Postharvest Biology and Technology 129 (2017) 52–63

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

1-Methylcylopropene and controlled atmosphere modulate oxidative stress metabolism and reduce senescence-related disorders in stored pear fruit

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ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form 16 March 2017
Accepted 16 March 2017
Available online xxx

Keywords:
γ-Aminobutyrate
Ascorbate
Controlled atmosphere
Glutathione
1-Methylcylopropene
Pear

ABSTRACT

European pears (*Pyrus communis* L.) are stored under low temperatures to extend postharvest life. Unfortunately, senescent scald and internal breakdown are likely to occur with prolonged exposure to storage. Both disorders can be reduced by controlled atmosphere (CA) and/or the ripening inhibitor, 1-methylcylcopropene (1-MCP). The principal aim of this study was to investigate the effect of 1-MCP and CA on fruit quality, including physiological disorders, and oxidative stress metabolites in stored 'Cold Snap' and 'Swiss Bartlett' pears. Freshly harvested pears were treated with or without 1-MCP, and then stored at 0 °C under refrigerated air or CA (18 kPa or 2.5 kPa O_2, and 2 kPa CO_2) for at least 167 d. 1-MCP and CA delayed and/or reduced the rates of ethylene production in stored fruit of both cultivars. 1-MCP and CA delayed fruit softening and peel yellowing in 'Swiss Bartlett' pears, but had negligible to slight effects with 'Cold Snap'. In both cultivars, high incidences of senescent scald and internal breakdown occurred in non-1-MCP-treated pears during refrigerated air storage. For the most part these symptoms were reduced by CA and 1-MCP, resulting in minimal to negligible incidence in 1-MCP-treated pears stored at 2.5 kPa O_2. γ-Aminobutyrate accumulated in stored pears, although 1-MCP and CA slightly reduced the levels in 'Cold Snap' fruit and 1-MCP increased levels in 'Swiss Bartlett' fruit. Ascorbate (total and reduced) levels were rapidly depleted in 'Cold Snap' fruit, regardless of treatment; these levels were better maintained in 1-MCP-treated 'Swiss Bartlett' fruit than control fruit across all storage atmospheres. In both cultivars, glutathione (total and reduced) concentrations and redox status fluctuated during storage, although these levels were generally higher in 1-MCP-treated fruit. Moreover, glutathione depletion occurred in advance of the development of senescence disorders in stored pear fruit.

1. Introduction

European pear (*Pyrus communis* L.) belongs to the Rosaceae family and is the fifth most cultivated tree fruit commodity in North America (FAOSTAT, 2016). The cultivar 'Bartlett' represents 75% of all pears that are grown in this region (Ingels et al., 2007). A major challenge for the production of 'Bartlett' and its strain 'Swiss Bartlett' is their susceptibility to fire-blight in the orchard (Bonn and van der Zweet, 2000; Hunter and Slingerland, 2008). Therefore, new cultivars with resistance to fire-blight have been developed over the last decade. The fire-blight resistant pear cultivar 'Cold Snap' (formerly 'Harovin Sundown'; Harris, 2016) was developed by crossing 'Bartlett' with US56112-146 (Hunter et al., 2009). To date, it is not known whether storability of pears differs between fire-blight resistant and susceptible cultivars. In order to extend the market for freshly harvested pear fruit, most European cultivars are stored under low temperature (-1 to 10 °C), including controlled atmosphere (CA) for periods of 3–10 months (Kader, 2007; Kupferman, 2003; Villalobos-Acuña and Mitcham, 2008). A consequence of prolonged storage can be the

