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Near-Freezing Temperature Storage Improves Peach Fruit Chilling Tolerance by Regulating the Antioxidant and Proline Metabolism

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Abstract: Chilling injury (CI) in peach fruit (*Prunus persica* cv. Yuhualu) is generally caused by long-time low temperature (5 °C or 0 °C) storage. However, peach fruit stored at near-freezing temperature (NFT in this research is −1 °C), defined as within 0.5 °C above the biological freezing point of biological tissue, does not exhibit CI symptoms. The effect of NFT on the CI, proline metabolism, and antioxidant capability of peach fruit during storage was studied and compared with 5 °C and 0 °C storage as controls. The results exhibit that NFT completely inhibited the occurrence of CI in peach fruit. NFT significantly ($p < 0.05$) enhanced the activities of superoxide dismutase, catalase, ascorbate peroxidase, and 1,1-diphenyl-2-picrylhydrazyl scavenging capacity. Moreover, the increase of malondialdehyde, ion leakage, and H_2O_2 accumulation were inhibited remarkably by NFT, and decreases in the contents of phenolics and ascorbic acid were slowed significantly in peach fruit stored at NFT ($p < 0.05$). Additionally, NFT storage enhanced proline accumulation by modulating the activity of proline metabolizing enzymes. In conclusion, the above results suggest that NFT storage can improve the chilling tolerance of peach fruit by regulating the antioxidant defense and proline metabolism, which might represent a potential novel method to store fruits and vegetables for longer storage times.

Keywords: near-freezing temperature storage; peach fruit; chilling injury; antioxidant defense system; proline metabolism

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1. Introduction

Peach fruit (*Prunus persica* cv. Yuhualu) is bright in color, rich in aroma and nutrients, sweet and sour, and well loved by consumers [1]. However, postharvest challenges arise due to the climacteric nature of peaches, leading to softening and decay when stored at room temperature. Although low-temperature refrigeration ranging from 0 °C to 8 °C is a widely employed strategy to extend peach storage, it often triggers chilling injury (CI). CI generally results in flesh browning, fibrillation, and aroma loss. Underlying these manifestations are disruptions in plant cell metabolism, including membrane damage, reactive oxygen disorder, and alterations in cell wall materials [2–4].

Membrane damage, caused by disturbance of oxidative stress metabolism, is a critical reason for CI [5]. The antioxidant defense system can reduce CI damage in plants by reducing damage to the plasma membrane caused by free radicals. Reactive oxygen species are essential redox signaling factors involved in developmental and physiological processes, and induce the expression of defense genes and adaptive response. Nitric oxide treatment can extenuate mitochondrial swelling, maintain mitochondrial membrane potential and membrane fluidity, and delay the decrease in activities of the cytochrome pathway and cyanide-insensitive pathway of the mitochondrial respiratory chain in peach

fruit [6]. Similarly, low temperature generally reduces the activities of catalase (CAT) and superoxide dismutase (SOD) in postharvest cherry fruit [7].

Various abiotic and biotic stresses generally lead to proline accumulation [8]. Both exogenous ethylene and glycine betaine treatments can delay plant chilling tolerance by promoting reactive oxygen species metabolism and proline accumulation [9]. Chitosan regulates proline metabolism and enhances the chilling tolerance of rice [10]. Generally, the accumulation of proline can maintain the structure and function of cells, which may improve the chilling tolerance of plants [11].

Near-freezing temperature (NFT) refers to the temperature range from the freezing point of the organism to 0 °C; the cells of the organism will not suffer freezing damage under this temperature range. NFT storage technology can inhibit the respiration and microbial metabolism of postharvest fruits while maintaining their quality [12,13]. Recent studies have suggested that NFT can significantly restrain CI occurrence and prolong the storage period of nectarines [14,15]. While this phenomenon shows that the chilling tolerance of the fruit is significantly enhanced at this temperature, the specific mechanism has not yet been elucidated.

This research delves into the repercussions of NFT storage on CI, quality, antioxidant properties, and the metabolism of reactive oxygen species and proline in peach fruits. The investigation seeks to unravel potential correlations between antioxidant and proline metabolism and chilling tolerance during NFT storage, presenting valuable insights for the preservation of fresh vegetables and fruits.

2. Materials and Methods

2.1. Plant Materials and Fruit Treatment

For this experiment, peach fruits (*Prunus persica* cv. Yuhualu), with commercial maturity (soluble solids content 8~9° brix), color, and size and absence of diseases and mechanical damage were collected and immediately transferred to the laboratory.

Following established protocols outlined in our previous research [14], the fruits underwent a precooling process at 5 °C ± 0.1 °C for 12 h. Subsequently, they were randomly divided into three groups: 5 °C (±0.1 °C), 0 °C (±0.1 °C), and Near-Freezing Temperature (NFT, −1 °C ± 0.1 °C). The storage duration was 28 days and the storage conditions included a relative humidity of 90%. Each treatment was replicated three times, with each replicate consisting of 100 peach fruits. Sampling was conducted at 0, 7, 14, 21, and 28 days, and fifteen fruits were sampled from each batch. Tissue slices of approximately 1 cm thick were rapidly frozen in liquid nitrogen and stored at −80 °C for subsequent analysis.

