Metabolomics reveals the effect of vacuum packaging combined with moderate-temperature preservation on quality changes of tender ginger

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ABSTRACT

Tender ginger is often used a fresh vegetable but hard to storage due to the delicate skin, high moisture content and prone to spoilage. In order to develop suitable preservation technology for tender ginger, the effects of vacuum packaging combined with different preservation temperatures (20–25 °C room temperature, 4 °C and 10 °C) on tender ginger shelf life were investigated. The results indicated that vacuum packaging combined with 4 °C (VP4) preservation could easily cause cold damage and postharvest physiological fluctuations. Vacuum packaging combined with 10 °C (VP10) inhibited moisture loss and physiological activities. Metabolomics analysis revealed 169 metabolites significantly differential regulated during VP10 preservation. The characteristic metabolites were primarily associated with amino acid, lipid and nucleotide metabolism. The metabolic pathways mainly involved linoleic acid metabolism; alanine, aspartate and glutamate metabolism; and purine metabolism. The above results indicated that VP10 effectively extended the preservation period of tender ginger with less damage.

1. Introduction

Vegetables are an integral part of the human diet and play an important role in human health because they can provide various nutrients and bioactive ingredients for human health, such as vitamins, minerals, dietary fiber, polyphenols, flavonoids, etc. (Chaudhary et al., 2025). However, fresh vegetables have high water content, and the skin is tender and easily damaged, making them prone to decay, so how to extend the shelf life of fresh vegetables is a critical issue (Khalid et al., 2024). Ginger is a perennial herbaceous plant with widely recognized culinary and medicinal value, because it contains various functional components, such as gingerols, polysaccharides, polyphenols, flavonoids, and dietary fiber, among others (Yeh et al., 2014). It is known for its diverse health benefits, including antioxidant, anti-inflammatory, chemopreventive, antipyretic, antimicrobial, and circulation-promoting properties (Stoyanova, Konakchiev, Damyanova, Stoilova, & Suu, 2006). Ginger is commonly used as a seasoning, traditional Chinese medicine, and spice, and the main edible and medicinal parts are underground rhizomes (Liu et al., 2014). Tender ginger is an

immature ginger that is often used for a vegetable. Tender ginger has similar nutritional components to ginger, but has higher water content and lower cellulose content than ginger. it is crispy, tender, tasty and refreshing, all of which make it highly favored by consumers (Liu et al., 2014). However, the high moisture content of tender ginger makes it prone to dehydration during preservation, leading to wilting and a decrease in quality. Additionally, the harvesting process can result in mechanical damage to rhizomes, creating opportunities for microbial invasion and causing decay (Khalid et al., 2024). These factors contribute to the short shelf life of tender ginger, which currently fails to meet consumer demands. Therefore, extending the preservation life of tender ginger is a challenge.

Traditional preservation methods for tender ginger mainly include sand preservation, cellar preservation, and pit burial. The aim of these methods is to extend the preservation period by controlling moisture loss and reducing the preservation temperature (Jia et al., 2021). However, it is hard to accurately control the relevant conditions, and these methods are also not suitable for preservation during the commercial sales process. Currently, the study of fruit and vegetable

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preservation has focused mainly on methods such as low-temperature preservation, antiseptic treatment and gas-conditioned packaging (Gomes et al., 2023). However, the excessive use of chemical preservatives may pose health risks to the human body that do not align with consumers' pursuit of green products. Moreover, the cost of controlled atmosphere preservation is relatively high.

Low-temperature preservation is a major preservation method for vegetables. Low temperatures reduce cell respiration rates, lower enzyme activity, decrease metabolic activity, and inhibit microbial growth (Goyer, Picard, Hellmann, & Mooney, 2019). Thus, controlling the temperature to extend the shelf life is the most widely used and effective method. However, if the preservation temperature exceeds the maximum tolerance threshold of the plant, cold damage may result, especially for tropical and subtropical products (Sevillano, Sanchez-Ballesta, Romojaro, & Flores, 2009). Therefore, selecting appropriate low-temperature conditions is particularly important for the preservation of fruits and vegetables. The exclusive use of low-temperature has limitations in terms of preservation effectiveness. Microbial growth and the metabolism of tender ginger are closely related to oxygen, as most microorganisms that cause food spoilage require oxygen for growth (Qadri, Yousuf, & Srivastava, 2015). In addition to temperature, appropriate packaging is also an effective way to extend the shelf life of fruits and vegetables. Packaging could protect food from decay and oxidation processes caused by various forms of contamination (including chemicals and microorganisms) to guarantee the quality of food for a long time (Azam et al., 2023). Although vacuum packaging materials may impact the environment and increase the cost of preserving tender ginger, the packaging could reduce oxygen exposure, inhibits browning, and prevents damage during transportation. As a result, vacuum packaging technology is widely used in the processing and preservation of most fruits and vegetables.

Although vacuum packaging technology has been widely applied in the fresh fruits and vegetables preservation, the application in tender ginger preservation has been less explored. Therefore, in the present study, the physiological and enzyme activity changes of tender ginger under vacuum packaging and different preservation temperature conditions were investigated. In addition, metabolomics combined with multivariate statistical analysis was employed to examine metabolite changes in tender ginger before and after preservation under optimal preservation conditions. The aim of this study was to explore the metabolic mechanisms involved in the postharvest preservation of tender ginger, providing a theoretical basis for the development of postharvest preservation processes for tender ginger.

2. Materials and methods

2.1. Chemicals and reagents

Sodium hypochlorite, methanol, sodium hydroxide, forintanol, ethanol and aluminum nitrate were purchased from Chron chemiscals (Chengdu, China). Gallic acid and thiobarbituric acid (TBA) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai China). Rutin, vanillin and trichloroacetic acid were purchased from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai China). Methanol and acetonitrile were of liquid chromatography-tandem mass spectrometry (LC–MS/MS) grade and purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Sample preparation

Fresh tender ginger was collected in early October from a farm in Chengdu, Sichuan Province (6-month growing cycle). The samples were firstly washed with water and dried, and then vacuum packaged and stored at 4 °C, 10 °C and room temperature (20–25 °C) for 0 days, 10 days, 20 days and 30 days, respectively. Each bag of ginger needs to be weighed before vacuum packaging. The samples were chopped and mixed, treated with liquid nitrogen, and then stored at −80 °C in a refrigerator.

2.3. Determination of the weight loss rate and moisture content

Three bags of tender ginger samples were taken and numbered for each treatment, and weighed at different preservation time: 0 days, 5 days, 10 days, 15 days, 20 days, 25 days, and 30 days. The weight loss rate during preservation was calculated as a percentage using the following Eq. 1, which is based on the initial weight at the beginning of each measurement period.

$$Weight\ Loss\ Rate = \frac{A - B}{A} \times 100\% \tag{1}$$

where A represents the initial weight and B represents the final weight.

