Characterization of browning during CO₂ deastringency treatment in astringent persimmon fruit

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Abstract

Deastringency treatment with CO₂ is an effective and convenient method for improving the marketability of persimmon fruit. However, the main persimmon cultivars in China turn brown very quickly following exposure to a high-CO₂ atmosphere, causing significant economic loss. However, the mechanisms of persimmon browning under CO₂ treatment remain largely unknown. In this study, we evaluated components and enzymes related to persimmon fruit browning. The results revealed that astringency was alleviated by simultaneous reduction of soluble tannin content and accumulation of insoluble tannin. During persimmon browning, fruit firmness, total phenolic content, and phenylalanine ammonia lyase (PAL) activity decreased significantly, whereas malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and polyphenol oxidase (PPO) activity increased significantly, suggesting that reactive oxygen species (ROS)-scavenging enzymes and non-enzymatic antioxidants cannot effectively maintain redox reaction balance to protect cell membranes from oxidative damage during CO₂ treatment. An untargeted metabolomics analysis identified 19 polyhydroxyphenols that were downregulated in CO₂-treated fruit, suggesting that phenolics may act as a substrate for persimmon browning. We also identified 11 metabolites associated with abiotic stress. Together, these results study provide valuable information on the mechanism of persimmon fruit browning induced by CO₂ treatment and will contribute to the ongoing development of the persimmon fruit industry.

Keywords Astringent persimmon · Browning · CO₂ deastringency · Enzyme activity · Metabolites

Introduction

Persimmon (*Diospyros kaki* Thunb.: Ebenaceae) is a deciduous plant that is well adapted to tropical, subtropical, and temperate areas. As a food source and an alternative to

the predominant fruit crops, persimmon is one of the most important fruit crops with high commercial value in Asian countries [1]. In pomology, persimmon cultivars are classified into four types: pollination-variant and astringent, pollination-variant and non-astringent, pollination-constant and astringent, pollination-constant and non-astringent [2]. Persimmon fruit are believed to have originated in China [3], and approximately 1,000 varieties of *D. kaki* have been found in China thus far. Interestingly, besides a few PCNA germplasms found in Luotian County, Hubei Province, the rest of the varieties in China are considered the PCA type [4]. In addition, astringent persimmon is the largest cultivated species of persimmon in the world because of its high yield, strong resistance, large fruit, and smooth flesh.

According to the Food and Agriculture Organization of the United Nations, the fruit production of persimmon in China was approximately 4,216,400 tons in 2018, accounting for 73.32% of total production worldwide. The majority of persimmon production is PCA cultivars, which

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are usually harvested for the fresh-fruit market when the fruit are still astringent [5]. It is necessary to remove the astringency of these fruit to make them palatable before consumption. Traditional methods to remove astringency at harvest involve over-ripening by treating the fruit with ethephon or ethylene, which is usually accompanied by a dramatic loss of fruit firmness. This handling limitation shortens the postharvest life of the fruit [6]. Thus, it is necessary and urgent to explore a novel and effective method to overcome the deficiencies of the traditional deastringency treatment in persimmon fruit.

Compared to traditional methods, CO₂ treatment is the most widely used deastringency technology due to its efficiency and convenience when preserving high levels of fruit firmness [7, 8]. The effectiveness of this treatment is based on its ability to insolubilize soluble tannins through the mediation of acetaldehyde generated during anaerobic respiration, which is triggered when fruit are exposed to a high-CO₂ atmosphere [9, 10]. However, our previous studies showed that most main persimmon cultivars in China, especially those with good flavor, such as ‘Mopan’, ‘Huo-jing’, ‘Zhongshi No. 1’, and ‘Gongcheng’, were susceptible and turned brown very easily after CO₂-deastringency treatment. The browning rate reached more than 50% when 100 astringent persimmon cultivars were treated under the optimal CO₂ deastringent conditions. Fruit browning is a physiological disorder that occurs in the fruit of many species such as apple, pear, pineapple, lychee, and persimmon [11–15]; it is characterized by darkened tissues near the fruit surface [13]. Browning significantly affects the economic value of the fruit and greatly limits the application of CO₂ deastringency technology in China.

