Postharvest Biology and Technology 147 (2019) 113–122

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Enhancement of quality and antioxidant metabolism of sweet cherry fruit by near-freezing temperature storage

Handong Zhao, Bangdi Liu, Wanli Zhang, Jiankang Cao, Weibo Jiang*

College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China

ARTICLE INFO

Keywords:
Sweet cherry
Near-freezing temperature storage
Postharvest quality
Antioxidant enzyme
Reactive oxygen species
Antioxidant capacity

ABSTRACT

Near-freezing temperature storage (NFTS) is a novel method to inhibit quality loss of fresh fruit. However, little information is available on NFTS delaying the onset of senescence in sweet cherry (*Prunus avium* L.) fruit and regulating the changes on antioxidative enzymes participated in the balancing of reactive oxygen system (ROS). Fruits were stored at NFT (between super-cooling point and freezing point), 0 °C and 5 °C, respectively, until fruits exhibited visually rot (sampled every twenty days). NFTS effectively slowed senescence process in sweet cherry fruit, as indicated by extending storage duration and improving the changes of firmness, anthocyanins, ion leakage, peel color and sugars content. Moreover, fruit stored at NFT had higher levels of ascorbic acid, phenolics and organic acids and lower accumulation of carotenoids, malondiadehyde, hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$). Additionally, NFTS maintained membrane integrity and prevented fresh browning of fruit by enhancing the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and gluta thione reductase (GR) and inhibiting the activities of guaiacol peroxidase (POD), polyphenol oxidase (PPO) and lipoxygenase (LOX). Furthermore, NFTS fruit exhibited higher level of antioxidant capacity as measured by radical scavenging activity and reducing power at the end of storage. These results indicate that the activities of antioxidant enzymes to scavenge superoxide anions and H_2O_2 during NFTS was implicated in the maintenance of membrane integrity, which might be a part of the mechanism associated with the delay of senescence in sweet cherry fruit.

1. Introduction

Sweet cherry (*Prunus avium* L.) has become one of the most important non-climacteric fruits worldwide for its satisfaction of quality attributes (Habib et al., 2015). Moreover, sweet cherry is becoming more and more popular due to its bioactive compounds with antioxidant characteristics, generally including polyphenols, vitamins, anthocyanins and carotenoids (Gonçalves et al., 2004; Serradilla et al., 2012; Usenik et al., 2008). Previous study has proved that cherry consumption was related with a lessening of several diseases, including cancer, cardiovascular, diabetes and inflammatory diseases, as a result of a decline in oxidant stress, tumour suppression, inflammation and glucose control (Mccune et al., 2011).

However, sweet cherry fruit is highly perishable owing to softening rate (Meheriuk et al., 1995) as a result of the high rate of transpiration and respiration, mechanical bruises and high susceptibility to fungal infections (Alique and Zamorano, 2005; Ceponis et al., 1987), which dramatically influence their storability and marketing acceptability after harvest. Basically, the main postharvest treatment to reduce the quality loss and extend storability of sweet cherry fruit is cold storage (Petriccione et al., 2015), but traditional cold storage method generally causes some physiological disorder, such as surface pitting and anthocyanins degradation (Correia et al., 2018; Wani et al., 2014). Near-freezing temperature (NFT), within the range of minimal non-frozen temperatures, is determined by freezing curve of the individual material (Supplementary material Fig. S1D), which originally been used to store fresh fish and animal organs (Okamoto et al., 2008; Zhu et al., 2016). Recent research exhibited that nectarine and apricot fruits also preferably preserved physiological and commercial qualities with the near-freezing temperature storage (NFTS) (Zhao et al., 2018; Fan et al., 2018). In our study, we have found NFTS could reduce the decay rate, delay the ripening process and maintain the higher content of bioactive compounds and antioxidant activity as compared with the traditional cold storage, which might associate to the changes in relative enzyme activity.

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot}-$), are inevitably originated in plant cell as a consequence of normal metabolism, which are mainly produced in

* Corresponding author.
E-mail address: jwb@cau.edu.cn (W. Jiang).

https://doi.org/10.1016/j.postharvbio.2018.09.013
Received 19 June 2018; Received in revised form 12 September 2018; Accepted 14 September 2018

reactions catalyzed by oxidase and lipoxygenase (LOX). Moreover, the producing and scavenging systems, including both non-enzymatic antioxidant and enzymatic mechanisms, are predicted the ROS content in plant cell (Apel and Hirt, 2004). Non-enzymatic compounds of sweet cherry fruit mainly include phenolics, anthocyanins, carotenoids and ascorbate acid, while the antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX), are principal active free radical scavenging enzymes (Valverde et al., 2015). SOD converts the $O_2^{\cdot}-$ free radical to O_2 and H_2O_2, then the CAT catalyses the decomposition of H_2O_2 to water and oxygen. Additionally, ascorbate and H_2O_2 are transformed to water and dehydroascorbate by APX, and H_2O_2 can be also reduced to water by using POD and phenols (Tareen et al., 2012). Although the presence of these antioxidant systems is dynamic and efficient, plant cells are still occurred by oxidative damages on account of uncontrolled production or incompetent scavenging of ROS. Thus, the process of fruit ripening and senescence associates with ROS accumulation (Hodges, 2004), so the content of antioxidant compounds and the activities of antioxidant enzymes could affect the fruit storability (Mondal et al., 2009; Kumar, 2014).

However, there is no literature about the effect of NFTS on the behavior of antioxidant enzymatic systems and membrane integrity in fresh fruit. The objective of our study was to research the effect of NFTS on antioxidant enzymatic system and membrane metabolism of sweet cherry fruit and to understand the inter-relationship between NFTS and oxidative stress as well as the association with postharvest senescence.

