

Postharvest Redox Potential in Relation to Ethylene Synthesis and Related Enzyme Activities in Persimmon Fruit: Postprint

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Abstract

Using the characteristic Guangxi persimmon cultivar (*Diospyros kaki* Thunb.) ‘Gongcheng Yueshi’ as experimental material, changes in oxidation-reduction potential (ORP), ethylene biosynthesis, firmness, color difference, antioxidant enzyme activities, and cell wall-degrading enzyme activities were measured and analyzed during postharvest storage to preliminarily investigate the relationship between persimmon fruit ORP and ethylene biosynthesis as well as related enzyme activities. The results showed that fruit firmness exhibited an overall decreasing trend during storage, with ethephon-treated fruit showing a rapid decline from 3 days postharvest, remaining significantly lower than the control until the end of storage. Color development was relatively slow during the first 14 days of storage; at 15 days postharvest, the total color difference ΔE value of ethephon-treated fruit rapidly increased to 29.6, indicating complete color development, which was significantly higher than the control value of 11.9. During the early storage period, ORP in both treated and control fruits remained stable at $7.5 \text{ mV} \cdot \text{g}^{-1}$; at 15 days postharvest, ORP in ethephon-treated fruit rapidly increased to $11.9 \text{ mV} \cdot \text{g}^{-1}$, representing 1.4 times that of the control. Ethylene biosynthesis in ethephon-treated fruit remained at a low level during the early storage period; at 15 days postharvest, ethylene production sharply increased to a peak of $0.372 \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, while the control maintained a low level of $0.033 \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. These results demonstrate that increased ethylene production is closely related to ORP elevation. Furthermore, the activities of peroxidase (POD) among antioxidant enzymes and -D-galactosidase (-D-Gal) among cell wall-degrading enzymes showed extremely significant positive correlations with ORP, indicating that both are influenced by changes in fruit ORP. Therefore, ORP in ethephon-treated persimmon fruit increased significantly at the end of storage, promoting a sharp increase in ethylene biosynthesis and inducing enhanced activities of the antioxidant enzyme POD and cell wall-degrading enzyme -D-Gal, leading to rapid post-ripening softening of the fruit.

Persimmon fruit ORP may function as a switch regulating the biochemical reaction of ethylene generation from 1-aminocyclopropane-1-carboxylic acid (ACC), i.e., disrupting the stable ORP state triggers the initiation of ethylene synthesis. This study provides a theoretical basis for postharvest storage and preservation techniques by investigating the relationship between oxidation-reduction potential and persimmon fruit ripening and softening.

Full Text

Preamble

Relationship Between Postharvest Oxidation-Reduction Potential and Ethylene Biosynthesis and Related Enzyme Activities in Persimmon Fruit

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Abstract

This study investigated the relationship between oxidation-reduction potential (ORP) and ethylene biosynthesis and related enzyme activities in ‘Gongcheng Yueshi’ persimmon (*Diospyros kaki* Thunb.), a characteristic variety from Guangxi, China. Changes in ORP, ethylene production, fruit firmness, color difference, and activities of antioxidant and cell wall-degrading enzymes were measured during postharvest storage. The results showed that fruit firmness decreased continuously throughout storage, with ethephon-treated fruit showing a rapid decline from day 3 postharvest, remaining significantly lower than the control until the end of storage. Color development was slow during the first 14 days, but on day 15, the total color difference (ΔE) in ethephon-treated fruit increased rapidly to 29.6, indicating complete color change, which was significantly higher than the control value of 11.9.

During the initial storage period, ORP remained stable at $7.5 \text{ mV} \cdot \text{g}^{-1}$ in both treated and control fruit. However, on day 15 postharvest, ORP in ethephon-treated fruit increased sharply to $11.9 \text{ mV} \cdot \text{g}^{-1}$, 1.4 times that of the control. Ethylene biosynthesis in ethephon-treated fruit remained low during early storage, but increased dramatically on day 15 to a peak of $0.372 \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, while the control maintained a low level of $0.033 \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. This demonstrates a close relationship between increased ethylene production and rising ORP. Additionally, peroxidase (POD) activity among antioxidant enzymes and -D-galactosidase (-D-Gal) activity among cell wall-degrading enzymes showed extremely significant positive correlations with ORP, indicating that both enzyme activities were influenced by changes in fruit ORP.