Fruit and vegetable browning can be divided mainly into two categories: non-enzymatic and enzymatic browning. Non-enzymatic browning is caused by various non-enzymatic chemical reactions, such as auto-oxidation and polymerization of polyphenols that produce brown tissues. Enzymatic browning is caused by the oxidation and polymerization of phenolic compounds produced by the catalysis of enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) [16, 17]. Although the effects of fresh-cut processing, mechanical damage, and hot water treatment have been well studied in persimmon fruit [5, 15, 18], the mechanisms of browning during CO₂ deastringency treatment have not yet been reported.

In this study, we evaluated the browning-related components and enzymes of the cultivar ‘Zhongshi No. 1’, which was found to be extremely susceptible to browning in our previous study. The results will provide valuable information on the mechanism of browning during CO₂ deastringency treatment.

Materials and methods

Plant materials

The persimmon cultivar ‘Zhongshi No. 1’ was harvested at the commercial maturity stage on October 8, 2018, in Mengzhou County (34°51′38″N, 112°42′58″E), Henan Province, China. We randomly collected 40 fruits from six healthy 10-year-old persimmon trees, with three biological replicates per sample. After harvest, each replicate was transported to the laboratory, and the samples were divided into control (CK) and CO₂-deastringency treatment groups. Fruit in the control group were stored at 25 °C without treatment for 48 h. Fruit in the CO₂ treatment group were placed in sealed containers containing 99.9999% food-grade CO₂ at 25 °C and 90% relative humidity for 24 h, subjected to deastringency treatment, and then maintained at 25 °C for another 24 h. Both the control and treated fruit were then peeled after fruit firmness measurement, and the flesh was immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

Determination of firmness and ascorbic acid, total phenolic, total flavonoid, tannin, and malondialdehyde (MDA) content

Before the fruit were peeled, sample firmness was measured using a firmness tester (GY-4; Zhejiang Tuopu, Ningbo, China) with a 8 mm plunger; the results are expressed as pressure (N). Ascorbic acid content was determined using high-performance liquid chromatography (HPLC) [19, 20]. Total phenolic content was determined according to the Folin–Ciocalteu method of Li et al. [21]. Total flavonoid content was measured using the AlCl₃ (HAc–NaAc) method according to Fan [22]. Soluble and insoluble tannin content was measured using the Folin–Ciocalteu method, as described by Oshida et al. [23]. Malondialdehyde (MDA) content was measured using the thiobarbituric acid method, as described by Cao et al. [24].

Assay of enzymatic activities related to fruit browning

For PPO and POD extraction, 5 g of frozen tissue was homogenized with 10 mL of sodium acetate buffer (0.1 mol/L, pH 5.5) containing 1 mmol/L polyethylene glycol (PEG), 1% Triton X-100, and 4% (w/v) polyvinyl-pyrrolidone (PVPP). For superoxide dismutase (SOD) and catalase (CAT) extraction, 5 g of frozen tissue was homogenized in 10 mL of cold 50 mmol/L K-phosphate buffer (pH 7.8) containing 5% (w/v) PVPP and 5 mmol/L DL-dithiothreitol (DDT). For PAL extraction, 5 g of

frozen tissue was homogenized in 10 mL 0.1 mol/L cold boric acid-borax buffer (pH 8.8) containing 4% (w/v) polyvinyl pyrrolidone (PVP), 2 mmol/L ethylene diamine tetraacetic acid (EDTA), and 5 mM β -mercaptoethanol. All homogenates were centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatants were used for enzyme assays.

PPO activity was monitored as previously described [24, 25], with slight modifications. The reaction solution consisted of a mixture of 4.0 mL sodium acetate buffer (50 mmol/L, pH 5.5) and 1.0 mL 4-methyl-catechol (50 mmol/L), and was incubated at 25 °C for 5 min. Then, 0.1 mL of the extracted enzyme was added to the reaction system, and the absorbance at 420 nm was measured. The results are expressed as U/g, where 1 U was defined as the amount of enzyme activity required to cause an absorbance change per 1 g of sample under assay conditions.