Samples from different preservation temperatures were sliced and placed in a 105 °C oven for drying until a constant weight was achieved. The moisture content was calculated using Eq. 2:

$$Moisture\ Content = \frac{A - B}{A} \times 100\% \tag{2}$$

where A represents the initial weight and B represents the final dry weight.

2.4. Determination of total polyphenol content (TPC) and total flavonoid content (TFC)

After washing and drying, the tender ginger was ground into powder in liquid nitrogen, and then the powder was stored immediately at −20 °C for subsequent analysis. The TPC was detected according to the method of Meng et al. (Meng et al., 2022) with slight modifications, 1 g of tender ginger powder was mixed with 10 mL of methanol, subjected to ultrasonic assisted extraction (KQ3200DB, Kunshan ultrasonic instrument, China) 45 min under the temperature of 45 °C, and then centrifuged at 4 °C (10,000 ×g, 10 min). The supernatant was collected, and methanol was added to make up to 10 mL of sample extract. For analysis, 0.5 mL of the sample extract was combined with 2 mL of 10 % Folin-phenol reagent. After thorough mixing, 2 mL of 7.5 % Na_2CO_3 solution was added, and the reaction was carried out in the dark at room temperature for 2 h. The absorbance was measured at a wavelength of 765 nm, and gallic acid used for the standard curve to calculate the TPC.

The TFC was determined according to the method of Meng et al. (Meng, Lei, et al., 2022) with slight modifications, 1 g of tender ginger powder was added to 25 mL of 80 % ethanol for ultrasonic extraction for 1 h. The extract was vacuum-filtered, and the supernatant was collected and diluted to 50 mL with distilled water. In a test tube, 1 mL of the sample to be tested was mixed with1 mL of 5 % (w/v) sodium nitrite solution and left to stand for 6 min. Then, 1 mL of 10 % aluminum nitrate solution was added, mixed and left to stand for an additional 6 min. Finally, 10 mL of 4 % sodium hydroxide solution and 50 % ethanol were added. After thorough shaking and standing for 10 min, the absorbance was measured at 510 nm, and rutin was used as a standard to calculate the TFC.

2.5. Gingerol content determination

Gingerol content was determined according to the methods of Zhang et al. (Zhang et al., 2020) with slight modifications, 5.0 g of tender ginger powder was accurately weighed, 45 mL of 95 % ethanol was added, and the suspension was incubated in a water bath for 1 h at 55 °C. After extraction, the solution was cooled and centrifuged at 4 °C (5,000 ×g, 10 min). Two milliliters of the supernatant were diluted with 95 % ethanol to a final volume of 10 mL. The absorbance at 280 nm was measured using a UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the content of gingerol was calculated

according to vanillin as a standard.

2.6. Malondialdehyde (MDA) content determination

The determination of the MDA content was based on the method of Hodges et al. (Hodges, DeLong, Forney, & Prange, 1999) with slight modifications. Five grams of tender ginger powder was mixed with 25 mL of 10 % (w/w) trichloroacetic acid (TCA) and then centrifuged at 16000 ×g for 10 min at 4 °C. Two milliliters of the supernatant were mixed with 2 mL of 0.6 % (w/w) TBA and heated in a boiling water bath for 20 min. After cooling to room temperature, the sample was centrifuged again. The absorbance (A) of the supernatant was measured at wavelengths of 450 nm, 532 nm, and 600 nm. The MDA concentration was calculated using the following equation (Eq. 3):

$$MAD = (6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}) \times 12.5 \tag{3}$$

2.7. Enzyme activity assay

Peroxidase (POD), catalase (CAT), phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO) in tender ginger were analyzed using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.8. Untargeted metabolomic analysis

2.8.1. Extraction of metabolites
Fifty milligrams of tender ginger were accurately weighed, 400 μL of extraction solution (methanol:water = 4:1) was added, the solution was mixed well, and the mixture was processed using a high-throughput tissue disruptor (Wonbio-96c, WONBIO, China) at 50 Hz for 6 min. After vortexing for 30 s, ultrasonic extraction was performed at low temperature for 30 min (5 °C, 40 kHz). Then, the samples were incubated at −20 °C for 30 min and centrifuged for 15 min (4 °C, 13,000 ×g), after which the supernatant was collected. The supernatant was dried with N_2, and 120 μL of reconstitution solution (acetonitrile: water = 1:1) was added. After ultrasonic extraction for 10 min (5 °C, 40 kHz), the sample was centrifuged again for 10 min (4 °C, 13,000 ×g). Finally, the supernatant was transferred to sample vials with insert tubes for LC–MS/MS analysis.

2.8.2. Quality control sample
Equal volumes of each sample were mixed evenly to create a quality control (QC) sample. Throughout the entire analytical process, a QC sample was inserted for every 10 samples to assess the reproducibility of the entire analysis procedure.

2.8.3. LC–MS/MS analysis
The analysis was conducted using a liquid chromatography-tandem mass spectrometry (LC–MS/MS) system (ExionLC AD, AB SCIEX, USA) equipped with a triple quadrupole mass spectrometer (Triple TOF5600, AB SCIEX, USA). A ACQUITY BEH C18 chromatographic column (100 mm × 2.1 mm i.d., 1.7 μm) (Waters, Milford, MA, USA) was used. Solvent A consisted of a 0.1 % formic acid aqueous solution, while solvent B consisted of acetonitrile and isopropanol (1:1) with 0.1 % formic acid. The gradient elution program was set as follows: 0–3 min, 5 % B; 3–9 min, 20 % B; 9–13 min, 95 % B; 13–13.1 min, 95 % B; and 13.1–16 min, 5 % B. The injection volume was 10 μL. The electrospray ionization (ESI) source parameters included scanning from 70 to 1050 *m/z* in positive or negative ion mode. The ion spray voltage was set to 4000 V (ESI+) or − 3000 V (ESI-); the declustering voltage was 80 V; the ion source temperature was 400 °C; the curtain gas pressure was 30 psi; the sheath gas pressure was 40 psi; and the auxiliary gas pressure was 50 psi at collision energies of 20–40-60 V.

2.9. Quantitative analysis of metabolites

The raw LC–MS data were processed using Progenesis QI metabolomics data processing software. Multivariate data analysis, including orthogonal projections to latent structures discriminant analysis (OPLS-DA) and principal component analysis (PCA), was performed using SPSS software. Significant differences in metabolites between different preservation times were identified based on statistical thresholds such as *P* values and variable importance in projection (VIP) values.