Therefore, ethephon treatment significantly increased persimmon ORP at the end of storage, promoted a sharp increase in ethylene biosynthesis, and en-

hanced POD and -D-Gal activities, leading to rapid postharvest softening. Persimmon fruit ORP may act as a switch regulating the biochemical reaction of 1-aminocyclopropane-1-carboxylic acid (ACC) conversion to ethylene, where breaking the stable ORP state triggers ethylene synthesis initiation. This study explores the relationship between oxidation-reduction potential and persimmon fruit ripening and softening, providing a theoretical basis for postharvest storage and preservation technologies.

Keywords: persimmon; oxidation-reduction potential; ethylene; enzyme activity; softening; preservation

Introduction

‘Gongcheng Yueshi’ is a distinctive astringent persimmon variety from Guangxi, China, characterized by the contradictory qualities of astringency versus sweetness and crispness versus softness. Physical and chemical damage from postharvest de-astringency treatments accelerates fruit ripening and softening, thereby affecting quality and shelf life. Rapid postharvest ripening and softening severely restrict the development of Guangxi’s persimmon industry. Persimmon is a climacteric fruit that is extremely sensitive to ethylene, as even trace amounts in the storage environment can induce endogenous ethylene production and subsequent postharvest softening. Some climacteric fruits do not ripen easily on the tree but rapidly enter the respiratory climacteric phase once detached.

Sun et al. (2009, 2010, 2013) found that ethylene synthesis initiation in persimmon fruit on the tree is controlled by both the “photosynthate flow factor” from phloem and the “water factor” from xylem, and that disrupting either pathway induces massive endogenous ethylene production. Postharvest studies have confirmed that water stress induces ethylene synthesis initiation in the fruit calyx, which subsequently triggers autocatalytic ethylene production in persimmon fruit. However, research has shown that exogenous antioxidants (vitamin C) can significantly inhibit the accumulation of ethylene synthesis precursors (ACC) and the increase in key enzyme (ACS, ACO) activities in postharvest persimmon and kiwifruit, thereby reducing endogenous ethylene production. Exogenous antioxidant application affects the organism’s oxidation-reduction potential (ORP) and related antioxidant indicators.

ORP characterizes the relative strength of oxidation-reduction status in organisms. Excessively high or low ORP levels can trigger stress responses and accelerate senescence. Current ORP research primarily focuses on environmental monitoring in water bodies, soil, and fermentation industries, while the relationship between postharvest ORP changes and fruit ripening-softening remains unexplored. This experiment investigated the relationship between ORP, ethylene biosynthesis, antioxidant enzymes, and cell wall-degrading enzymes during persimmon postharvest storage to elucidate the role of ORP in postharvest ripening and softening, providing a theoretical foundation for fruit preservation

technologies.

Materials and Methods

1.1 Materials and Treatments

The experimental material was ‘Gongcheng Yueshi’ persimmon. Fruit were harvested on October 29, 2016, in Gongcheng County, Guilin City, Guangxi, and transported to the laboratory of the Horticulture Department at Guangxi University on the same day. Uniform fruit (180–200 g) with consistent color and maturity, free from pests, diseases, and mechanical damage, were selected and stored at 25 ± 1 °C and 60–70% relative humidity for the experiment from October 29 to November 13, 2016.

Two treatments were established, each with 350 fruit. For the ethephon treatment, fruit were immersed in $1000 \text{ mg} \cdot \text{L}^{-1}$ ethephon solution for 5 min, air-dried in a ventilated shade, sealed in polyethylene bags (80 cm \times 80 cm) for 24 h, and then placed in cold storage. Deionized water treatment served as the control. Fruit parameters were measured every 3 days, and equatorial flesh tissue was sampled, snap-frozen in liquid nitrogen, and stored at -80 °C for further analysis.