POD activity was monitored according to Cao et al. [24], with a slight modification. The POD extract (0.5 mL) was mixed with 3 mL of 25 mmol/L guaiacol solution. Then, 0.2 mL H₂O₂ (0.5 mol/L) was added to the reaction system and the absorbance at 470 nm was measured. The results are expressed as U/g.

SOD activity was assayed according to Kumar et al. [26], with some modifications. The reaction mixture consisted of 1.2 mL sodium phosphate buffer (50 mmol/L, pH 7.8), 0.3 mL MET (0.13 mol/L), 0.3 mL EDTA (0.1 mmol/L), 0.3 mL nitroblue tetrazolium (NBT; 0.75 mmol/L), 0.3 mL riboflavin (0.02 mmol/L), and 0.5 mL of the SOD extract. After exposure to white light for 15 min at 30 °C, the absorbance of the reaction mixture at 560 nm was measured. The results are expressed as U/g.

CAT activity was determined as the decrease in absorbance at 240 nm for 1 min following the decomposition of H₂O₂, in accordance with Duan et al. [27], with some modifications. The reaction mixture consisted of 2.5 mL of 50 mmol/L phosphate buffer (pH 7.0) containing 15 mmol/L H₂O₂ and 0.5 mL of CAT extract. The results are expressed as U/g.

PAL activity was determined as described previously [24, 28]. Briefly, 3 mL of 0.1 mol/L boric acid-borax buffer (pH 8.8), 500 μ L of 20 mol/L L-phenylalanine, and 500 μ L of extracted enzyme were incubated at 37 °C for 60 min. The reaction was terminated by the addition of 0.1 mL of 6 mol/L HCl, and the absorbance of the reaction mixture at 290 nm was measured. The results are expressed as U/g.

Extraction parameter settings for untargeted metabolomic analysis

A portion (100 mg) of each collected sample was extracted with 120 μ L of precooled 50% methanol, vortexed for 1 min, and incubated at room temperature for 10 min. The

extraction mixture was then stored overnight at – 20 °C. After centrifugation at 4000 $\times$ g for 20 min, the supernatants were transferred into new 96-well plates. The samples were stored at – 80 °C prior to liquid chromatography–mass spectrometry (LC–MS) analysis. In addition, pooled quality control (QC) samples were prepared by combining 10 μ L of each extraction mixture.

A 4- μ L sample was injected into an ultra-performance liquid chromatography (UPLC) system (SCIEX, Macclesfield, UK) equipped with an ACQUITY UPLC T3 column (100 mm $\times$ 2.1 mm, 1.8 μ m; Waters, Milford, MA, USA) for the reversed-phase separation. The column oven was maintained at 35 °C. The flow rate was 0.4 mL/min, and the mobile phase consisted of solvent A (water, 0.1% formic acid) and solvent B (acetonitrile, 0.1% formic acid). Gradient elution conditions were set as follows: 0–0.5 min, 5% B; 0.5–7 min, 5–100% B; 7–8 min, 100% B; 8–8.1 min, 100–5% B; 8.1–10 min, 5% B.

A high-resolution tandem mass spectrometer TripleTOF-5600plus (SCIEX) was used to detect metabolites in both positive and negative ion modes. The QC sample was used to evaluate the stability of the LC–MS system throughout data acquisition.

Data preprocessing and pretreatment

LC-MS raw data files were converted into mzXML format after the pretreatments using XCMS software and then processed by the XCMS, CAMERA and metaX toolbox implemented with the R software [29–31]. Each ion was identified by combining retention time (RT) and *m/z* data. The online KEGG and HMDB databases were used to annotate the metabolites. An in-house fragment spectrum library of metabolites was used to validate the metabolite identification.