2.2. Confirmation of Near-Freezing Point Temperature of Peach Fruit

After calibration by a 0 °C of water and ice, the probe of the high-precision temperature tester (Elitech RC-4HC, JiangChuang, Xuzhou, China) was used for testing the freezing point temperature [14]. According to the freezing curve (Supplementary Material Figure S1), the super-cooling point was −2.3 °C and the freezing point was −1.1 °C. Thus, to prevent freezing damage, the near-freezing storage temperature was set at −1 °C and the temperature fluctuation was controlled within ±0.1 °C.

2.3. Measurement of Incidence and Incidence Index of CI in Peach Fruit

Subjective assessment of CI development was performed with 15 fruits from each replicate according to the previous method [15]. The CI degree was investigated by comparing the internal browning area of peaches transferred to room temperature for three days after storage [16]. The degree of internal browning was calculated using a 0 to 4 rating, as follows: 0, no browning; 1, browning area less than 20%; 2, browning area 20~40%; 3, browning area 40~60%; and 4, browning area greater than 60%. The CI index = $\sum$ (browning degree × number of fruits with browning in each grade)/(4 × total number of fruit) × 100%.

2.4. Determination of Ion Leakage and Contents of Malonaldehyde and H_2O_2 in Peach Fruits

Ion leakage was determined using 15 pieces of pulp (3 mm thickness × 8 mm diameter) from 15 fruits [17]. The pulp was allowed to stand in 30 mL of double-distilled water for 30 min. After shaking, the electrical conductivity of the mixed solution was detected by an electrical conductivity meter (DDS-12B, Beijing, China), then the mixed sample was warmed in boiling water for 15 min. After cooling, the final conductivity of the mixed sample was recorded. The relative conductivity is generally used to represent the amount of ion leakage (conductivity of mixed solution/final conductivity) × 100%.

To test the malonaldehyde (MDA) content, tissue (10 g) was placed in a system of thiobarbituric acid as previously reported [18]. After chromogenic reaction, the absorbance was recorded at 532 nm and 600 nm and the accumulation was described in μmol kg^{-1}. Moreover, flesh tissue (2.0 g) was used for measuring the H_2O_2 content as previously reported [19], and the H_2O_2 content was recorded as mmol kg^{-1}.

2.5. Determination of Proline Level and Related Enzyme Activities in Peach Fruits

The proline content was measured by adding sulfosalicylic acid to ground flesh in an ice bath, followed by boiling and centrifugation [20]. The absorbance of the supernatant was tested at 520 nm, with the proline content expressed in mg kg^{-1}.

We modified a previous method for measuring the enzyme activities of pyrroline-5-carboxylate reductase (P5CR) and Δ^1-pyrroline-5-carboxylate synthetase (P5CS) [21]. Fruit tissue (1.0 g) was mixed with formulated extraction buffer (pH 7.5, 2 mmol L^{-1} phenylmethyl sulfonyl fluoride, 10 mol L^{-1} $MgC1_2$, 0.5 mol L^{-1} Tris-HCl, 2% Polyvinyl pyrrolidone). The resulting homogenate was cooled in an ice bath, followed by centrifugation at 20,000× *g* for 20 min at 4 °C. The residue was repeatedly extracted once, and the collected supernatant from the centrifugation was combined to form the enzyme extract.

The reaction solution, including 10 mmol L^{-1} ATP, 20 mmol L^{-1} $MgCl_2$, 50 mmol L^{-1} L-glutamate, and 100 mmol L^{-1} hydroxylamine hydrochloride, was mixed with 1 mL of the preceding enzyme extract. The reaction termination buffer, containing 5% $FeCl_3$ and 12% trichloroacetic acid in 5 mol L^{-1} HCl, was added to this system, then the mixture was reacted in a water bath at 37 °C for 15 min. After centrifugation, the supernatant was used to test the absorbance at 535 nm and the blank control without ATP was used as a reference. The unit of P5CS (U) is defined as the quantity of enzyme required to produce 1 μmol of γ-glutamine every 60 s, and results are described in U g^{-1} protein.

The activity of P5CR was determined by the previous method [22]. Reaction solution buffer, including 0.1 mmol L^{-1} NADPH, 0.56 mmol L^{-1} P5C, was reacted with enzyme solution (20 μL). The oxidation of 1 μmol of NADPH per minute is defined as one unit activity of P5CR.

We modified the proline dehydrogenase (PRODH) activity determination method proposed previously [23]. Fruit tissue (3.0 g) was blended at low temperature with potassium phosphate buffer (100 mmol L^{-1} 5 mL pH 7.4) containing 1.0% (*w*/*v*) PVPP, 1.0 mmol L^{-1} EDTA, 5.0 mmol L^{-1} $MgCl_2$, 10 mmol L^{-1} β-mercaptoethanol, and 60 mmol L^{-1} KCl. After centrifugation (10,000× *g*, 15 min, 5 °C), the enzymatic activity was estimated using the obtained supernatant.