2.10. Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The differentially regulated metabolites from the two groups were summarized using the KEGG database (https://www.kegg.jp/kegg/pathway.html), where significantly enriched pathways in which the metabolites were involved were compared with the background pathways and defined by the hypergeometric test and a threshold of *P*-value <0.05.

2.11. Data statistics and analysis

The experimental design included three biological replicates, and all measurements were conducted with three technical replicates (6 biological replicates of the metabolome). Statistical analysis was performed using SPSS version 27. Single-factor analysis of variance (ANOVA) was used to analyze significant differences between the experimental and control groups. Finally, Origin 2022 was used for data analysis and visualization.

3. Results and discussion

3.1. Weight loss rate and moisture content

The weight loss rate is a crucial indicator for evaluating the freshness of fruits and vegetables. The reduction in mass reflects the loss of water and nutrients to some extent. As shown in Fig. 1A, all the experimental groups experienced a certain amount of mass reduction. Compared to the weight loss rate of 6.29 % after 30 days of VP10 preservation, weight loss rate was significantly greater under RT preservation, reaching complete decay after 15 days. The weight loss rate VP4 samples was also greater than that VP10 samples, which indicated that lower temperatures are not suitable for the preservation of tender ginger.

After harvest, fruits and vegetables continue to undergo respiration during preservation. With continuous changes in respiration, the internal moisture content also undergoes constant changes. A decrease in moisture can lead to wilting and browning of fruits and vegetables, affecting the weight loss rate. As shown in Fig. 1B, with increasing preservation time, the moisture content of tender ginger under different preservation temperature conditions tended to decrease. During the first 5 days of preservation, there was no significant change in moisture content for all samples, but the moisture content of samples stored at RT sharply decreased from the 10th day preservation. This suggests that RT conditions exacerbate metabolic activity, leading to rapid water loss and structural degradation. During VP4 preservation, there was no significant change in the moisture content within 20 days preservation, and the moisture content significant decreased after 25 days preservation. This can be attributed to VP4 potentially causing cold-induced stress in tender ginger cells, disrupting membrane integrity and accelerating water loss in later stages. In contrast, at VP10, there was no significant change in moisture content throughout the preservation period. These findings highlight VP10 as the optimal preservation condition for tender ginger, balancing reduced respiratory activity and avoiding cold damage.
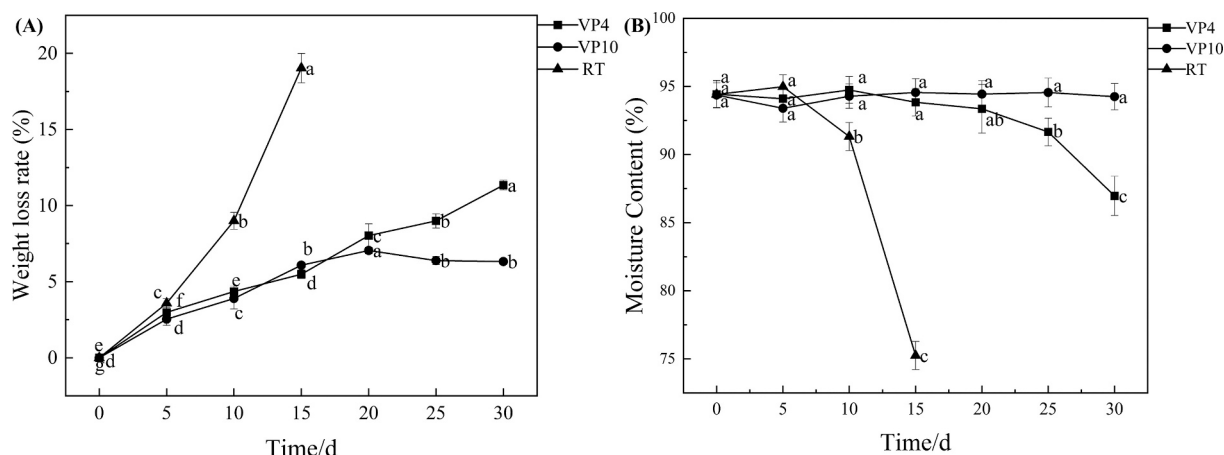
Fig. 1. Physical changes of tender ginger under vacuum packaging combined with 4 °C (VP4), 10 °C (VP10), and room temperature (RT) preservation. (A) Weight loss, (B) moisture content. Different letters indicate a significant difference among groups ($p < 0.05$).

3.2. Changes in the chemical composition of tender ginger during preservation

As shown in Fig. 2A and B, the changes in TPC and TFC exhibited an initial increase followed by a subsequent decrease. The TPC and TFC of VP4 samples reached their maximum values on the 10th and 15th day, respectively, and then began to decrease. VP10 samples reached the maximum values of TPC and TFC on the 15th and 20th day of preservation, respectively, and then began to decrease. The maximum values of TPC and TFC for VP10 samples were 1.01 times and 1.08 times higher,

respectively, than those for VP4 preservation. Phenolic compounds and flavonoid compounds typically possess potent antioxidant capabilities, mitigating the detrimental effects of free radicals on plants (Bagheri, Al Lawati, & Hassanzadeh, 2021). Studies also suggest that secondary metabolites such as phenols, phenolic acids, and flavonoids play a positive role in plant resistance to pathogenic microorganisms from the perspective of plant immunity (Asem, Imotomba, Mazumder, & Laishram, 2015).