The pre-processed dataset was used to perform principal component analysis (PCA) for outlier detection and batch effects evaluation. Supervised PLS-DA was conducted to discriminate the different variables between groups through metaX, and the VIP value was calculated. Student's *t*-tests were conducted to detect differences in metabolite concentrations between two phenotypes, adjusting *P* for multiple tests according to the false discovery rate, as determined by the Benjamini–Hochberg test.

Statistical analyses

All experiments were performed using an absolutely randomized design and the results are expressed on a fresh weight basis. All data were analyzed using the SPSS v23.0 software (SPSS Inc., Chicago, IL, USA). Duncan's

multiple comparisons test was used to test for significant differences. Significance was evaluated at a level of $P < 0.05$.

Fig. 1 Phenotype of CO₂-treated persimmon fruit

Results

Physiological parameters related to browning caused by CO₂ deastringency treatment

The phenotype of CO₂-treated persimmon fruit is shown in Fig. 1. Visible browning on the fruit surface demonstrated that ‘Zhongshi No. 1’ was susceptible to browning due to CO₂ deastringency treatment. Fruit firmness was also affected by CO₂ treatment (Fig. 2A), with significant differences detected between the control (74.14 ± 5.88 N) and treated (11.77 ± 2.60 N) fruit.

Tannins are substrates in enzymatic browning reactions with many biological activities for fruit and vegetable quality, and they are closely associated with astringency and enzymatic activity [32–35]. Soluble tannin content decreased from 14.31 ± 0.06 to 1.19 ± 0.02 g/kg after deastringency treatment (Fig. 2B, C), perhaps because soluble tannins were accumulated and converted to insoluble tannins, followed by acetaldehyde generation within the fruit during CO₂ deastringency treatment [10, 36]. These results also demonstrate that the CO₂ deastringency treatment used in this study was effective, as the threshold soluble tannin content value for astringency is 2 g/kg [37]. The insoluble tannin contents of the control and treated samples were 1.64 ± 0.13 and 9.22 ± 0.22 g/

Fig. 2 Physiological parameters of control (CK) and CO₂-treated (TB) persimmon fruit. Data are mean $\pm$ standard deviation (SD). Different letters indicate significant differences between CK and TB ($P < 0.05$)

kg; this increase in insoluble tannins is likely attributable to the transformation of soluble to insoluble tannins.

Phenolics are substrates of enzymatic browning reactions; PAL is involved in the biosynthetic pathway of phenolic compounds and may accelerate browning [16, 38, 39]. In this study, the total phenolic content showed a downward trend after CO₂ treatment, from 13.19 ± 0.90 to 7.24 ± 0.047 g/kg (Fig. 2D), and PAL activity decreased significantly from 175.33 ± 4.03 U/g in the control group to 136.83 ± 5.06 U/g in the treatment group fruit (Fig. 2E).

In normal cell tissues, PPO is located in the organelles and polyphenol compounds are located in vacuoles; compartmentalization of the intracellular membrane plays an important role in the browning process. PPO activity was 3.94 ± 0.33 U/g in the control group and 22.22 ± 0.14 U/g in the treatment group (Fig. 2F). MDA is an indicator of cell membrane integrity [40]. In this study, MDA content increased from 3.08 ± 0.38 μ mol/kg in the control group to 6.09 ± 0.41 μ mol/kg in the treatment group (Fig. 2G), suggesting that browning caused by CO₂ deastringency treatment damaged cell membrane structure and intracellular membrane compartmentalization, leading to the reaction of phenolic substrates and PPO.

CO₂ deastringency treatment affects oxidative stress in fruit, and has been reported to rapidly increase fruit ROS levels [41]. Effective ROS destruction requires the participation of several ROS-scavenging enzymes, such as SOD, POD, and CAT [14], as well as non-enzymatic antioxidants such as ascorbic acid and flavonoids [41]. In this study, CO₂ deastringency treatment induced significant increases in persimmon fruit SOD (1.12 ± 0.024 to 2.91 ± 0.074 U/g), POD (0.49 ± 0.011 to 22.79 ± 0.91 U/g), and CAT (1.33 ± 0.05 to 53.00 ± 0.99 U/g) activity (Fig. 2H, I, J). Ascorbic acid

and flavonoid levels were significantly increased following CO₂ deastringency treatment, by approximately 1.93- and 1.28-fold, respectively (Fig. 2K, L).