The reaction mixture (2.5 mL pH 10.3) contained 1.6 mL 0.15 mol L^{-1} Na_2CO_3-$NaHCO_3$ buffer, 0.2 mL 0.1 mol L^{-1} L-proline, and 0.2 mL 0.9 mmol L^{-1} 2,6-dichlorophenol indiophenol. After 5 min warming at 30 °C, 0.5 mL reaction mixture was mixed with 0.2 mL enzyme extract and 9 mg mL^{-1} phenazine methyl sulfate reagent. The absorbance change at 600 nm was marked immediately. The enzyme activity unit (U) was clarified as 0.01 ΔA600 nm g^{-1} min^{-1}.

2.6. Measurement of Enzyme Activities of Antioxidant in Fruit

Fresh pulp (10 g) was used for determining the SOD activity of peach according to a previous method [24]. The amount of enzyme required to inhibit the tetrazolium photoredox reaction by 50% was defined as one enzyme unit (U). Following a previous method [25], 2.0 g pulp was used to prepare the crude enzymatic extract of CAT and ascorbate peroxidase (APX). Following reported methods, we utilized the enzymatic extract to calculate the estimated activities of CAT and APX [8]. The enzyme reaction system of peroxidase (POD) contained 2.5 mL of 0.2 mol L^{-1} phosphate buffer (pH 5.0), 150 µL of 0.08% H_2O_2, 0.1 mL of 0.1% guaiacol, and 10 µL enzyme solution. After adding the hydrogen peroxide for 5 min, the absorbance at 460 nm was recorded. Each enzyme activity unit was defined as every 0.01 change in absorbance [26].

2.7. Determination of Phenolics, Ascorbic Acid, and Free Radical Scavenging Capacity

The level of phenolics in the fruits was measured by the Folin–Ciocalteu method [27], with the phenolics content expressed in gallic acid equivalents and the unit expressed in g kg^{-1}. In addition, flesh tissue (1.0 g) was used to estimate the ascorbic acid content in peach fruits following previously reported methods [14]. The volume of the titrated solution was recorded, with the ascorbic acid content expressed as mg kg^{-1}.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is generally utilized to evaluate the antioxidant capacity of tissue [28]. Fruit extract (10 µL) was thoroughly mixed with distilled water (90 µL) and DPPH methanol (3.9 mL 0.0250 g L^{-1}), then the mixture was placed in the dark for 30 min. The absorbance was tested at 515 nm without DPPH. The calculation formula of the clearance rate was as follows: (%) DPPH clearance = $(A_0 - A_1)/A_0 \times 100\%$; in the above formula, A_0 represents the Abs control and A_1 represents the Abs sample.

2.8. Statistical Analysis

The experiment used a completely randomized design. Analysis of variance was performed among different groups, and Duncan's multiple interval test was used to compare the mean values with a significance level of 0.05 (SPSS 11.0 Inc., Chicago, IL, USA). Final values represent the mean ± SE (standard error) of three replicates. Figures were prepared using Origin Program 2016 (OriginLab Co., Northampton, MA, USA).

3. Results

3.1. NFT Storage Enhances the Chilling Tolerance of Peach Fruit

Both the CI incidence and CI incidence index of peach fruits stored at 5 °C and 0 °C showed an upward trend with the prolongation of storage time. However, the NFT group did not have any CI symptoms during the whole storage time (Figure 1A,B). On Day 7, for the 5 °C group the CI incidence was 12.61% and the CI incidence index was 5.31%. On Day 28, for the 0 °C group the CI incidence and CI incidence index were 20.93% and 10.52%, respectively. The above results, as exhibited in Figure 1, indicate that NFT was able to reduce the occurrence of CI. Figure 1C shows that the degree of browning inside the peach fruits in the 5 °C and 0 °C groups increased sharply, while the peach fruits at NFT kept their original luster and internal color during the whole storage time. This phenomenon suggests that NFT storage was able to remarkably alleviate the internal browning of peach fruits.

