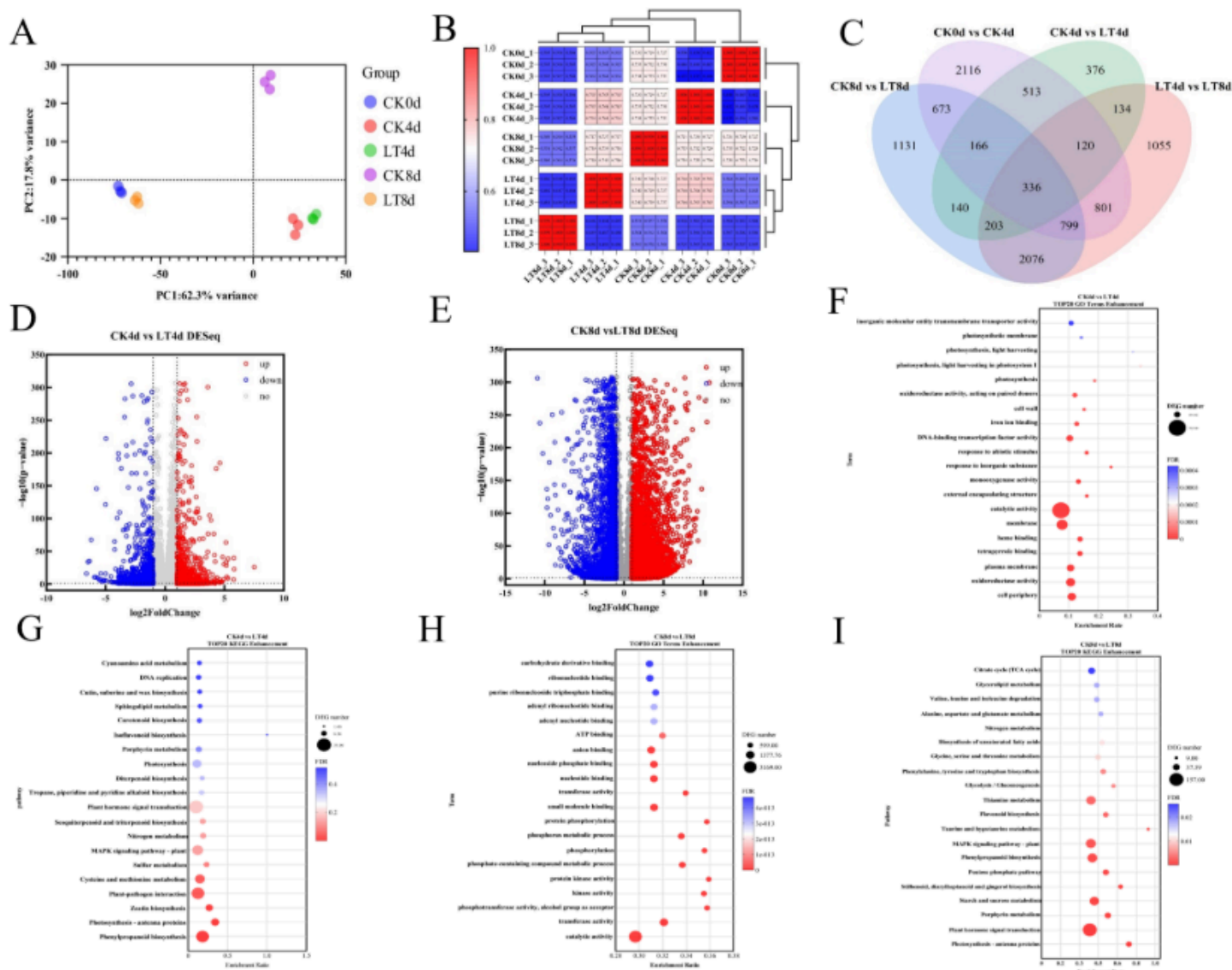


Figure 4

Transcriptomic profiling of fresh-cut stem lettuce under control (CK) and combined L-cysteine–trehalose (L+T) treatments. (A) Principal component analysis (PCA) showing transcriptional divergence among CK0d, CK4d, LT4d and LT8d groups. (B) Pearson correlation heatmap verifying the reproducibility of biological replicates. (C) Venn diagram presenting unique and shared differentially expressed genes (DEGs) across four comparative groups. (D-E) Volcano plots illustrating DEG profiles for CK4d vs LT4d, and CK8d vs LT8d, where red, blue and grey dots represent significantly up-regulated, down-regulated and non-differentially expressed genes, respectively. (F, G) GO and KEGG enrichment bubble plots for CK4d vs LT4d; (H, I) for CK8d vs LT8d.