Gingerol, which is a general term for spicy substances such as gingerols and shogaols in ginger is known for its anti-inflammatory,
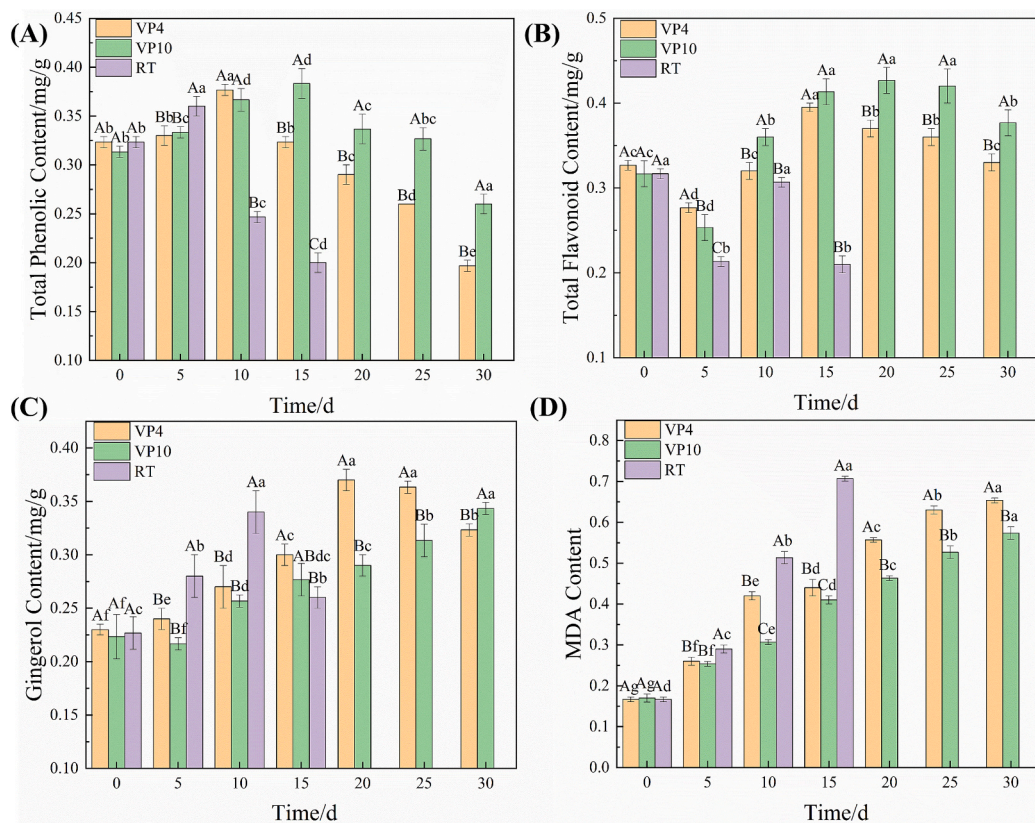

Fig. 2. Chemical changes of tender ginger under vacuum packaging combined with 4 °C (VP4), 10 °C (VP10), and room temperature (RT) preservation. (A) Changes in total phenolic content, (B) changes in total flavonoid content, (C) changes in gingerol content, (D) changes in malondialdehyde (MDA) content. Capital letters (A-C) represent differences between different preservation conditions at the same time, and small letters (a-g) represent differences between different times for the same preservation conditions. Different letters indicate a significant difference among groups ($p < 0.05$).

antioxidant, and antibacterial properties (Johnson, Mani, White, Brown, & Naiker, 2021). However, gingerols are prone to oxidation and hydrolysis, leading to a gradual reduction in their content. The results demonstrated that gingerol of RT preservation firstly increased within 10 days preservation and then decreased. During VP4 preservation, the gingerol content increased within 20 days preservation and then decreased (Fig. 2C). However, the gingerol content gradually increased through the whole preservation period, which indicated that the physiological metabolism of tender ginger is relatively stable during VP10 preservation, and the content was higher than that of the VP4 samples. The accumulation of gingerol might be due to the significant inhibition or stimulation of related enzyme activities in tender ginger during preservation (Ramirez-Ahumada, Timmermann, & Gang, 2006).

The MDA content is widely used as an indicator of the degree of membrane damage in plants (Morales & Munné-Bosch, 2019) and reflects the extent of damage to cell membranes during preservation. The results showed in Fig. 2D indicated that with increasing preservation time, the content of MDA in tender ginger stored at different temperatures significantly increased. However, the growth rate of MDA at VP10 was significantly lower than that at the other preservation temperatures (Fig. 2D). Elevated MDA levels indicate severe oxidative damage to cell membranes (Li, Han, Wang, Zhang, & Nie, 2022). MDA can directly participate in the regulation of defense genes in plants and provide cell protection under stress conditions (Morales & Munné-Bosch, 2019).

3.3. Changes in the enzyme activity of tender ginger during preservation

The infection of plants with pathogens produces reactive oxygen species (ROS), and increased levels of ROS affect a range of biochemical reactions in plants. However, enzymes can maintain proper ROS levels,

in turn affects plant disease resistance (Akbar et al., 2023). To study the effect of changes in antioxidant enzymes during preservation on tender ginger, POD, CAT, PPO, and PAL enzyme activities were measured to reveal the changes in enzyme activity during preservation (Fig. 3). POD is widely present in plant cells and catalyzes the oxidation of various important phenolic substances in the presence of deoxidases. It is also a crucial endogenous reactive oxygen species (ROS) scavenger that is closely related to plant disease resistance. Compared with RT samples, the growth rate of POD in VP4 and VP10 samples were lower and exhibited a later inflection point (Fig. 3A). During VP4 and VP10 preservation, the CAT content in tender ginger initially increased and then decreased (Fig. 3B), and the increase level in VP10 was significantly greater than that in VP4. CAT is a catalase that accelerates the detoxification of reactive oxygen species (ROS). It decomposes accumulated hydrogen peroxide into water and molecular oxygen, alleviating potential damage caused by substances such as hydrogen peroxide. Simultaneously, it enhances plant cell apoptosis to limit settlement by necrotic pathogens, reducing the harm caused by pathogens to plants (Blackman & Hardham, 2008). In the late preservation stage, POD and CAT activities of VP10 samples were significantly higher than that in VP4 samples, suggesting that VP10 could better maintain the enzyme activities to decompose hydrogen peroxide of tender ginger, thus effectively alleviating oxidative damage and prolonging the preservation quality. The lower POD and CAT activities in VP4 indicated that cold stress or metabolic inhibition induced by low temperature decreased the enzyme activities, which in turn weakened the detoxification ability of ROS.