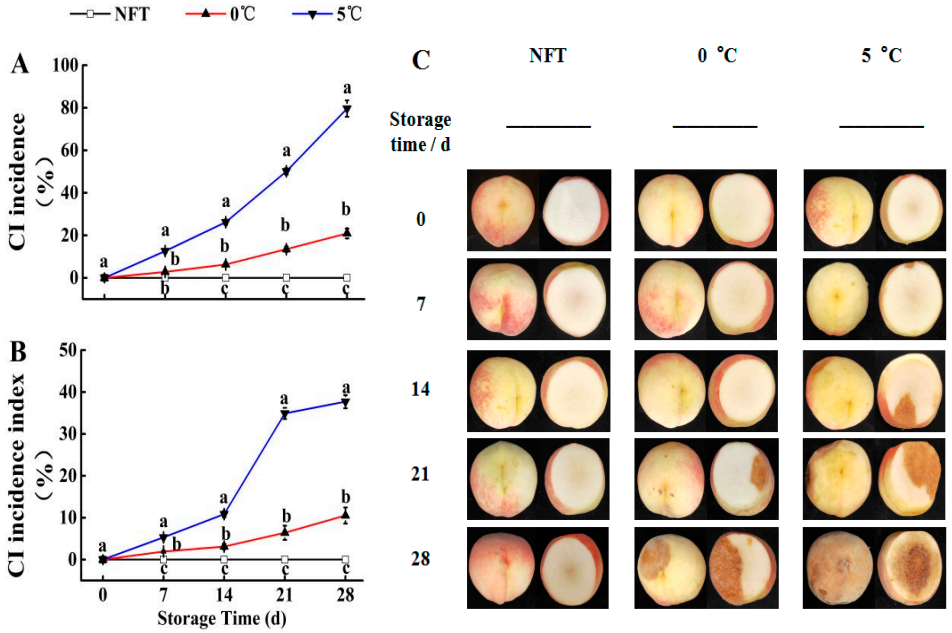

Figure 1. Effects of different temperature treatments on CI incidence and CI incidence index of peach fruits. CI incidence (**A**), CI incidence index (**B**), and photographs of stored peach fruits (**C**). Data are expressed as the mean ± SE (n = 3). Vertical bars represent the standard error of the mean ($p < 0.05$). Duncan's test letters represent the difference among the different temperatures within the same days.

3.2. NFT Can Reduce Ion Leakage and Contents of MDA and H_2O_2

Figure 2A shows that the ion leakage gradually emerged as an incremental trend in all groups with the extension of storage time. At 5 °C, the ion leakage increased rapidly by 28.40% within 28 days. This increase in ion leakage was significantly suppressed when decreasing the storage temperature. The ion leakage level of peach fruits stored at NFT was significantly ($p < 0.05$) lower than those of the 0 °C and 5 °C groups.

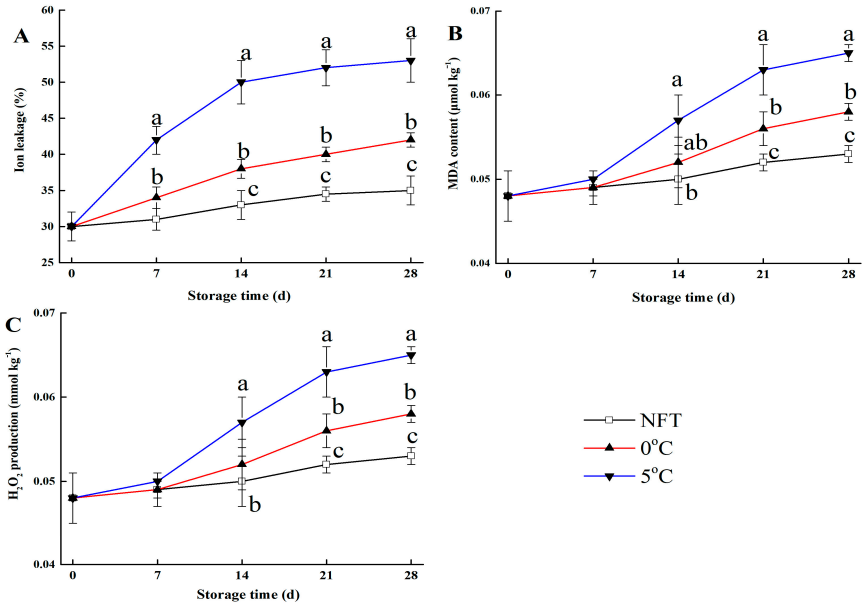

Figure 2. Effects of NFT storage on ion leakage (**A**), MDA content (**B**), and H_2O_2 (**C**) content of peach fruits. Data are expressed as the mean ± SE (n = 3). Vertical bars represent the standard error of the mean ($p < 0.05$). Duncan's test letters represent the difference among the different temperatures within the same days.

During the first of 14 days of storage, NFT inhibited the increase of MDA content, with a level of 0.048 μmol kg^{-1}. The MDA content of the 5 °C group increased rapidly after Day 7, an abnormal situation that may have been caused by severe fruit rot. Moreover, the MDA content of the NFT and 0 °C groups decreased slowly during the storage period, and the MDA value of the NFT group increased to 0.591 μmol kg^{-1} until the end of storage, which is remarkably lower than the 0 °C and 5 °C groups (Figure 2B).

As Figure 2C shows, the highest H_2O_2 contents appeared on Day 28 in the 0 °C and 5 °C groups, respectively. Compared with the 0 °C and 5 °C groups, the content of H_2O_2 in the NFT increased mildly and remained at a lower level ($p < 0.05$) in the whole storage time.