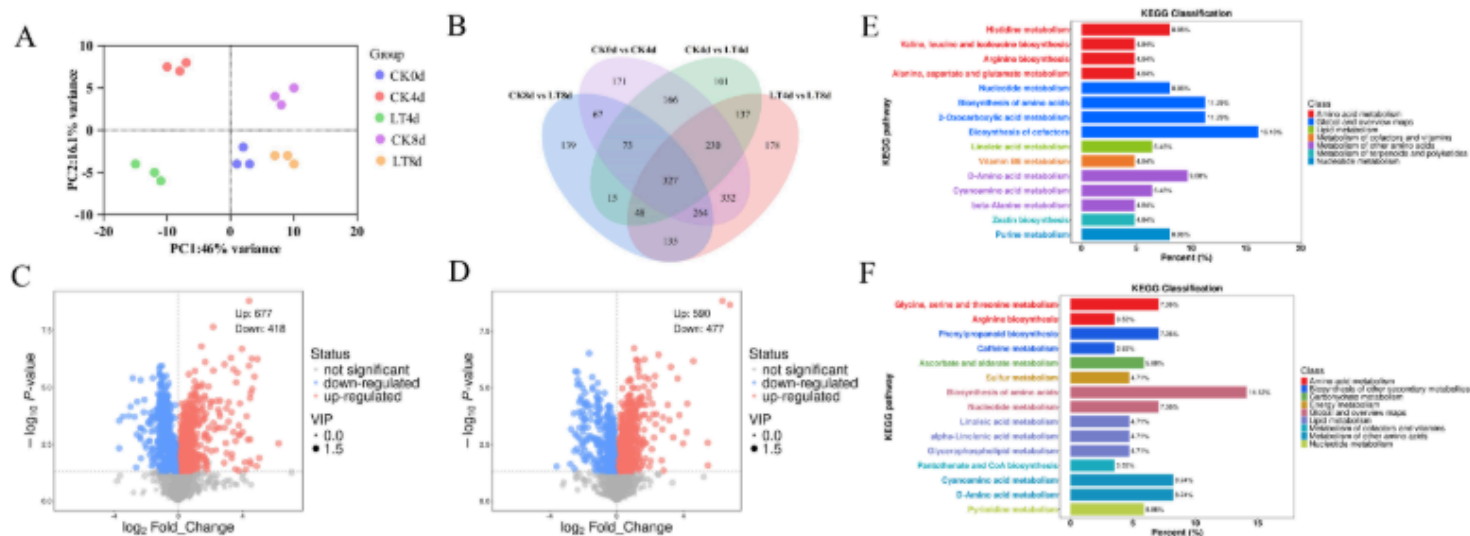


Figure 5

Metabolomic profiling of fresh-cut stem lettuce under control (CK) and combined L-cysteine–trehalose (L+T) treatments. (A) PCA score plot showing metabolic separation among CK0d, CK4d, LT4d, CK8d and LT8d groups. (B) Venn diagram illustrating shared and unique differentially accumulated metabolites (DAMs) across four comparative groups. (C-D) Volcano plots of DAMs for CK4d vs LT4d and CK8d vs LT8d, where red and blue dots represent significantly up-regulated and down-regulated metabolites, respectively. (E-F) Top enriched KEGG pathways of DAMs derived from control and L+T-treated comparisons.