Previous research has shown that reactive compounds produced in PPO-catalyzed reactions can inhibit microbial activity (Wang, Pian, Chen, & Zhang, 2021). Therefore, a significant increase in PPO activity
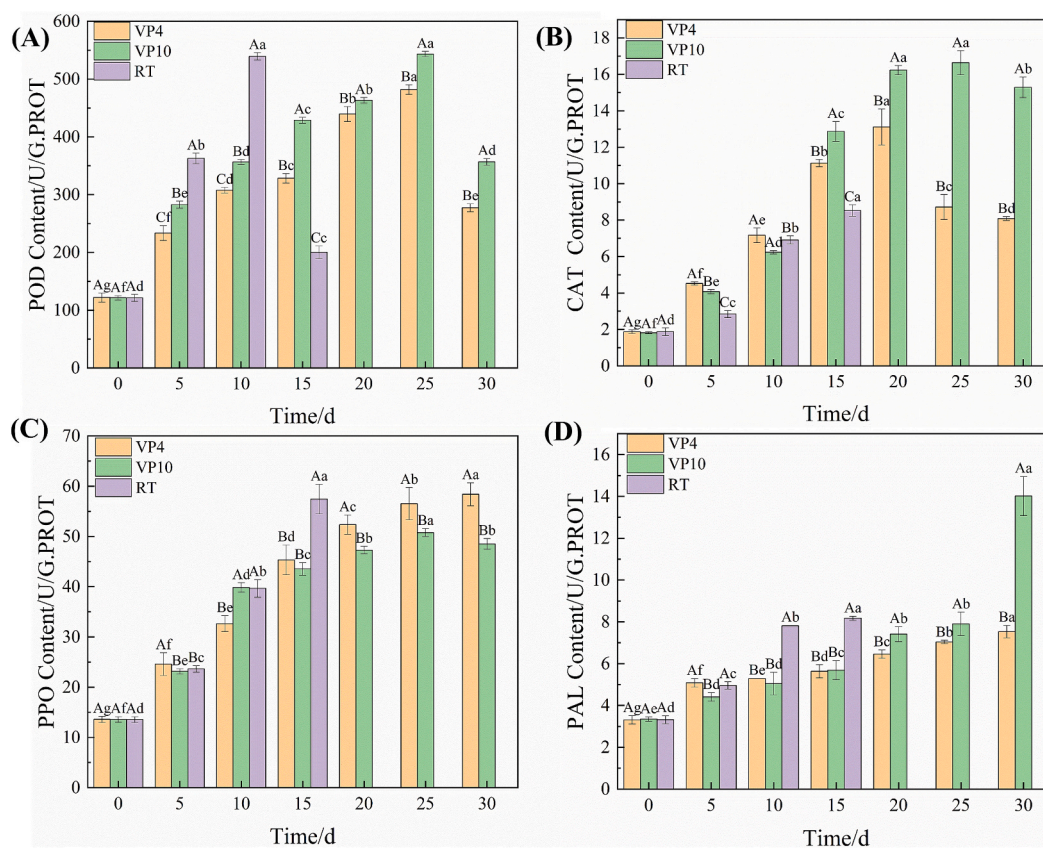

Fig. 3. Changes in the enzyme activities of tender ginger under vacuum packaging combined with 4 °C (VP4), 10 °C (VP10), and room temperature (RT) preservation. (A) Peroxidase (POD) activity, (B) catalase (CAT) activity, (C) polyphenol oxidase (PPO) activity, (D) phenylalanine ammonia-lyase (PAL) activity. Capital letters (A-C) represent differences between different preservation conditions at the same time, and small letters (a-g) represent differences between different times for the same preservation conditions. Different letters indicate a significant difference among groups ($p < 0.05$).

helps enhance the inherent resistance of plant tissue, slowing the damage incurred. However, substances produced by PPO-catalyzed oxidation can lead to browning of vegetables and fruits, thereby reducing product quality. Under various preservation conditions, the activity of PPO in tender ginger was significantly greater than that on Day 0. After 15 days preservation at VP10 and VP4, the PPO activity continued to increase, indicating the ongoing increase in the resistance of the tender ginger plants (Fig. 3C). At RT, the activity reaches its maximum after 15 days. However, subsequent measurements were not possible due to the decay of the tender ginger. In the later stages of preservation, the PPO content at VP10 was lower than that at VP4, suggesting that tender ginger at VP10 experiences less tissue disease and external stimuli than at VP4, while browning due to overactive PPO was reduced.

PAL is the rate-limiting enzyme for the metabolism of phenylalanine in plants. Its activity is positively correlated with disease resistance and is induced by infection from pathogens and their toxins (Akbar et al., 2023). High PAL activity is associated with phenylalanine synthesis, which affects plant growth, development, and tissue differentiation and is influenced by external stimuli (Bagal, Leebens-Mack, Lorenz, & Dean, 2012). Additionally, PAL activity affects the production of precursors of lignin, plant flavonoids, and flavonoid-like substances. In the present study (Fig. 3D), the PAL activity in all the groups gradually increased with increasing preservation time. From Day 25 to Day 30, the PAL content in VP10 samples increased from 7.9 to 14.02, which was 12.2 times higher than the change in VP4 samples. The above results indicated that VP10 preservation could effectively protect the tender ginger from postharvest decay.

3.4. Quantitative analysis of metabolites

To further compare and analyze the changes in ginger before and after preservation, a replicated untargeted metabolomics analysis was performed on each sample. Based on the analysis of the PCA score plot, all samples were located within the 95 % confidence interval, and the quality control (QC) samples also showed significant clustering on the PCA score plot (Fig. 4A), suggesting that the instrumental signals were very stable at different detection time points.

Metabolites differences in ginger were investigated under VP10 preservation via LC–MS combined with multivariate statistical methods. A total of 653 metabolites were detected throughout the preservation process (Fig. 4B), including carbohydrates and their derivatives (37 species), organic nitrogen compounds (11 species), amino acids and their derivatives (66 species), lipids and lipophilic compounds (180 types), alkaloids and their derivatives (8 species), nucleotides and their derivatives (21 species), organic acids and their derivatives (42 species), phenylpropanoids and polyketides (26 species), organic heterocyclic

compounds (58 species), benzenoids and their derivatives (30 species), flavonoids (20 species), phenols and acids (26 species), terpenoids (103 species), and others (25 species), as shown in Fig. 4B. Among them, lipids and lipophilic compounds are the largest category of metabolites (terpenoids are also lipids and lipophilic compounds), accounting for 43.3 % of the total metabolites (283 species).

3.5. Differentially abundant metabolite analysis using OPLS-DA

Normally, the PCA method cannot remove random errors that are irrelevant to the research purpose, but OPLS-DA can effectively filter out noise that is irrelevant to the classification information (Meng, Lei, et al., 2022). By analyzing the OPLS-DA score plots (Fig. 5A), a significant separation between ginger stored for 0 days and 30 days could be observed, suggesting that ginger metabolites change significantly with preservation time. In addition, the evaluation metrics of the model were obtained by an iterative replacement test: $R_2X = 0.542$, $R_2Y = 0.992$, and $Q_2 = 0.935$, which demonstrated the excellent predictive ability of the OPLS-DA model and further confirmed the feasibility of exploring metabolite changes before and after preservation (Meng et al., 2022).

3.6. Differentially abundant metabolite analysis during preservation

The variable importance in projection (VIP) values can be utilized to identify metabolites that exhibit differential expression via OPLS-DA. Statistically explicit thresholds based on p values <0.05 and VIP > 1 were utilized to identify significantly different metabolites between preservation periods. The volcano plot (Fig. 5C) (117 upregulated, 52 downregulated) illustrates the disparity in the number of different metabolites between samples before and after preservation (Table S1). The top 100 different metabolites of ginger before and after preservation were subjected to hierarchical clustering analysis (HCA), which revealed four major clusters (Fig. 6A). These metabolites significantly impact the quality of ginger during preservation. The variations in these metabolites play a crucial role in determining the quality changes in ginger during preservation.

