Food Chemistry 264 (2018) 263–269

Contents lists available at ScienceDirect

Food Chemistry

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

Low temperature conditioning alleviates loss of aroma-related esters of 'Nanguo' pears by regulation of ethylene signal transduction

Miaomiao Yao[1], Xin Zhou[1], Qian Zhou, Fei Shi, Baodong Wei, Shunchang Cheng, Zhuo Tan, Shujuan Ji*

College of Food, Shenyang Agricultural University, No.120 Dongling Road, Shenhe District, Shenyang City 110866, People's Republic of China

ARTICLE INFO

Keywords:
'Nanguo' pear
Aroma
Ethylene signaling
Low-temperature conditioning

ABSTRACT

The optimum harvest time for 'Nanguo' pears lasts for only 20 days. Refrigeration is a common technique used to extend the fruit supply. However, with low-temperature storage, the proper aroma of the pear is lost when it matures during shelf-life at room temperature. In this study, we investigated the ability of low-temperature conditioning (LTC) to regulate aroma esters of 'Nanguo' pears, and identified the key genes in the ethylene signal transduction pathway based on RNA-seq analysis. LTC caused higher ethylene production and effectively alleviated the loss of aroma-related esters in 'Nanguo' pears during refrigeration and during the subsequent shelf-life at room temperature. Furthermore, the expression levels of *PuERS1*, *PuEIN4*, *PuEIN2*, and *PuERF* were increased under LTC treatment. Thus, we speculate that the alleviation effect of LTC on fruit aroma esters is closely related to ethylene signal transduction, including up-regulated expression of *PuERS1*, *PuEIN4*, *PuEIN2,* and *PuERF*.

1. Introduction

'Nanguo' pears (*Pyrus ussuriensis* Maxim.) are native to Liaoning province, China. They are normally harvested in early September, and after an appropriate maturation period the fruit become a golden color with a sour-sweet taste, being particularly well-known for their attractive fragrance. This combination of flavor and aroma makes the 'Nanguo' pears a highly popular fruit among the Chinese population (Wang et al., 2017; Zhou et al., 2015). However, as a climacteric fruit, the relatively short shelf-life at ambient temperatures has negatively influenced the market value of 'Nanguo' pears. Thus, cold storage is widely used to extend the marketing time by inhibiting the physiological activities in 'Nanguo' pears (Wang et al., 2017); however, long-term refrigeration may cause the fruit to be susceptible to chilling injury (CI) and gradually develop CI symptoms, including the low production of aroma esters, which is a serious problem (Zhou et al., 2015).

Fruit aroma plays a well-established role in determining the final sensory quality and is a large determinant of consumer preference of a fruit. The aroma of pear cultivars is attributed to several volatile compounds that have been extensively studied, including esters, aldehydes, alcohols, ketones, and hydrocarbons (Willner, Granvogl & Schieberle, 2013). Esters are the most important volatile compounds contributing to the typical aroma of the ripe fruit of 'Nanguo' pears.

Fruit ripening is a rather complex process (Kuang et al., 2017), which depends on multifarious pre- and post-harvest elements. 'Nanguo' pear is a typical climacteric fruit, characterized by an increase in the production of ethylene and a respiration rate burst at the onset of ripening (Paul, Pandey & Srivastava, 2012). As a gaseous plant hormone, ethylene plays a critical regulatory role in the growth and development of climacteric fruits (Liu, Pirrello, Chervin, Roustan & Bouzayen, 2015). Indeed, a previous report confirmed that kiwifruit is very sensitive to ethylene, and ethylene production is accompanied by the emergence of maturity features (Chiaramonti & Barboni, 2010), which influences the formation of aroma-related esters (Guenther et al., 2015). Tomato is the primary model of climacteric fruits, and quality of the fruit has been shown to be largely dependent on the plant hormone ethylene (Alexander & Grierson, 2002), which regulates several aspects of tomato quality, including fruit color, texture, aroma, and flavor (Karlova et al., 2011). Ethylene functions as a developmental driver, and the ethylene production peak is accompanied by a burst of volatiles, pulp softening, and a pericarp color change from green to yellow during banana fruit ripening (Hong et al., 2015). Moreover, several studies have demonstrated that ethylene may be a modulator of volatile biosynthesis during fruit ripening (Sdiri, Rambla, Besada, Granell & Salvador, 2017; Li et al., 2014; Garcia-Rojas et al., 2016).

