Postharvest Biology and Technology 113 (2016) 8–16

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Effects of hot air and methyl jasmonate treatment on the metabolism of soluble sugars in peach fruit during cold storage

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ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 23 October 2015
Accepted 25 October 2015
Available online 9 November 2015

Keywords:
Prunus persica
Hot air
Methyl jasmonate
Sugar metabolism

ABSTRACT

Soluble sugar metabolism affects the quality and chilling resistance of postharvest peach fruit. Although hot air (HA) and methyl jasmonate (MeJA) treatments are often effective in reducing chilling injury (CI), little is known about the relationship between sugar metabolism and HA or MeJA treatments in peach fruit. In this study, peach fruit was treated with hot air at 37 °C for 3 days or MeJA vapor at 10 μmol/L for 24 h before storage at 5 °C. Soluble sugar content, gene expression and enzyme activities associated with sugar metabolism were measured. Both treatments resulted in an initial increase, then a decrease in sucrose content over the course of storage time. Sucrose levels at every time point, but one, throughout the experiment were significantly higher than in control fruit, paralleled by higher gene expression and activity of *SPS* (sucrose phosphate synthase) and lower expression and activity of *AI* (acid invertase). HA-treated fruit had the highest sucrose content at the end of storage and the mildest CI symptoms. All treated fruit had higher sorbitol content and lower levels of *SDH* (sorbitol dehydrogenase) gene expression than control fruit. After 21 days in cold storage, sucrose content had decreased sharply in the control group, hexose content was not markedly affected, perhaps due to the increased expression of *PFK* (phosphofructokinase), resulting in more glucose entering the Embden–Meyerhof–Parnas pathway (EMP). These results suggest that the increase in sucrose observed during cold storage, associated with higher *SPS* and lower *AI* levels, enhances the chilling tolerance observed in HA- and MeJA-treated fruit.

1. Introduction

Because peaches (*Prunus persica* L. Batsh) ripen and deteriorate quickly at ambient temperature, cold storage is used to slow the ripening process and the development of decay. Peaches are however sensitive to low temperature, and symptoms of chilling injury (CI) develop within 1 or 2 weeks when fruit is stored at 2–5 °C; these symptoms include wooliness, flesh browning, reddening and leatheriness (Lurie and Crisosto, 2005). Hot air treatment (HA), an environmentally friendly technology, has proven to be an effective method for reducing CI in peach fruit by enhancing antioxidant system activity, stimulating the accumulation of heat shock proteins, and maintaining membrane integrity (Cao et al., 2010; Murray et al., 2007; Wang et al., 2014). Methyl jasmonate (MeJA), a natural plant regulatory compound, can trigger defense mechanisms against chilling stress and plays an important role in alleviating CI in peaches (Meng et al., 2009) by enhancing cell membrane integrity and antioxidant system activity (Cao et al., 2009), increasing the polygalacturonase/pectin methylesterase ratio (Jin et al., 2009) and increasing the content of proline and γ-aminobutyric acid (Cao et al., 2012).

Sugars, including soluble sugars and sugar alcohols, are significant sources of energy, contribute to the quality and taste of fruits (Cai et al., 2015), and affect fruit stress resistance (Der Agopian et al., 2011). Puig et al. (2015) put forward that sugar partitioning and demand during cold storage may play a role in the tolerance mechanism of peach fruit. The soluble sugars in peach fruit include sucrose, sorbitol, glucose and fructose. Sucrose is well known as the major carbohydrate in peach fruit, and it is hypothesized that high sucrose and glucose content can alleviate CI symptoms in peaches (Abidi et al., 2015). Wang et al. (2013) demonstrated that sucrose plays a more important role than glucose or fructose in protecting peach fruit and alleviating CI during cold stress. Jiang et al. (2013) have suggested that sucrose is

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http://dx.doi.org/10.1016/j.postharvbio.2015.10.013
0925-5214/

more important than hexose in protecting grape branches from cold injury under low temperature conditions. In potato (*Solanumtuberosum* L.) microtubers and field-grown tubers, higher sucrose levels were observed at 4 °C than at 10 °C (Pathirana et al., 2008). Sucrose regulates osmotic pressure (Anchordoguy et al., 1987), stabilizes cell membrane structure (Oliver et al., 2002), activates the anti-oxidization system to eliminate free radicals (Nishizawa et al., 2008), and regulates metabolic pathways (Lalonde et al., 1999).

Researchers have demonstrated that HA treatment can modify sugar metabolism in postharvest fruit at chilling and non-chilling temperatures. Lara et al. (2009) showed that during storage at ambient temperature, peach fruit treated at 39 °C for 3 days prior to storage has significantly decreased sucrose content and increased levels of reducing sugars, compared to non HA treated peach fruit. Shao et al. (2013) found that heat treatment increased levels of reducing sugars in loquat fruit under chilling stress. Holland et al. (2002) demonstrated that heat treatment prevents the decline of sucrose content in citrus fruit stored at chilling temperature. In toto, research indicates that sucrose is a necessary factor in heat-induced chilling tolerance. In addition, treatment with MeJA at low concentrations significantly increases the sucrose content in two guava fruit cultivars during storage for various times at 5 °C, followed by two days at 25 °C (González-Aguilar et al., 2004).

To our knowledge, no studies have focused on the effect of HA and MeJA treatments on sugar metabolism in peaches exposed to low temperatures. Therefore, the objective of our study was to examine the effect of HA and MeJA treatments on sugar content in peach fruit and associated changes in gene expression and enzyme activity during cold storage.