3.6.1. Lipid and lipid compounds
Among all differentially abundant metabolites, lipids and lipid compounds were the most predominant, totaling 52 (Table S2). The content of lipids and lipid compounds generally showed an increasing trend from the pre-storage to post-storage periods. This might be attributed to the continuous apoptosis of tender ginger cells over time, leading to the gradual release of lipids and lipid molecules bound to proteins. Therefore, the upregulated quantity of lipids and lipid-like substances exceeded the downregulated quantity. PA (20:2(11 Z,14
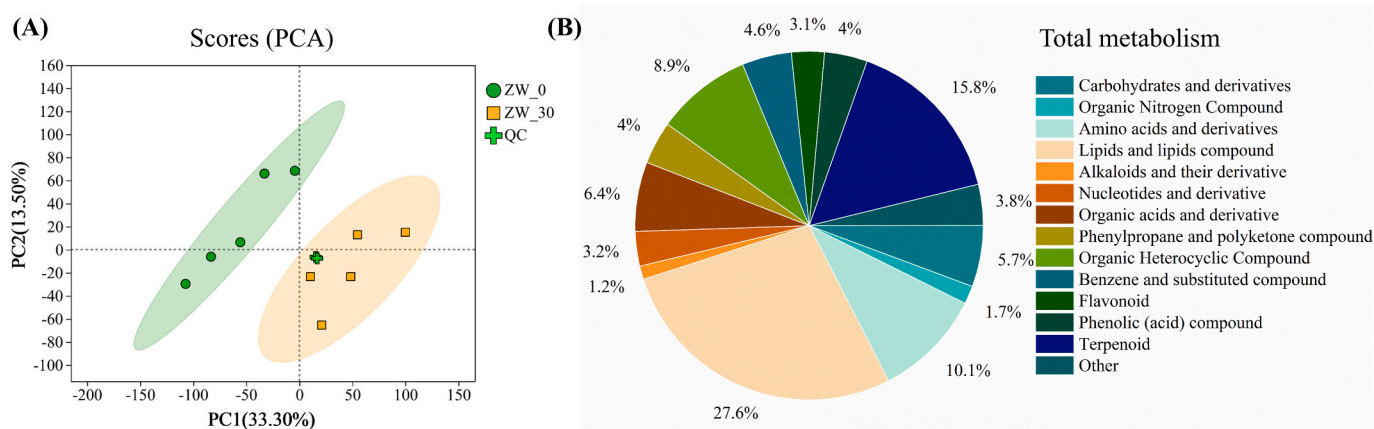

Fig. 4. Metabolism of tender ginger under 10 °C preservation. (A) Principal component analysis (PCA) for the detected metabolites, (B) classification of all the detected metabolites.
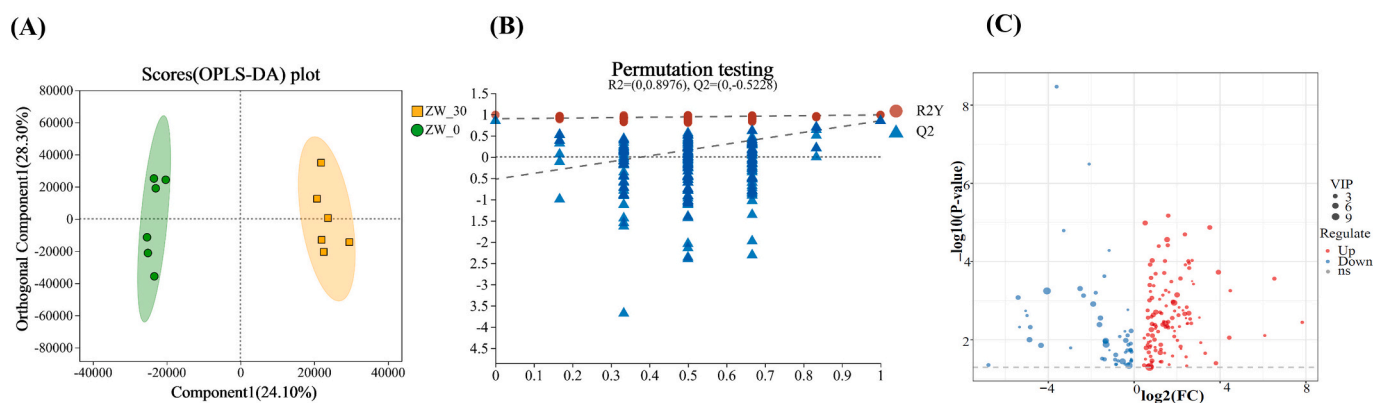
Fig. 5. (A) The score plots and model verification of OPLS-DA analysis, (B) volcano map, (C) the significantly differential regulated metabolites.
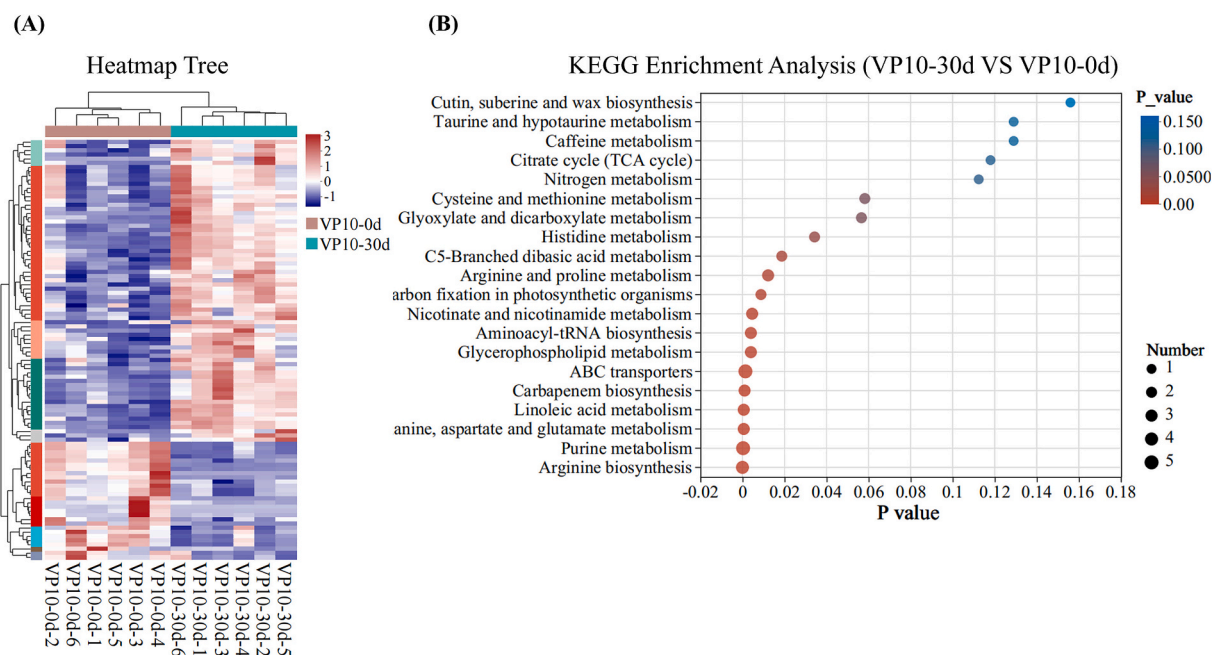

Fig. 6. (A) Heatmap analysis, (B) KEGG enrichment analysis of the significantly differential regulated metabolites.