2. Materials and methods

2.1. Plant material and NFT determination

Sweet cherry (Tieton) was obtained from an experimental orchard in Beijing at commercial maturity stage (firmness was about 3.9 N and SSC was about 18.3%). Fruit trees were maintained with standard cultivation, fertilizer, herbicide and pesticide practices. Fruits, with uniformity in shape, size, color and physical integrity without visual defects, were harvested randomly and transported to the laboratory immediately. Fruit was pre-cooled in an experimental temperature-controlled wind tunnel at 5 °C for 24 h. After pre-cooling, fruits were randomly separated into 5 °C, 0 °C or NFT (-3..0 ± 0.1 °C) for the sweet cherry group × 3 replications = 9 lots (5 kg per lot). All fruits were stored with a relative humidity (RH) of 90 ± 2% in darkness. Samples were removed on every twenty days during storage to evaluate the physiological and antioxidant capacity changes. 50 fruits (about 500 g fruit flesh) were used in each replicate (per lot), and there were about 150 fruits used for each sampling date in each temperature group for 3 replicates. Flesh sample was immediately used or frozen in liquid nitrogen and stored at –80 °C. All experiments were performed in triplicate.

To ensure the storage temperature precise, the new storage equipment, including refrigerated storage system, microcontrollers, temperature sensors, alarm device and temperature-controlling, was designed (Fan et al., 2018). The temperature control mode could display and set the box storage temperatures in real-time (Fig. S1 A). Once the temperature exceeded the setting range, the temperature control mode could regulate the fans or electric heater to keep the temperature within the designed parameters (Fig. S1B).

To confirm the biological freezing point and super-cooling point of sweet cherry fruit, previous method (Jie et al., 2003) was modified. After calibrated the thermocouple by the mixture of 0 °C water and ice, twenty fruits were selected randomly to determine the freezing curve by the HP34970 A data collector. Then, to obtain biological freezing curve, samples with thermocouples were situated in a freezer (− 20 °C) and the temperature data was recorded every 10 s. The biological freezing point and super-cooling point of sweet cherry fruit were determined based on the biological freezing curve. The freezing temperature curve and the NFT determination of sweet cherry were exhibited in supplementary material (Fig. S1C and D). The curve displayed that the super-cooling point and freezing point of fruit were –3.3 °C and–2.8 °C, respectively (Fig. S1D). To avoid freezing damage, the NFT of sweet cherry fruit was controlled at − 3.0 ± 0.1 °C which is slight higher than supper-cooling point.

Previous research confirmed that there was a high negative correlation between soluble solids and freezing point (Jie et al., 2003), and the higher levels of soluble sugars in fruit prevent water transferring out of the cells, which enhance the osmotic potential and make it difficult to form the ice crystals. Thus, higher soluble solids could enhance the NFT tolerance and osmotic potential of fruit.

2.2. Evaluation of firmness, ascorbic acid, total anthocyanins, total caroteniods and color changes

Fruit firmness was measured as penetration force on the fruit flesh. A penetrometer (Effegi pressure tester, Facchini 48011, Alfonsine, Italy) with a 3.5 mm probe was used to test the firmness and the results were expressed in N. Ascorbic acid was extracted and analyzed by Xi's method (Xi et al., 2017). Anthocyanins content was estimated by a pH-differential method (Albishi et al., 2013) and expressed as cyanidin-3-glucoside equivalent (CGE) per kilogram of fresh weight (molar extinction coefficient of 26,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}). Absorbance measurements were conducted at 520 and 700 nm. Total carotnoids were extracted according to previous method (Giménez et al., 2016).

The peel color change was determined using a reflectance spectrophotometer (Model NF333, Nippon Denshoku Industries, Tokyo, Japan). For color measurement, thirty fruits were randomly picked from each replicate, and color was expressed according to the CIE Lab system (a-red/green and b-yellow/blue), and the value of a, b were converted to Hue angle (a relative ratio of the yellow intensity to red intensity) and Chroma (an indicator of redness) [Hue angle ($\tan^{-1}$ (b/a)) and chroma, a measure of color clarity $(a^2+b^2)^{1/2}$].

2.3. Analysis of sugars, organic acids and phenolic compounds

Extraction and determination of sugars was performed according to previous condition with some modifications (Fan et al., 2017). 1 g sample was ultrasonic extracted at 30 °C for 1 h and centrifugated at 10,000*g* for 20 min. Sugar compounds, including fructose, sorbitol, glucose and sucrose, were identified and quantified by comparing relative retention time and peak area of standard substance (Fig. S2). For testing organic acids, 1 g fresh sample was ground in 20 mL deionized water and shaken at room temperature for 1 h. The organic acids was analyzed according to (Fan et al., 2017) method, and identified and quantified by comparing relative retention time and peak area of samples and standard substances (Fig. S3). To evaluate phenolic compounds, a HPLC method (Liu et al., 2015), with a Shimadzu liquid chromatograph, was used (Fig. S4).

To test soluble solid content (SSC), 50 fruits from each replication were randomly selected. SSC was tested by a digital refractometer (PR-101, Spectrum Technologies, Plainfield, IL), and results were expressed in percentage (%). Titratable acid (TA) was determined by titrating 25 mL of cherry juice to pH 8.5 with 0.1 mol L^{-1} NaOH, and results were expressed as the percentage of malic acid. To investigate total phenolic compounds, sample was extracted from 8.0 g frozen sweet cherry flesh as previous research (Pérez-Jiménez and Saura-Calixto, 2005). Total phenolics (TP) concentration was measured by the Folin-Ciocalteu method and expressed as gram gallic acid equivalents per kilogram fresh weight.