2. Materials and methods

2.1. Plant material and experimental design

Peach fruits (*Prunus persica* L. Batsch) were harvested from a commercial orchard in Fenghua, Zhejiang Province, China. Fruits were immediately transported to our laboratory, selected for uniformity of color and the absence of physical damage, and randomly divided into three groups (HA treated, MeJA treated and non-treated control) of 105 peaches each. The first group was heat-treated at 37 °C for 3 days, after which fruits were stored at 5 °C for 5 weeks. The second group was treated with 10 μmol/L MeJA (Sigma-Aldrich, Madrid, Spain) vapor in a sealed container for 24 h at 5 °C. After treatment, the container was opened and ventilated for 1 h, and then fruits were stored at 5 °C for 5 weeks. The last group (control) was stored at 5 °C for 5 weeks immediately after selection. After cold storage for 0, 7, 14, 21, 28, or 35 days, CI indexes were assessed immediately after removal from cold storage, and then samples of five fruits from each replicate were mixed and frozen immediately in liquid nitrogen, and then stored at −80 °C. Each treatment was replicated three times and the experiments were conducted twice.

2.2. Evaluation of CI index

Internal browning (IB) is the visual characteristic symptom of CI. CI was assessed the visually IB on five fruits from each replicate after cutting the fruits along their axial diameters. The severity of CI was scored on a scale ranging from 0 to 4: 0 = none of IB, 1 = slight, 2 = moderate, 3 = moderately severe, 4 = severe. The results were expressed as the CI index, calculated using the following formula: CI index = [(CI score)(number of fruits with that CI score)]/(4 × total number of fruits in each treatment).

2.3. Measurement of soluble sugars

The method of Shao et al. (2013) was used to determine the soluble sugar content of the frozen fruit. Briefly, 5 g frozen peach tissue was ground with 0.5 ml of solution I [5.48% (w/v) zinc acetate: glacial acetic acid (97:3)] and 0.5 ml solution II [potassium ferrocyanide 2.65% (w/v)]. The homogenate was diluted to 25 ml with deionized water and passed through a 0.2 μm filter. Soluble sugars were measured using a high performance liquid chromatography (HPLC) system (Model 2695, Waters, USA), an X Brige™ Amide Column (3.5 μm, 4.6 × 250 mm, USA), and a refractive index (RI) detector (Model 2414, Waters, USA). A sample of 20 μl was injected into the HPLC system for analysis. Acetonitrile/water (80:20, v/v) was used as the solvent at a flow rate of 1 ml min^{-1} at 35 °C.

2.4. RNA isolation and real-time PCR analysis

Total RNA from frozen peach samples was isolated from 2 g of tissue according to the method described by Meisel et al. (2005). First, samples were subjected to several extractions with chloroform:isoamyl alcohol (24:1) to remove protein. Samples were then treated with RNase-free DNase (TaKaRa, Japan) to eliminate genomic DNA, following the manufacturer's instructions. RNA integrity was assessed by agarose electrophoresis. The concentration of DNA-free RNA was determined using a spectrophotometer (NanoDrop 1000, Thermal, USA). RNA samples were stored at −80 °C.

First-strand cDNA was synthesized from 2 μg of treated total RNA using the SYBR PrimeScript RT-PCR kit II reverse transcriptase (TaKaRa, Japan) following the manufacturer's instructions. The cDNA was diluted 10-fold with DEPC treated water and stored at −20 °C prior to RT-qPCR analysis.

Relative gene expression levels were determined by RT-qPCR using the SYBR Green kit II (Takara, Japan) as a fluorescent reporter. RT-qPCR primers sequences are shown in Table 1, which was according to the design of Wang et al. (2013) and the *P. persica* expressed sequence tag (EST) database (TIGR Plant Transcript Assemblies; http://plantta.Tigr.org) (Childs et al., 2007). These gene included: *AI* (GenBank ID KC905744), *NI1* (GenBank ID AM409095), *NI2* (GenBank ID XM_007221355), *SS* (GenBank ID JQ412752), *SPS* (GenBank ID JQ412751), *SDH* (GenBank ID AB025969), *PFK* (GenBank ID KC700019) and *G6PDH* (GenBank ID XM_007215059). PCR reactions were performed in a total volume of 20 μl, which included 1.6 μl of cDNA, 0.8 μl of each primer (10 μM), 10 μl of SYBR Green kit II, and 6.8 μl of RNase-free water. The real-time PCR program was initiated with a preliminary step of 2 min at 95 °C, followed by 40 cycles of 94 °C for 15 s, 55 °C for 20 s and 72 °C for 20 s. Relative gene expression was calculated using the 'Comparative $2^{-\Delta\Delta CT}$' method (Livak and Schmittgen, 2001). Translation elongation factor 2 (TEF2, JQ732180.1) was used as an internal control, and cycle threshold (Ct) numbers were extracted for both the reference and target genes. Each RNA sample

Table 1
Real-time PCR primer sequences for genes related to sugar metabolism.

Gene	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
AI	TCATACGCCCATACCACCAG	CGAAATCGGAATCGAATAGC
NI1	TGCTCTGGAGTATGAAGAATGG	ATCCACTGCCTTTTGTGCTAAC
NI2	CTATGACACCAAAAGGGGTAGG	GCTTTCTTCTTGGGTTAGCACT
SS	ATGAGGAGAAGGCTGAGATGAAG	CAAGTAGCGAATGTTGGAAGTC
SPS	TTGAGGCTACAGGAAAGGAAAG	GGACGCTCCTCTGAATGAATAG
SDH	GCAGACTTTGTTGTTCAAGAGC	TCATGTCAGGGCAGAGATTG
PFK	TCTTGCATCGACGAACCAGC	AAGCCGTAAGTCATGTCACCTTG
G6PDH	GGTCCAGCAGAAGCCGATG	CGTTATGGTATATGGCACACACTG
TEF2	TGAAGGAGAGGGAAGGTGAAAG	TGAAGGAGAGGGAAGGTGAAAG