Metabolite identification

To explore the changes in metabolites after browning due to CO₂ treatment, we performed untargeted metabolomic analysis in both positive (ESI⁺) and negative (ESI⁻) modes. PCA was performed to evaluate the repeatability and stability of the metabolic profiles obtained by UPLC–MS, and to detect differences among samples. Both positive and negative electron spray ionization (ESI) models clearly divided the samples into two groups, and grouped samples from the same treatment together, demonstrating the high quality of the data (Fig. 3). This result indicates that CO₂ deastringency treatment had a significant effect on metabolite variation.

Partial least-squares discriminant analysis (PLSDA) clearly distinguished between the control and treatment groups (Fig. 4). No significant outliers were found according to the PLSDA scores, suggesting that the samples were of high quality. The R²Y and Q²Y data supported the predictive accuracy of the model and reliability of the data.

We obtained 12,009 and 8,012 ion features using the positive and negative models, respectively; among these, 8271 and 4205 were annotated. The important metabolites were identified based on thresholds of the variable importance in projection score (VIP > 1.0), fold change (FC > 2.0 or FC < 0.5), and *P* < 0.05. A total of 689 and 1573 metabolites were upregulated and downregulated, respectively, in positive mode, and 588 and 1,182 metabolites were upregulated and downregulated, respectively, in

Fig. 3 Principal component analysis (PCA) of control (CK) and CO₂-treated (TB) persimmon fruit in **A** positive and **B** negative ion modes

Fig. 4 Partial least-squares discriminant analysis (PLSDA) score plots of control (CK) and CO₂-treated (TB) persimmon fruit in **A** positive and **B** negative ion modes

Table 1 Statistical results of metabolite annotation analysis

Mode	All features	High-quality features	All annotated	Up	Down
Pos	12,009	10,434	8271	689	1573
Neg	8012	7369	4205	588	1182

Mode indicates that the mode of MS analysis is mainly divided into a positive ion modes and a negative ion mode; all features indicates the number of substances extracted by XCMS software (3.2.0 version, UC, Berkeley, CA, USA); high-quality metabolites indicates CV ≤ 30% of metabolites intensity in all QC samples, which were used for further analysis. all annotated indicates the amount of metabolites annotated by level-one and level-two MS data. Up and down indicates that compared to control (CK), the number of up-regulation and down-regulation for CO₂-treated (TB) sample

negative mode (Table 1). Moreover, 30 metabolites were found to be associated with abiotic stress and phenolics (Table 2). Browning is caused by the enzymatic oxidation of phenolic compounds by PPO to *o*-quinones, which are very reactive, forming brown polymers [12]. The levels of 19 polyhydroxyphenols including manniflavanone, quercitrin, procyanidin_B4, proanthocyanidin_A2, and sciadopitysin, are listed in Table 2. Polyhydroxyphenols clearly decreased following treatment; these results were consistent with those for total phenolics (Fig. 2D); enzymatic reaction of phenolic substances produce substrates with PPO, which were significantly more abundant in the treatment group. We also identified 11 compounds associated with abiotic stress: valine, tyrosine, lysine, succinic acid, phenylalanine, trehalose, allose, D-glucose, mannitol, D-sorbitol, and spermidine.

Discussion

When persimmon fruit were exposed to an atmosphere with high CO₂ and low O₂ concentrations, the local anoxic conditions caused oxidative stress and changes in normal cellular metabolism, impairing fruit quality and leading to browning [6, 41, 42]. Browning induced by CO₂ deastringency treatment of ‘Zhongshi No. 1’ fruit is a physiological disorder of the internal flesh that is visible at the fruit surface, significantly impacting the economic value of the fruit.