These biological effects of ethylene on fruit ripening are largely achieved *via* the ethylene signaling pathway. There are five main components involved in the ethylene signaling pathway: ethylene

* Corresponding author.
E-mail address: jsjsyau@yeah.net (S. Ji).
[1] Miaomiao Yao and Xin Zhou are co-first authors and they have contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2018.05.024
Received 22 January 2018; Received in revised form 24 April 2018; Accepted 1 May 2018
Available online 08 May 2018

receptors; CTR1, a negative regulator; EIN2, a positive regulatory factor in the endoplasmic reticulum (ER); EIN3, a transcription factor located in the nucleus; and the transcription factor ERF. The ethylene receptors, as the first components of the ethylene signal transduction pathway, can be divided into two subfamilies according to the ethylene-binding domain sequence: the first subfamily comprises ETR1 and ERS1 and the second subfamily comprises ETR2, EIN4, and ERS2. The next ER-localized downstream component associated with ethylene receptors is the Raf-like Ser/Thr kinase CTR1, which plays a negative regulatory role in ethylene signaling. Another protein located in the ER membrane, EIN2, plays a pivotal role in the ethylene signaling cascade and consists of 12 transmembrane helices at the *N*-terminal, which is regulated by ethylene and a hydrophilic *C*-terminus (Wen et al., 2012; Bisson, Bleckmann, Allekotte & Groth, 2009); ethylene sensitivity will be lost without this component. Within the nucleus, the EIN3 and EIL (EIN3-like) proteins belong to a small family involved in the regulatory cascade and function downstream of EIN2 in the signaling pathway (Li et al., 2012). Moreover, members of the EIN3/EIL family can stimulate the expression of other transcription factors (such as the ethylene transcription factor ERF1, which is a member of the large ERF transcription factor family), control the final step of the ethylene signal pathway and are thus directly involved in regulating fruit ripening and quality (Xie et al., 2014).

In recent years, many studies have revealed a relationship between the above-mentioned ethylene signaling elements and low temperature, indicating that the ethylene signaling pathway is modulated by temperature. For example, an increase in expression levels of ethylene receptor and response regulator genes such as *CTR1* was detected in response to cold storage in tomato (Rugkong, McQuinn, Giovannoni, Rose, & Watkins, 2011), 'Conference' pears (Chiriboga et al., 2013), and kiwifruit (Yin et al., 2009). In peach (Wang et al., 2017), cold storage induced the expression of several downstream signaling elements, including *CTR1*, *EIN2,* and *EIN3/EIL*, but resulted in overall reduction of the levels of ethylene receptor genes. Some studies showed that the expression of *ERFs* was induced in response to low temperature and then decreased during cold storage, including in papaya (Zou et al., 2014) and kiwifruit (Yin et al., 2012). However, there is no information available on the complete expression profiles of the genes involved in the ethylene signaling pathway in response to low-temperature stress in 'Nanguo' pears.

Many studies have suggested that the reduced release of aroma compounds in fruit during cold storage is due to the low production of ethylene in response to reduced temperature. For example, in peach, the changes in ethylene production caused by cold storage appear to play a role in regulating the biosynthesis of volatile compounds after cold storage (Zhang et al., 2011). Similar conclusions have been reached in the fruits of kiwi (Guenther et al., 2015) and pear (Lia, Jia, Li, Li, & Teng, 2016). These findings suggest an association between the changes of genes involved in ethylene signaling and the decline of aroma-related esters during cold storage; however, there is limited information available on this issue with respect to pears.