Z)/18:2(9 Z,12 Z)), phosphatidic acid (PA), is a major component of cell membranes. LysoPE (18:2(9Z,12Z)/0:0) is a lyso-phosphatidylethanolamine (LPE), and LPE treatment can delay the aging of plant leaves and fruits, prolong the shelf life of products and alleviate the ethylene-induced process (Cowan, 2009). Moreover, LPEs enhance phenylalanine ammonia-lyase activity and can activate the plant immune system (D'Arrigo & Servi, 2010). Several lyso-phosphatidylcholines (LysoPC(18:2(9Z,12Z)), LysoPC(18:1(9Z)), and LysoPC(18:1(11Z))) all tended to be upregulated. LysoPC is a bioactive lyso-phospholipid that can mediate various cell responses, including plant growth and development and systemic responses after wounding (Sun et al., 2023). Previous study indicated that the connection between the response mechanisms of plants to internal and external stimuli is achieved through the conversion and metabolic changes of phospho-lipids, especially changes in lipid signaling components such as phospholipids, phosphatidic acid, lyso-phospholipids, and phospholipases, which are significantly influenced in this process (Cowan, 2009). In the late stage of preservation, a large amount of cell apoptosis may lead to an increase in most lipid metabolites in response to external stimuli and defense against pathogens.

3.6.2. Amino acids and their derivatives

During the preservation of tender ginger, due to enzymatic hydrolysis and microbial action, proteins in tender ginger cells are hydrolyzed into various amino acids and their derivatives. The production of these substances is closely related to the changes in appearance and flavor of tender ginger during preservation. Seventeen amino acid compounds were identified among the differentially abundant metabolites, 13 of which were positively correlated with each other (Table S3). L-Glutamate, phenylbutyrylglutamine, and 4-methylene-L-glutamine are glutamate derivatives, and they are upregulated throughout the preservation period. Glutamate is a unique primary metabolite in plant metabolism; it not only serves as a central hub connecting carbon and nitrogen metabolism but also regulates root development and defense responses. Glutamate is a component of plant signal transduction during stress (Liao, Chung, & Hsieh, 2022). Additionally, L-glutamate and L-aspartic acid are important umami substances. AK-toxin I was detected during preservation, and AK-toxin I is a pathogenic fungal toxin; its presence may indicate that the tender ginger became infected with some fungus during preservation, leading to the production of AK toxin. D-Pipecolic acid accumulates in plants starting from bacterial contamination, and this accumulation is associated with the acquisition of systemic resistance (Al-Rooqi et al., 2022). Additionally, D-Pipecolic acid plays a

crucial role in the growth, development, nutrition, signaling, and defense systems of certain plants, exhibiting antimicrobial activity and functions in toxin defense (Al-Rooqi et al., 2022). Moreover, D-pipecolic acid combined with vacuum packaging and 10 °C may inhibit microbial growth and reproduction, effectively reducing protein degradation.

3.6.3. Terpenoids

Similar to those of lipids and lipophilic compounds, the contents of terpenoid compounds mostly increase with increasing preservation time. Terpenoids exhibit significant antibacterial and antifungal effects. The heartwood of woody plants, which contains high levels of terpenoids, is known for its strong resistance to decay (Chen et al., 2020). In this study, 37 different terpenoid compounds (Table S4) were detected during the preservation period. Hydroxysintaxanthin 5,6-epoxide and 3-hydroxysintaxanthin, both of which are carotenoids and their derivatives, were positively correlated during preservation. Research has indicated that carotenoids have antioxidant activity, serving as antioxidants with inhibitory effects on reactive oxygen species (ROS). Carotenoids can quench lipid peroxides and react with free radicals in cell membranes, protecting them from oxidative damage. Citronellyl-β-sophoroside (8.23-fold increase) imparts a rose-like fragrance to tender ginger in the late preservation period, and also have antibacterial effects, demonstrating rapid bactericidal effects against *Escherichia coli* (Oka et al., 1998). Sesquiterpenoids in plants, such as deoxynivalenol, have a significant inhibitory effect on fungi. Additionally, sesquiterpene derivatives such as batatasin III play an antifungal role as fungicides (Toffolatti et al., 2021). Triterpenoids in plants from the Mahogany family exhibit broad-spectrum antifungal and antibacterial properties (Petrović, Stojković, & Soković, 2019).

3.6.4. Phenylpropanoids and quinones

Phenylpropanoids and quinones are commonly found in plants. These secondary metabolites share the common feature of a hydroxyl aromatic ring and play crucial roles in plant growth and development, response to environmental stress, enhancement of disease resistance, prevention of stress-induced damage to plants, action of signaling molecules, and contribution to the color and aroma of flowers and fruits (Parthasarathy, Borrego, Savka, Dobson, & Hudson, 2021). Throughout the entire preservation period, significant changes were observed in phenylpropanoids and quinones (Table S5), particularly in compounds such as 4,8,9-trihydroxy-17-methoxy-2-oxatricyclo[13.2.2.1,]icosa-1(17),3,5,7(20),15,18-hexaen-10-one and 6-[(E)-2-(2,4-dihydroxy-phenyl)ethenyl]-3-(3-methylbut-2-en-1-yl)benzene-1,2,4-triol. In the comparison between Day 30 and Day 0, the former increased by 2.78 times, and the latter increased by 2.82 times. The increase in content after the preservation period may be in response to external stresses, avoidance of pathogen growth and antioxidant responses, etc., which contribute to the protection of ginger itself from harmful effects.

3.6.5. Benzene and derivatives

In this study, a total of 56 benzene and its derivatives were detected, including 26 belonging to the phenol (acid) compound class. Among the differentially abundant metabolites, 17 were identified (12 upregulated, 5 downregulated) (Table S6). Research indicates that some biologically active compounds with antioxidant activity have beneficial effects on human health (Asem et al., 2015; Bagheri et al., 2021). In this study, the levels of most of the detected phenolic substances were increased. The levels of 2-(3-methylbut-2-en-1-yl) benzene-1,3,5-triol, 4-gingerol, and sinapyl alcohol were increased by 11.49-, 1.69-, and 1.54-fold, respectively, between the preservation periods. The content of Apocynin, a phenolic compound, in tender ginger increased by 1.7 times before and after preservation. Apocynin is an effective NADPH oxidase inhibitor and has been shown to possess anti-inflammatory properties (Ximenes, Kanegae, Rissato, & Galhiane, 2007). (10*S*,11*S*)-Pterosin C, classified as a flavonoid substance, increased by 3.42 times after 30 days of preservation compared to before preservation. Flavonoids and derivative

compounds play crucial roles in plant defense responses against pathogens and have functions such as antioxidant and anti-inflammatory properties (Akbar et al., 2023).