3.3. Effect of NFT Storage on Proline Metabolism in Peach Fruit

Compared with the beginning, the proline level of the 5 °C storage group decreased by 7% after 14 days of storage (Figure 3A). During the first 21 days of storage, the proline value of the 0 °C storage group was consistently lower than that of the 5 °C and NFT groups. On Day 28 of storage, the proline content of the NFT group was similar to that of the 0 °C group, and was 16% higher than that of the 5 °C group.

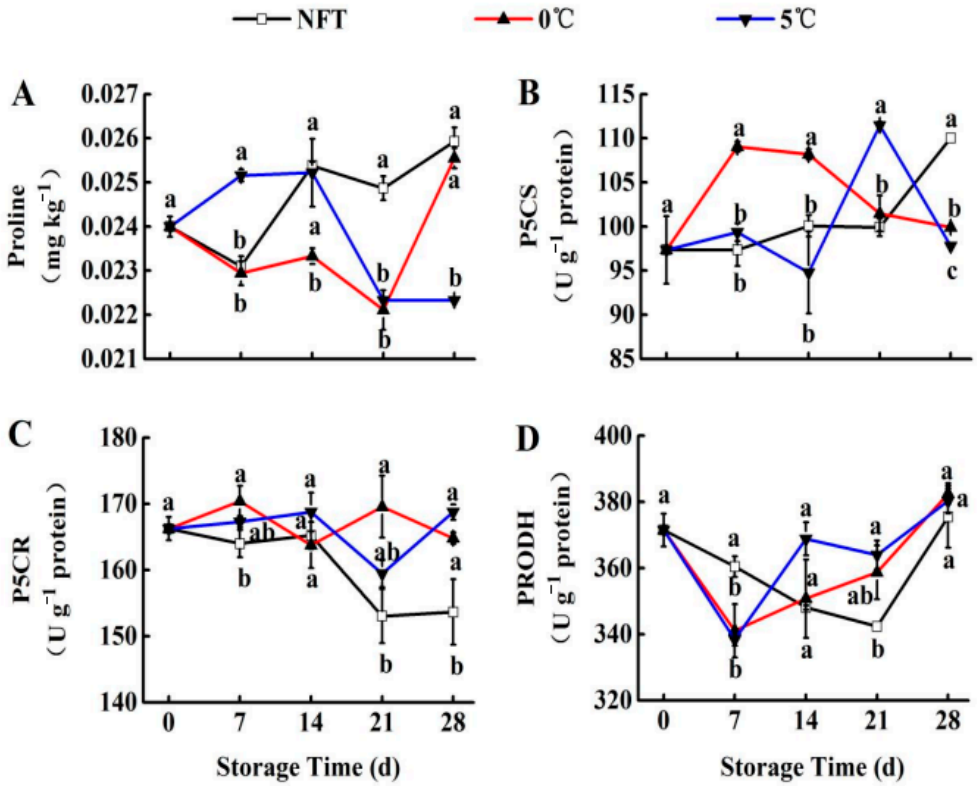

Figure 3. Effects of NFT storage on proline content and related enzyme activities in peach fruits. Proline content (**A**) and activities of P5CS (**B**), P5CR (**C**), and PRODH (**D**). Data are expressed as the mean ± SE (n = 3). Vertical bars represent the standard error of the mean ($p < 0.05$). Duncan's test letters represent the difference among the different temperatures within the same days.

The P5CS activity of fruits stored at NFT showed a gradual increase throughout the storage period. The P5CS activity of the NFT group was significantly higher ($p < 0.05$) on Day 28 than that of the 5 °C and 0 °C groups (Figure 3B). In the whole storage time, there were some fluctuations in the activity of P5CR in the 0 °C and 5 °C treatment groups, while the overall change was not significant (Figure 3C). The P5CR activity of the NFT group was always lower than that of the other two groups during the whole storage process.

During storage, the activity in the different temperature groups first decreased and then increased. On Day 7, the activity of PRODH in the 0 °C and 5 °C groups decreased rapidly to the lowest level and then gradually increased. PRODH activity in the NFT group

decreased laxly and arrived at the lowest value on Day 21, maintaining a lower level than the 0 °C and 5 °C groups (Figure 3D).

3.4. NFT Enhances the Antioxidant Defense System

The SOD activities at different storage temperatures showed signs of a mild retreat at the beginning, then SOD activities increased and peaked in all groups on Day 21 (Figure 4A). Compared with the 0 °C and 5 °C groups, the SOD activity of the NFT group was the highest, and showed an upward trend during storage. These results indicate that NFT storage was able to effectively improve the SOD activity of peach fruits.