Low temperature conditioning (LTC) is a valuable post-harvest strategy to remarkably alleviate the CI of vegetables and fruit (Chaudhary, Jayaprakasha, Porat, & Patil, 2014; Wang et al., 2017; Zhang et al., 2017), and is widely carried out to alleviate the loss of aroma-related esters and peel browning induced by low-temperature stress in 'Nanguo' pears (Zhou et al., 2015; Wang et al., 2017). Based on this background, we hypothesized that LTC would prevent the loss of aroma, through regulation of ethylene signal transduction in 'Nanguo' pears. Accordingly, the aim of the present study was to determine the effect of LTC on preventing the loss of aroma esters in 'Nanguo' pears during storage and identify the underlying regulatory mechanism.

RNA-sequencing (RNA-seq) is an efficient method to identify differentially expressed genes in different samples and has been used to reveal a large number of genetic resources of 'Nanguo' pears. On the basis of transcriptome analysis, we used RNA-seq to investigate the regulation of refrigeration for 90 days (the LTC treatment) on six crucial genes involved in ethylene signal transduction in 'Nanguo' pears at the mRNA level during cold storage. We further determined the effects of LTC on the subsequent shelf-life at room temperature, with a focus on ripening and aroma-related esters changes, to gain a better understanding of the role of ethylene signal transduction in the release of 'Nanguo' pears aroma esters under LTC treatment.

2. Materials and methods

2.1. Plant material and treatment

The fruit of 'Nanguo' pears (*Pyrus ussuriensis* Maxim.) were harvested at maturity (fruit weight: 75 ± 5 g and firmness: 19.5 ± 0.5 N) from trees grown in a commercial orchard located in Anshan, Liaoning Province, China (41.07 °N, 123.00 °E) on September 17, 2016. The picked fruit were carefully packed in plastic boxes and transported to the laboratory of Shenyang Agriculture University within 3 h. Fruit of uniform size and ripeness without insect pests or mechanical injury were selected as the experimental materials, and were randomly divided into two groups of 600 fruit each. First, both groups were placed in unsealed polyethylene bags (200 fruit placed in each bag, 6 bags in total) with 0.04-mm thickness, to alleviate the loss of water, and were stored at room temperature (20 ± 1 °C) for 5 days during the pre-ripe stage.

The first group (control) was maintained at 0 ± 0.5 °C with 80–85% relative humidity for 90 days, and then transferred to room temperature (20 ± 1 °C) for 12 days to simulate shelf storage. The second group was subjected to the LTC treatment in the following stages. First, the fruit were exposed to 10 °C for 3 days and then the temperature was subsequently reduced to 5 °C for 2 days, and then finally stored at 0 ± 0.5 °C to 90 days.

2.2. Fruit sampling

The fruit were first sampled at the day after pre-ripeness, and then the fruit of the control group and the LTC-treated group were respectively sampled at 0, 3, 6, 9, and 12 days of shelf life for subsequent ripening followed by 90 days of cold storage at 0 °C. Three independent biological replicates were established for both groups, and 30 fruit were sampled for each replicate at each sampling point.

The fruit firmness, ethylene production, aroma esters production, and gene expression levels of *PuERS1, PuEIN4, PuCTR1, PuEIN2, PuEIN3,* and *PuERF* were tested for the same sample at each time point. All of the collected samples were immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation and analysis.

2.3. Measurement of fruit maturity

Fruit firmness (Zhou et al., 2015) was measured destructively during ripening using a TA-XT2i Plus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a 2-mm plunger tip, and the results are expressed in newtons (N). The rate of penetration was 3 mm/s with a final penetration depth of 5 mm. Four measurements were taken on opposite sides of the equator of each fruit after the removal of a slice of the skin.

For measuring ethylene production, four fruits from each treatment were weighed and enclosed in a sealed 1.2-L plastic container for 5 h at 20 ± 1 °C. Then 1 cm^3 of headspace gas was sampled; the concentration of ethylene (μL kg^{-1} h^{-1}) in a 1-cm^3 headspace sample measured using a gas chromatograph (CP-3800; Varian, Palo Alto, CA) equipped with a flame ionization detector. The injector, detector, and oven temperatures were respectively 110 °C, 140 °C, and 90 °C.