3.6.6. Others

Apart from the mentioned substances, changes also occurred in heterocyclic compounds. Compared to those on Day 0, nine heterocyclic compounds were significantly differentially expressed on Day 30, all of which were upregulated. Xanthine and theobromine are crucial intermediates in purine degradation metabolism. The abnormal concentrations of these compounds in the human body can serve as biochemical indicators for clinical diseases (Wei et al., 2023). Additionally, theobromine can interact with certain amino acids (L-glutamate and L-aspartic acid), influencing the flavor of tender ginger. 1-Carbapen-2-em-3-carboxylic acid is a naturally occurring carbapenem compound. Carbapenems vital as an antibiotic. However, most carbapenems are produced by bacteria; its increase may reflect significant microbial proliferation during tender ginger preservation (Huang, Yang, Leighton, & Li, 2023). Docosahexaenoyl ethanolamide (DHEA) is a bioactive lipid product derived from ω-3 fatty acids through oxidative and nonoxidative pathways. DHEA has been suggested to have anti-inflammatory effects (Ghanbari, Loron, & Sayyah, 2021). Carissanol, which is upregulated on Day 30, is known to exist in ginger as an antioxidant (Idris, Yasin, & Usman, 2019). Over the preservation period, significant changes in 18 heterocyclic compounds (Table S7), such as pyrrolidine and jasmine lactone, play a crucial role in influencing the flavor of tender ginger.

3.7. KEGG pathway enrichment

Metabolite pathway enrichment analysis is a valuable tool for elucidating the mechanisms of metabolic changes in different experimental samples. Based on the KEGG database, we analyzed the enrichment of metabolic pathways for different metabolites throughout the entire preservation process. Compared with those on Day 0, 37 metabolic pathways involving three modules were identified on Day 30: metabolism (35 pathways), genetic information processing (1 pathway), and environmental information processing (1 pathway). Fig. 6B shows the top 20 metabolic pathways. Through KEGG enrichment analysis, all metabolic pathways were screened to determine the final relevant pathways. Using *p* values and metabolite enrichment, five most relevant metabolic pathways were identified: arginine biosynthesis, purine metabolism, linoleic acid metabolism, arginine, aspartic acid, and glutamic acid metabolism, and carbapenem biosynthesis.

Arginine, during its synthesis, influences the generation of various substances, further affecting the metabolism of other substances, such as nitrogen metabolism, polyamines, and glutamic acid. Research has confirmed that arginine is a key factor influencing the inhibition of oxidative stress and the induction of endogenous antioxidation (Liang et al., 2018). Linoleic acid is a major component of cell membranes and maintains their fluidity and stability. In plants, linoleic acid is used to produce jasmonic acid (Carvalhais et al., 2013), a signaling molecule for plant defense and development. Additionally, linoleic acid and its metabolites have fungicidal properties and play a role in physiological processes such as cell signal transduction (Yoon, Ahn, Hwang, Kang, & Chung, 2021). Carbapenem biosynthesis can produce relevant antibiotics (1-carbapen-2-em-3-carboxylic acid), demonstrating efficient resistance against various bacterial infections. This process is crucial for ginger preservation, serving as an important antimicrobial compound with significant value in medical and biological research (Huang et al., 2023). During the preservation period, ginger's purine metabolism is closely related to the metabolism of arginine, aspartic acid, and glutamic acid, which may be contribute to the formation of ginger's flavor. Compounds involved in purine metabolism, such as xanthine, hypoxanthine, guanine, and guanosine, may synergistically influence the flavor of ginger in addition to amino acids such as glutamic acid (Xie et al., 2019). However, purine compounds not only are critical

components of nucleic acids and nucleotides but also act as regulators of metabolism and intermediates in metabolic pathways. For instance, purine metabolism produces purine alkaloids (caffeine), which contribute to its associated metabolism.

4. Conclusions

In this study, we investigated the changes in the physicochemical indicators and enzyme activity of ginger buds after vacuum packaging at different preservation temperature. The results of the study showed that VP10 preservation significantly reduced mass loss, delayed the rate of reduction in total phenols and total flavonoids, and effectively enhanced antioxidant enzyme activities. Metabolomic assessment revealed 169 different metabolites before and after VP10 preservation. The primary metabolites were lipids and lipid-like compounds, which are potentially associated with lipid release triggered by cell apoptosis. Among these metabolites, PA and LPEs, which play crucial roles in plant growth, development, and defense systems, were significantly upregulated. During the process of storing tender ginger, flavor-related substances such as glutamate, aspartate, and xanthine are significantly upregulated. In addition, compounds such as PA, alpha, gamma-onoceradienedione, hydroxysintaxanthin 5,6-epoxide, citronellyl beta-sophoroside, xanthine, and 4-methylene-ʟ-glutamine can serve as characteristic metabolites during the preservation of tender ginger. These metabolites are influenced by glycerol metabolism, amino acid metabolism, and purine metabolism. Metabolic pathway enrichment analysis indicated that pathways such as arginine biosynthesis, purine metabolism, linoleic acid metabolism, alanine, aspartate, and glutamate metabolism, and carbapenem biosynthesis were significantly affected. This study provides a new theoretical basis for understanding the changes in metabolites and metabolic pathways before and after the preservation of tender ginger, creating opportunities for extending the preservation strategy for tender ginger. However, the volatile flavor of tender ginger is also important, but this study did not focus on the changes in flavoromics. In addition, although vacuum packaging effectively extends the shelf life of tender ginger, the shelf life not sufficient for commercial applications. Therefore, in the future, it is necessary to explore the combination of vacuum packaging and other preservation technologies to extend the shelf life of tender ginger, such as vacuum packaging combined with biological antibacterial agents.

CRediT authorship contribution statement

Ying-Ying Jing: Writing – original draft, Methodology. **Fan-Bing Meng:** Writing – original draft, Formal analysis, Data curation. **Zhen-Yu Peng:** Supervision, Resources. **Qing-Zhou Li:** Visualization, Funding acquisition. **Ya-Ting Lei:** Validation, Project administration. **Yun-Cheng Li:** Writing – review & editing.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102168.

Data availability

No data was used for the research described in the article.

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