1.2.1 Determination of Firmness and Total Color Difference

Firmness was determined following Wang et al. (2010). Twelve fruit per treatment were randomly selected. After removing small pieces of peel from opposite sides of the equatorial region, a texture analyzer TA.XT.plus (Stable Micro Systems, UK) equipped with a C002ST probe (5 mm diameter cylinder) was used to puncture the fruit to a depth of 10 mm at a speed of $1.5 \text{ mm} \cdot \text{s}^{-1}$. Each measurement included three biological replicates per treatment, with four fruit per replicate.

Total color difference was measured according to Fan et al. (2017). Nine fruit per treatment were randomly selected, and the equatorial region was measured once using a GR-10 colorimeter (KONICA MINOLTA, Japan). Each measurement included three biological replicates per treatment, with three fruit per replicate.

1.2.2 Determination of Oxidation-Reduction Potential

ORP was determined following Chen et al. (2005) with modifications. Twelve fruit per treatment were randomly selected. From each fruit, 10 g of equatorial flesh was sliced ($\text{Ø} = 12 \text{ mm}$, $t = 5 \text{ mm}$) and placed in 150 mL of phosphate buffer ($0.1 \text{ mol} \cdot \text{L}^{-1}$, pH 7.0) for 30 min. After filtration, ORP was measured for 2 min using a metal platinum electrode 600s-ORP (JENCO, USA) calibrated with quinhydrone saturated solution. Each measurement included three biological replicates per treatment, with four fruit per replicate.

1.2.3 Determination of Ethylene Biosynthesis

Five fruit per treatment were randomly placed in a 4 L sealed container at 25 °C for 6 h. Five gas samples (5 mL each) were collected from the headspace and analyzed using a gas chromatograph GC-17A (Shimadzu, Japan). GC conditions: column—stainless steel packed column with Porapak Q stationary phase (2 m × 3 mm od); detector—flame ionization detector (FID); gases—carrier N₂ (flow rate 50 mL · min⁻¹), fuel H₂ (30 mL · min⁻¹), and air (300 mL · min⁻¹); temperatures—detector 200 °C, injector 110 °C, column 90 °C; manual injection using a microsyringe (Hamilton, USA) with 1000 µL injection volume.

1.2.4 Enzyme Extraction and Activity Assay

For superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), enzyme extraction followed Aebi (1984) and Chance & Maehly (1995) with modifications. Flesh tissue (3.0 g) was ground in liquid nitrogen and mixed with 5.0 mL extraction buffer (0.1 mol · L⁻¹ phosphate buffer, pH 7.8; 5 mmol · L⁻¹ DTT; 5% PVP), then centrifuged at 4 °C for 30 min. The supernatant was used for enzyme activity assays. Enzyme activities were determined colorimetrically: SOD activity followed Li et al. (2016), while CAT and POD activities followed Khademi et al. (2014).

For polygalacturonase (PG), cellulase (Cx), and -D-galactosidase (-D-Gal), enzyme extraction followed Andrews & Li (1995) and Zhou et al. (1999) with modifications. Flesh tissue (3.0 g) was ground in liquid nitrogen, mixed with 6.0 mL of 0.2% sodium sulfate solution, and centrifuged at 4 °C for 15 min. After discarding the supernatant, 6 mL of 100 mmol · L⁻¹ sodium acetate buffer (containing 100 mmol · L⁻¹ NaAc, 1% (V/V) β-mercaptoethanol, and 1.5% PVP (K-30), pH 5.2) was added, mixed in an ice bath, and centrifuged at 4 °C for 30 min. The supernatant was used for enzyme activity assays. PG activity was determined following Jiang et al. (2010), Cx activity by the 3,5-dinitrosalicylic acid method (Opigo & Ying, 2010), and -D-Gal activity following Itamura et al. (2013). All physiological measurements included three biological replicates per treatment, with four fruit per replicate.

1.3 Data Analysis

Data were plotted and analyzed using Excel 2010 (Microsoft, USA) and SPSS 17.0 (IBM SPSS, China). Significant differences were determined using the least significant difference (LSD) test at $P < 0.05$.