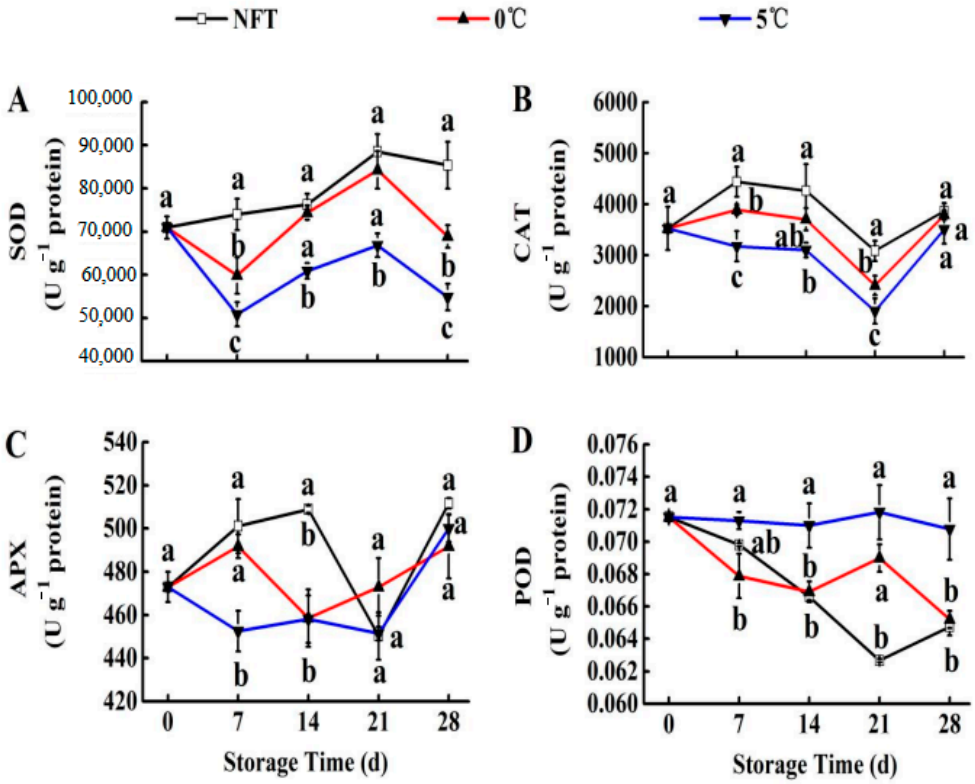

Figure 4. Effects of NFT storage on reactive oxygen species metabolism in peach fruit. Measurement of SOD (**A**), CAT (**B**), APX (**C**), and POD (**D**) activities. Data are expressed as the mean ± SE (n = 3). Vertical bars represent the standard error of the mean ($p < 0.05$). Duncan's test letters represent the difference among the different temperatures within the same days.

The activities of CAT in all group exhibited a slight fluctuation at the end of storage. CAT activity in the 0 °C and NFT groups decreased to the lowest value at Day 21, then increased again. NFT treatment delayed the decrease in CAT activity and maintained a higher level than in the other groups during the whole storage period ($p < 0.05$, Figure 4B).

Figure 4C shows that APX activity exhibited an increase at beginning of storage, followed by a decrease; moreover, the APX activity in the NFT storage group was higher than that of the 0 °C and 5 °C groups, and was 1.1-fold higher on Day 14. In the whole storage time except for Day 21, the APX activity of peach fruits stored at NFT was higher than in the 0 °C and 5 °C groups.

According to Figure 4D, the POD activity of the peach fruits in the 5 °C group remained unchanged throughout the storage time and was remarkably loftier ($p < 0.05$) than that of the other two groups. The POD activity of peach fruits stored at NFT gradually decreased, and was lower than that of the 0 °C and 5 °C groups on Day 27.

3.5. Effects of NFT Storage on Phenolics Content, Ascorbic Acid Content, and DPPH Scavenging Capacity of Peach Fruit

According to Figure 5A, the contents of peach phenolics at different temperatures showed a decreasing trend at first and then increasing trend during storage. Among the different groups, the contents of phenolics at NFT was significantly higher than that of the 5 °C and 0 °C groups ($p < 0.05$). During storage, the ascorbic acid level exhibited a declining trend at all temperatures, with that of the 5 °C group being significantly ($p < 0.05$) lower than those of the other two groups. There was no significant difference in ascorbic acid level between the 0 °C and NFT groups ($p > 0.05$), indicating that both the 0 °C and NFT treatments inhibited the decrease in ascorbic acid content (Figure 5B).