SOD, POD, and CAT are ROS-scavenging enzymes in plants. SOD constitutes the first enzymatic line of defense against ROS, catalyzing the dismutation of O₂⁻ to non-toxic O₂ and less toxic H₂O₂, whereas POD and CAT remove H₂O₂. Ascorbic acid and flavonoids are potent and versatile antioxidants in plants, effectively quenching free radicals and protecting cell membranes and intracellular proteins from oxidative damage [43, 44]. In this study, persimmon fruit SOD, POD, and CAT activity rapidly increased following CO₂ treatment. CO₂ deastringency treatment increased SOD activity by 2.60-fold compared with the control; a similar study reported higher SOD activity in the ‘Rojo Brillante’ persimmon cultivar following CO₂ deastringency treatment [42]. Under oxidative stress conditions, the contents of ascorbic acid and flavonoids, which function as antioxidants in persimmon fruit, increase to maintain the redox reaction balance within the fruit.

MDA is the main product of membrane lipid peroxidation; its accumulation level can be used as an indicator of tissue cell damage and physiological aging [40]. In this study, the MDA content of browning fruit increased following treatment with high concentrations of CO₂, enhancing

Table 2 Metabolites showing significantly different content levels between CO₂-treated (TB) and control (CK) persimmon fruit

Metabolites	Fold change	P value	VIP	RT	M/Z	KEGG ID	Up/Down
Valine	2.14	1.67E-03	1.09	1.01	118.09	C00183	Up
Tyrosine	3.11	1.57E-08	1.48	1.114	182.08	C00082	Up
Lysine	4.61	9.46E-07	1.75	0.771	147.11	C00047	Up
Succinic acid	3.44	1.53E-06	1.5	1.122	117.02	C00042	Up
Phenylalanine	2.26	0.001047	1.21	2.432	166.09	C00079	Up
Trehalose	4.23	2.87E-08	1.62	0.899	387.11	C01083	Up
Allose	2.33	3.73E-06	1.2	0.863	225.06	C01487	Up
D-Glucose	3.21	1.77E-03	1.4	2.0891	203.05	C00031	Up
Mannitol	0.14	3.75E-05	1.72	0.919	181.07	C00392	Down
D-sorbitol	0.05	3.49E-08	2.26	0.972	183.09	C00794	Down
Spermidine	0.42	2.22E-02	1.17	8.387	146.16	C00315	Down
Manniflavanone	0.01	7.80E-07	2.89	0.94	613.10	C09763	Down
Cyanidin-3-O-(6"-O-malonyl-2"-O-glucuronyl)glucoside	0.02	6.24E-06	2.74	1.95	729.17	C16373	Down
Delphinidin 3-glucoside 5-caffoyl-glucoside	0.04	1.01E-03	2.40	1.16	788.18	C16313	Down
Cyanidin 3-O-(6-O-malonyl-beta-D-glucoside)	0.12	9.03E-05	1.98	2.48	553.14	C12643	Down
Delphinidin 3-O-(6"-O-malonyl)-beta-glucoside-3'-O-beta-glucoside	0.15	2.06E-06	1.94	1.11	714.17	C16304	Down
Sciadopitysin	0.14	3.84E-05	1.88	0.92	579.13	C10239	Down
Cinnamtannin_A1	0.13	1.36E-04	1.87	2.55	889.19	C17624	Down
Delphinidin-3-(p-coumaroyl)-rutinoside-5-glucoside	0.19	5.73E-06	1.80	1.11	942.24	C16294	Down
Asebogenin	0.14	2.45E-07	1.74	0.91	287.09	C09471	Down
Procyanidin_B4	0.19	7.85E-06	1.65	0.92	549.12	C10238	Down
ent-Fisetinidol-(4beta->8)-catechin-(6->4beta)-ent-fisetinidol	0.24	5.81E-04	1.63	1.05	873.18	C10225	Down
Proanthocyanidin_A2	0.25	1.26E-04	1.59	2.49	577.14	C10237	Down
Cyanidin 3-O-(6-O-p-coumaroyl)glucoside	0.31	3.70E-04	1.47	2.47	596.15	C12095	Down
Quercitrin	0.33	3.67E-03	1.43	2.02	471.09	C01750	Down
Robinin	0.33	4.61E-04	1.39	2.47	763.20	C10178	Down
Cyanidin 3,5,3'-tri-O-glucoside	0.42	1.66E-03	1.27	1.56	791.24	C08629	Down
Gallocatechin-(4alpha->8)-epigallocatechin	0.44	7.31E-04	1.17	1.82	611.14	C10227	Down
Delphinidin_3-O-sophoroside	0.45	1.44E-02	1.06	1.02	666.12	C16307	Down
6-Prenylnaringenin	0.40	4.27E-06	1.02	2.61	339.12	C09832	Down