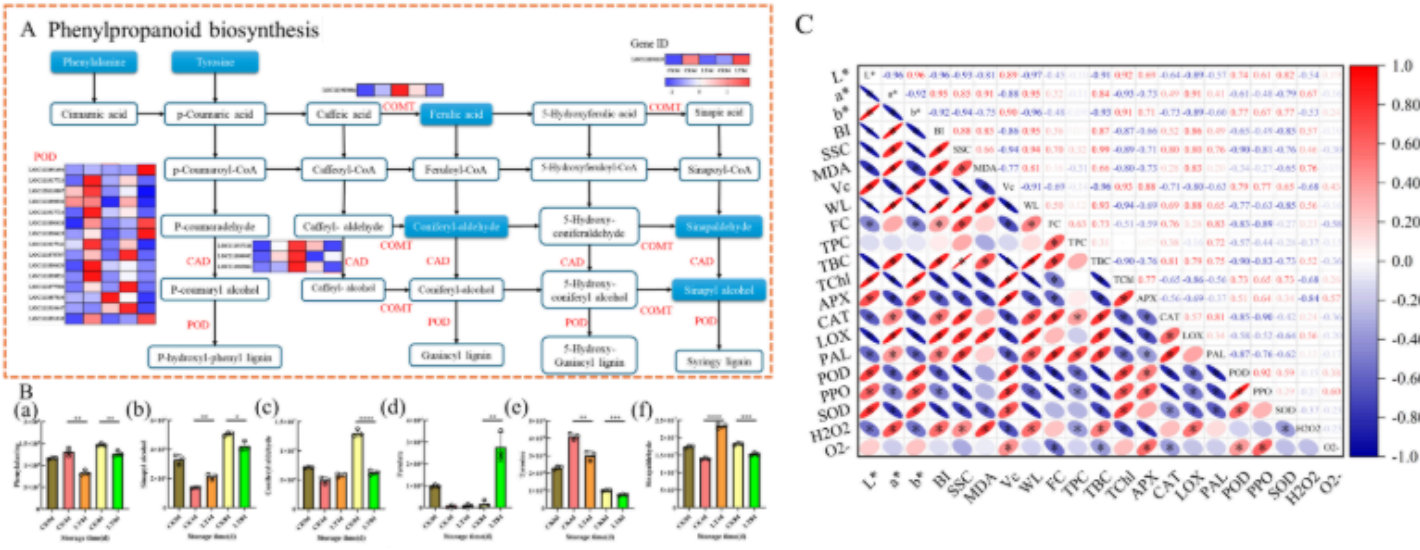


Figure 6

Regulatory mechanism of combined L-cysteine-trehalose (L+T) treatment on phenylpropanoid biosynthesis in fresh-cut stem lettuce. (A) Schematic diagram of the phenylpropanoid biosynthesis pathway, with core intermediate metabolites highlighted in blue boxes; heatmaps display the FPKM z-score of key enzyme-encoding genes. (B) Bar charts illustrating the dynamic accumulation of pivotal metabolites.

phenylpropanoid-derived metabolites during cold storage. Asterisks denote significant differences among treatments ($p < 0.05$). (C) Correlation heatmap showing relationships between browning-related quality indices, physiological parameters, and enzyme activities. Red and blue circles indicate positive and negative correlations, respectively, with color intensity reflecting correlation strength.