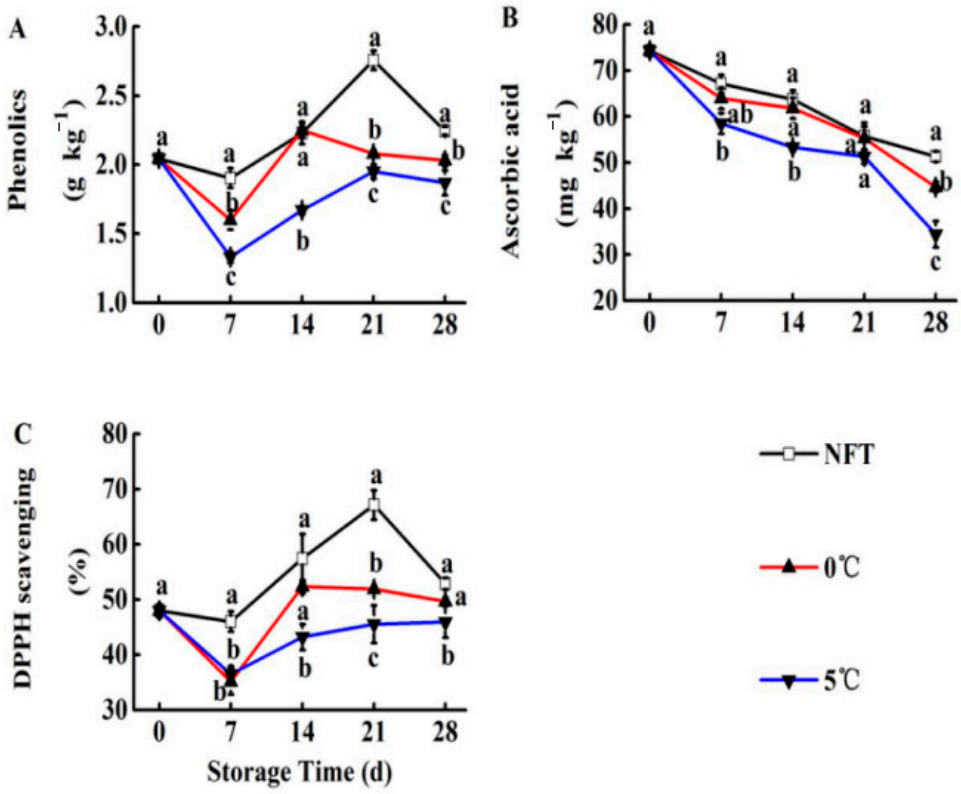

Figure 5. Effects of NFT storage on phenolics content (**A**), ascorbic acid content (**B**), and DPPH scavenging capacity (**C**) of peach fruits. Data are expressed as the mean ± SE (n = 3). Vertical bars represent the standard error of the mean ($p < 0.05$). Duncan's test letters represent the difference among the different temperatures within the same days.

DPPH scavenging and phenolics exhibited similar trends (Figure 5C). The DPPH scavenging capacity in the 5 °C and 0 °C groups showed a decreasing trend at first, followed by an increase during storage. Moreover, the scavenging rates on Day 28 and Day 0 were almost the same. The free radical scavenging rate of the NFT group was considerably higher ($p < 0.05$) than those of the 5 °C and 0 °C groups during the whole storage time.

4. Discussion

The symptoms of CI in peach fruits are browning and softening, which seriously affect fruit quality and commercial value. In recent years, methods such as treatment with heat, 1-Methylcyclopropene, glycine betaine, melatonin, and other methods have been used to inhibit CI [29–32]. In our study, both the CI incidence and CI index of peach fruits at NFT did not exhibit growth during the whole storage time, indicating that NFT completely inhibited the occurrence of CI. Ion leakage is an important physiological indicator for judging membrane damage, and can accurately reflect its development [33]. The increase

of ion leakage in the NFT group was very slow, indicating that the fluidity of the cell membrane was maintained, meaning that the cell membrane remained almost undamaged.

Similarly, low temperature stress can enhance membrane peroxidation and reactive oxygen metabolism in plant cells; the accumulation of MDA and H_2O_2 disrupts the plasma membrane and affects cell membrane fluidity, leading to the development of CI [18,34]. In our study, the results showed that NFT storage significantly decreased both MDA and H_2O_2 contents, thereby reducing the toxic effects of free radicals on the cell membrane and maintaining cellular homeostasis. These results indicate that NFT can enhance chilling tolerance in fruit.

Proline regulates cell osmotic balance and protects proteins, helping to stabilize the cell structure when plants are under abiotic stress [35,36]. At the same time, proline can scavenge free radicals and protect the antioxidant enzyme system [37]. The synthesis of proline in plants is catalyzed by P5CS and P5CR. For example, P5CS plays a critical role in the synthesis of proline when plants suffer stress [38]. As the key enzyme in proline synthesis, the activity of P5CS was reinforced by NFT storage in peach fruits while the activity of P5CR was weakened, indicating that NFT accelerated proline synthesis. Proline metabolizing enzymes can coordinate with PRODH to degrade the accumulated proline in plants, which might result in the breakdown of osmotic balance and cause damage [39]. NFT reduced the activity of PRODH and inhibited the degradation of proline in peach fruits. The above results suggest that NFT storage can improve proline accumulation by regulating the activities of enzymes involved in proline metabolism, ultimately enhancing the chilling tolerance of fruit (Figure 6).