VIP, variable importance for the projection; RT, retention time; KEGG (Kyoto Encyclopedia of Genes and Genomes)

membrane lipid peroxidation. PPO is found in most higher plants and plays a central role in fruit browning [45]; the levels of total phenolics and 19 phenolic compounds decreased significantly as those of PPO activity significantly increased in treated fruit, suggesting that browning damages cell membrane structure and of intracellular membrane compartmentalization, leading to the reaction of phenolic substrates and PPO. These results suggest that ROS-scavenging enzymes and non-enzymatic antioxidants in 'Zhongshi No. 1' fruit could not effectively maintain the redox reaction balance to protect cell membranes and intracellular proteins from oxidative damage during CO₂ deastringency treatment.

Plant metabolomics examines the role of metabolite change in various tissues [46]. As metabolites are the final products of cell biological regulation process, metabolite changes and their levels are regarded as the ultimate response of plants to environmental stresses [47]. Phenolics

act as substrates of enzymatic browning reactions; in this study, the levels of total phenolics and 19 phenolic compounds decreased significantly in the treated samples, suggesting that phenolics similarly acted as a substrate for browning in 'Zhongshi No. 1' persimmon fruit.

Many amino acids also play significant roles in plant growth and various stress responses, including as regulatory and signaling molecules [48, 49]. Several studies have demonstrated that amino acids accumulate to high levels under abiotic stress [50–53]. In this study, the levels of valine, tyrosine, lysine, succinic acid, and phenylalanine were increased under high concentration CO₂; a possible reason for this is that high-concentration CO₂ could induce the synthesis of amino acids and the degradation of proteins in persimmon fruit. In addition, the results also indicated that soluble sugars such as trehalose, allose, and D-glucose, which accumulate under stress responses caused by abiotic

stress, may also contribute to sustaining osmotic pressure and protecting the plasma membrane and proteins, as previously reported [53–55].

Polyamines belonging to the aliphatic series can also protect plant cell membranes and reduce oxidative damage of plant cells [56]. Sugar alcohols have been reported to sustain the stability of macromolecules, scavenge ROS in cells, and prevent peroxidation of fatty acids in cell membranes [57]. In this study, the levels of spermidine, mannitol, and D-sorbitol were decreased in CO₂-treated ‘Zhongshi No. 1’ fruit, which may have led to browning.

Conclusions

High CO₂ concentrations induced oxidative stress, and damaged cell membrane structure and intracellular membrane compartmentalization, leading to reactions among phenolic substrates and PPO. ROS scavenging enzymes (SOD, POD, and CAT) and non-enzymatic antioxidants (ascorbic acid and flavonoids) were unable to effectively maintain redox reaction balance and protect cell membranes from oxidative damage during CO₂ treatment. We identified 19 polyhydroxyphenols that showed decreased content in treated fruit, suggesting that phenolics act as a substrate in the enzymatic browning reaction of ‘Zhongshi No. 1’ persimmon fruit. We also identified 11 metabolites associated with abiotic stress. The findings of this study provide valuable information on the mechanism of browning under CO₂ deastringency treatment, and will contribute to the ongoing development of the persimmon fruit industry.

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Declarations

Conflict of interest There are no competing interests in this paper, and the authors do not have any possible conflicts of interest.

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