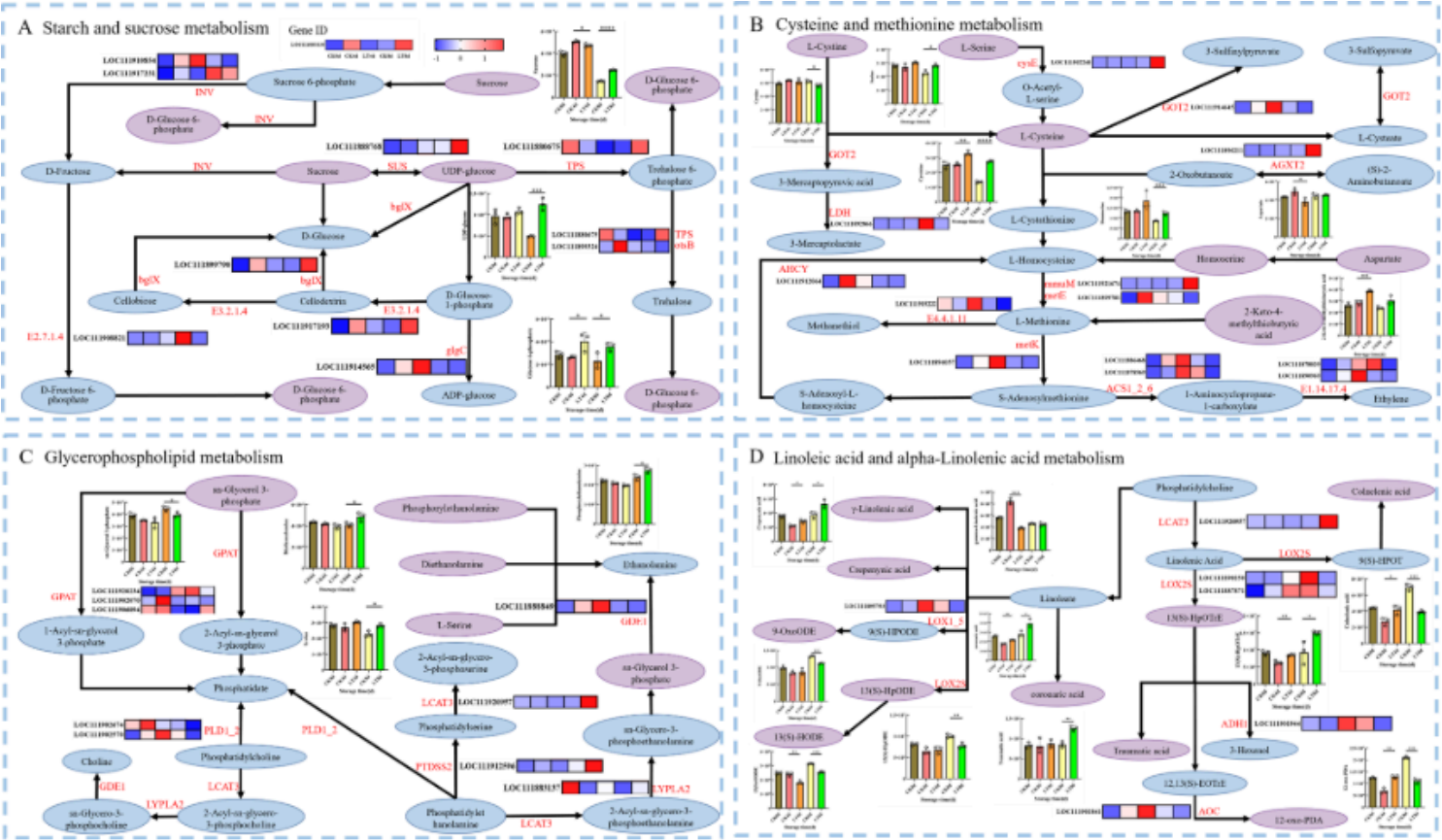


Figure 7

Schematic regulatory diagrams of four core metabolic pathways modulated by combined L-cysteine-trehalose (L+T) treatment in fresh-cut stem lettuce.(A) Starch and sucrose metabolism; (B) Cysteine and methionine metabolism; (C) Glycerophospholipid metabolism; (D) Linoleic acid and α -linolenic acid metabolism. purple boxes represent key functional metabolites, heatmaps show the FPKM z-score of pivotal enzyme-encoding genes, and bar charts display the dynamic accumulation of core metabolites under different treatments during cold storage. Bar graphs display metabolite contents with statistical significance ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$). CK, untreated control; LT, L+T treatment.

Supplementary Files

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- [Supplementarymaterial.docx](#)