Results

2.1 Changes in Firmness and Total Color Difference During Storage

Postharvest fruit firmness and color changes directly reflect ripening status. Figure 1 [Figure 1: see original paper]A shows that fruit firmness decreased continuously during storage. Ethephon-treated fruit firmness declined rapidly from day

3 postharvest, decreasing from an initial 13.2 N to 9.96 N, and remained significantly lower than the control throughout storage, while control fruit softened slowly. Figure 1B shows that total color difference (ΔE) increased continuously during storage. In ethephon-treated fruit, ΔE changed minimally during the first 14 days, with slow color development. On day 15 postharvest, ΔE increased rapidly to 29.6, indicating complete color change, which was significantly higher than the control (11.9). These results demonstrate that ethephon treatment accelerated fruit softening and color development, promoting postharvest ripening.

2.2 Changes in Oxidation-Reduction Potential and Ethylene Biosynthesis During Storage

ORP characterizes fruit oxidation-reduction status. Figure 2 [Figure 2: see original paper]A shows that ORP remained stable at $7.6 \text{ mV} \cdot \text{g}^{-1}$ during the first 14 days of storage. On day 15 postharvest, ORP in ethephon-treated fruit increased rapidly to $11.9 \text{ mV} \cdot \text{g}^{-1}$, significantly higher than the control value of $8.7 \text{ mV} \cdot \text{g}^{-1}$. Ethylene, one of the five major plant hormones, accelerates fruit ripening. Figure 2B shows that ethylene biosynthesis in ethephon-treated fruit remained higher than the control throughout storage. On day 15 postharvest, ethylene production increased sharply to a peak of $0.372 \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, while the control maintained a low level of $0.033 \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. ORP showed an extremely significant positive correlation with ethylene biosynthesis during storage (Table 1), indicating a close relationship between increased ethylene production and rising ORP.

2.3 Changes in Antioxidant Enzymes During Storage

SOD, CAT, and POD are important enzyme systems in reactive oxygen metabolism that scavenge reactive oxygen species (ROS) and delay fruit ripening and senescence. Figure 3 [Figure 3: see original paper] shows that antioxidant enzyme activities in ethephon-treated fruit were generally higher than in the control. SOD activity decreased continuously during storage. On day 15 postharvest, SOD activity in ethephon-treated fruit decreased from an initial $5.25 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ to $4.6 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, higher than the control value of $3.56 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$. Figures 3B and 3C show that CAT activity in ethephon-treated fruit changed minimally during the first 9 days, but exhibited a sharp rise-decline trend from days 9–15, peaking at $347.47 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ on day 14, while control CAT activity remained stable at $7.9 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$. Notably, POD activity remained below $0.035 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ with no significant changes during the first 14 days, but increased sharply to $0.504 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ on day 15 in ethephon-treated fruit, significantly higher than the control ($0.005 \text{ U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$). During storage, SOD and CAT activities showed no significant correlation with ORP, ethylene production, firmness, or total color difference, whereas POD activity showed extremely significant positive correlations with ORP, ethylene production, and color difference, and a significant negative correlation

with fruit firmness (Table 1). These results indicate that increased POD activity is closely associated with rising ORP, increased ethylene biosynthesis, and fruit ripening-softening.

2.4 Changes in Cell Wall Degrading Enzymes During Storage

PG, Cx, and β -D-Gal are key enzymes that promote cell wall component hydrolysis and regulate fruit ripening-softening. Figures 4 [Figure 4: see original paper]A and 4B show that PG activity increased initially then decreased during storage, with ethephon-treated fruit showing slightly higher PG activity than the control, while Cx activity showed no significant difference between treatments. Figure 4C shows that β -D-Gal activity increased continuously during storage, with minimal changes in ethephon-treated fruit during the first 14 days. On day 15 postharvest, β -D-Gal activity increased sharply to a maximum of $60.64 \text{ mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, remaining significantly higher than the control until the end of storage. PG and β -D-Gal activities showed significant negative correlations with fruit firmness, while β -D-Gal activity showed extremely significant positive correlations with ORP, ethylene biosynthesis, and total color difference (Table 1). These results demonstrate that increased β -D-Gal activity is closely associated with rising ORP, increased ethylene biosynthesis, and fruit ripening-softening.