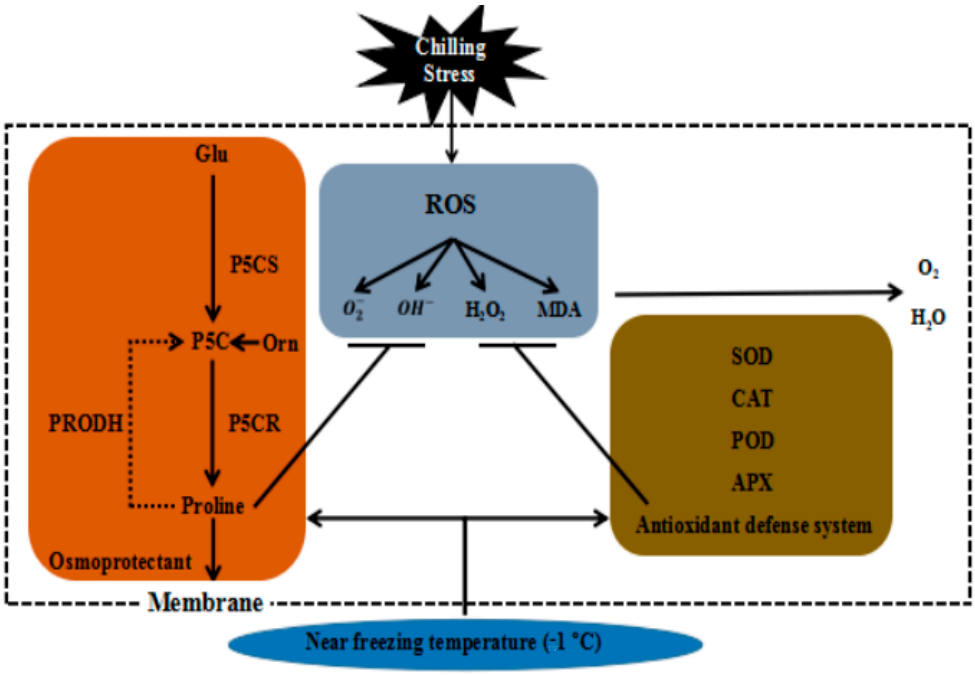

Figure 6. Schematic presentation of NFT inhibiting the occurrence of CI.

Excessive concentrations of reactive oxygen species can lead directly to plant senescence and even death; the antioxidant defense system, including CAT, SOD, APX, and POD, is critical in regulating the purging of reactive oxygen [40]. When plants suffer from abiotic stress, the metabolic balance of reactive oxygen species is broken and a large number of free radicals is generated in the cell, which damages the cell membrane and eventually induces CI. SOD, as a key enzyme in preventing oxidative stress, catalyzes the formation of H_2O_2 and O_2, after which H_2O_2 is converted into H_2O and O_2 under the action of CAT and POD, retarding the toxic effect of reactive oxygen species [41]. As an enzyme that catalyzes the breakdown of peroxides, CAT reduces the toxic effects of H_2O_2 on metabolic tissues [42]. In

addition, APX catalyzes the redox reaction between ascorbic acid and H_2O_2 so that ascorbic acid is oxidized to form hydroascorbic acid, with H_2O_2 decomposed in the process of maintaining the balance of free radical metabolism in cells [43]. Furthermore, both phenolics and ascorbic acid can scavenge free radicals and improve antioxidant capacity [44]. Zhao et al. found that NFT can effectively promote the accumulation of phenolics in peach fruits and slow the decline in ascorbic acid content [8]. The changes in the activities of CAT, SOD, and APX in our research indicate that NFT storage helped to promote scavenging of reactive oxygen radicals, prevent the accumulation of high oxygen concentrations, maintain the balance of reactive oxygen species, and re-establish cellular redox homeostasis, thereby enhancing the chilling tolerance of peach fruits (Figure 6).

5. Conclusions

In conclusion, this study highlights intriguing findings regarding the efficacy of NFT storage in mitigating chilling injury in peaches, especially when compared to storage at higher temperatures. Notably, NFT storage exhibited consistent preservation of elevated levels of antioxidant enzyme activities, ascorbic acid, phenolics, and DPPH scavenging capacity. In addition, it effectively mitigated the accumulation of malondialdehyde and inhibited the activity of proline dehydrogenase (PRODH). Finally, NFT storage facilitated increased proline production and bolstered the overall antioxidant defense system.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10040337/s1, Figure S1. Freezing point curve of peach fruit.The temperature initially decreased to the super-cooling point of peach fruit during the freezing process. Owing to exothermic phenomena, the temperature curve raised and temporarily formed distinct plateaus, which correspond to the biological freezing point of peach fruit. According to the curve, the super-cooling point and freezing point of fruits were –2.3 °C and –1.1 °C, respectively. Moreover, temperature fluctuation was confirmed within a small region (± 0.1 °C) to avoid freezing damage.

Author Contributions: H.Z.: funding acquisition, writing and editing, investigation, parameter testing. S.M.: methodology establishment, parameter testing, funding acquisition. M.F.: experiment administration, validation. Q.C.: writing—review. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original date presented in the research are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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