Discussion and Conclusion

Ethylene is a confirmed key factor regulating fruit ripening, softening, and senescence. Synthetic ethephon releases ethylene gas upon contact with water and is widely used for artificial ripening of climacteric fruits such as banana, jackfruit, tomato, cherry, pear, and fig. This study found that ethephon treatment significantly promoted fruit firmness decline and color development, demonstrating clear ripening effects. Concurrently, ethephon-treated fruit showed a sharp increase in ethylene production. During storage, ethylene production showed a significant negative correlation with fruit firmness and an extremely significant positive correlation with total color difference, confirming ethylene as a key regulator of persimmon fruit ripening and softening.

ORP represents the relative strength of oxidation-reduction status in organisms and characterizes the free energy and direction of redox reactions. Redox reactions consist of two half-reactions—oxidation and reduction—each with its corresponding potential. In living organisms, respiration and various metabolic pathways depend on redox reactions, which not only provide energy but also determine senescence and death. Ethylene synthesis from ACC and O₂ by ACC oxidase (ACO) is essentially a redox reaction, where electron transfer and continuous changes in oxidation-reduction status affect the reaction direction. In this study, persimmon ORP remained relatively stable during the first 14 days of storage. On day 15 postharvest, ORP in ethephon-treated fruit increased rapidly and significantly above the control, accompanied by massive ethylene production. ORP showed an extremely significant positive correlation with ethylene production, indicating a close relationship between ethylene synthesis and

ORP steady state in persimmon fruit. ORP may act as a switch regulating the biochemical reaction of ACC conversion to ethylene, where breaking the ORP steady state triggers ethylene synthesis initiation and accelerates postharvest ripening.

Cells maintain a complete oxidation and reduction system that is normally in dynamic balance for proper cellular function. Imbalance between these systems triggers stress responses and accelerates senescence. Plants produce reactive oxygen species (ROS) such as superoxide anion ($O \cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) during normal metabolism. ROS are the primary factors disrupting the dynamic balance between oxidation and reduction systems. SOD, CAT, and POD are important enzyme systems in the ROS defense system that scavenge ROS, reduce ROS accumulation-induced cellular damage, and delay senescence. This study found that SOD activity remained at high levels without significant changes throughout storage, while CAT and POD activities remained low during early storage. POD activity showed an extremely significant positive correlation with ORP. These results suggest that SOD plays an important role in scavenging ROS and maintaining redox balance throughout storage, while CAT and POD may be activated only after H_2O_2 accumulates to certain levels, with CAT acting earlier than POD. The coordinated action of SOD, CAT, and POD maintains relatively stable ORP to delay rapid fruit senescence and softening. Meanwhile, increased POD activity is closely related to ORP steady state disruption, which may be the primary reason for the rapid POD activity increase.

Cell wall degradation is the direct cause of fruit softening. The interaction of various cell wall-degrading enzymes (PG, Cx, β -D-Gal, etc.) modifies pectin structure, degrades cell wall components, and ultimately destroys the overall cell wall structure. The roles of PG and Cx in promoting fruit softening have been demonstrated in blue honeysuckle, watermelon, and apple. Qi et al. (2015) found that β -D-Gal had a closer relationship with ripening-softening than PG and Cx in non-storable 'Jingbaili' pear fruit, with similar reports in strawberry, peach, and tomato. In this study, β -D-Gal activity showed an extremely significant negative correlation with fruit firmness and extremely significant positive correlations with ethylene production and color difference. The correlation coefficients between β -D-Gal activity and ethylene production, firmness, and color difference were much greater than those for PG and Cx, consistent with Qi et al. (2015) in pear fruit. Additionally, β -D-Gal activity showed an extremely significant positive correlation with ORP, indicating that β -D-Gal activity is affected by ORP changes during storage, where increased ORP induces higher β -D-Gal activity, promoting cell wall degradation and accelerating fruit softening.

In summary, oxidation-reduction potential plays a key role in persimmon postharvest ripening and softening. Exogenous ethylene breaks the stable oxidation-reduction state in fruit, triggers endogenous ethylene synthesis initiation, promotes massive ethylene production, and enhances POD and

-D-Gal activities, thereby accelerating postharvest ripening and senescence during storage.

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