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http://dx.doi.org/10.1016/j.postharvbio.2017.03.008
0925-5214/

development of internal breakdown and senescent scald in 'Bartlett' and 'Cold Snap' pear fruit (DeEll and Ehsani-Moghaddam, 2011; Lum et al., 2016b). Symptoms of internal breakdown (also known as internal browning and core breakdown) first appear as browning of the core tissue followed by cortical browning (Lum et al., 2016b; and references therein). Senescent scald is browning of the peel surface that can extend into the underlying flesh, and is etiologically distinct from superficial scald (Lum et al., 2016b; Lurie and Watkins, 2012). Superficial scald develops during the post-storage warming period and occurs as irregular brown patches of the peel that can become rough; it is associated with accumulation of the volatile α-farnesene and its subsequent oxidation (Lurie and Watkins, 2012). Interestingly, senescent scald is not associated with the accumulation of these metabolites in 'Abbé Fétel' pears (Vanoli et al., 2010). Both internal breakdown and senescent scald coincide with ethylene production in stored pears (Argenta et al., 2016; DeEll and Ehsani-Moghaddam, 2011; Ekman et al., 2004).

Ethylene-mediated ripening and senescence of pear fruit are effectively reduced by CA, specifically the use of low O_2 partial pressures of 1–2.5 kPa and elevated CO_2 partial pressures of 0.5–5 kPa (Drake, 1994; Kader, 2007; Kupferman, 2003). Unfortunately, CA can promote core breakdown in 'Conference' and 'Rocha' pears (Deuchande et al., 2016; Lum et al., 2016b). A complementary strategy to CA is the pre-storage application of the ripening inhibitor 1-methylcyclopropene (1-MCP) (Watkins, 2006, 2015). 1-MCP-treated 'Bartlett' pears are less susceptible to internal breakdown and senescent scald during low temperature storage than non-1-MCP-treated fruit (DeEll and Ehsani-Moghaddam, 2011; Wang and Sugar, 2015). Pear fruit, including 'Cold Snap', are free of these senescence disorders when 1-MCP treatment is combined with CA; a drawback of these postharvest treatments is the dramatic reduction or inhibition of ethylene-mediated ripening (Gago et al., 2015; Lum et al., 2016b; Ma and Chen, 2003; Villalobos-Acuña and Mitcham, 2008). The biochemical mechanisms associated with the development of senescent scald and internal breakdown in 'Bartlett' type pear fruit, and their control by 1-MCP and CA, are not well understood.

In stored pear fruit, a temporal reduction in respiration-derived ATP coincides with an increase in fermentation-related metabolites and NAD(P)H (Lum et al., 2016b). NADPH is used to generate reactive oxygen species and for the catabolism of γ-aminobutyrate (GABA) (Foyer and Noctor, 2011; Lum et al., 2016b). Low temperature and hypoxia cause the accumulation of GABA in apple and pear fruits, which coincides with physiological disorders (Leisso et al., 2015; Lum et al., 2016a, 2016b). Alternatively, GABA levels are rapidly elevated in CA-treated plant tissues and this can occur in the absence or prior to the appearance of visible injury symptoms (Lum et al., 2016b; Zhou et al., 2016).

In stored pome fruit, a shift in cellular NADPH redox status culminates in a decline in the antioxidant capacity within the fruit, as this cofactor is used to power the ascorbate-glutathione recycling pathway to generate reduced glutathione (GSH) and reduced ascorbate (AA) required for sequestering reactive oxygen species (Lum et al., 2016a, 2016b). Decreased ascorbate levels occur in advance of core breakdown and internal browning in pears, but in some cultivars this is evident in the absence of disorders (Deuchande et al., 2016; Lum et al., 2016b). Similarly, glutathione levels and its redox balance tend to fluctuate in CA-stored pear fruit, and can contribute to the occurrence of physiological disorders, including CA-related injury in 'Honeycrisp' apples (Lum et al., 2016a, 2016b). To date, no information exists on whether shifts in ascorbate, GABA and glutathione are linked to the development of senescent scald and internal breakdown.

In this study, we investigated the effect of 1-MCP and CA (18 kPa or 2.5 kPa O_2 in the presence of 2 kPa CO_2) versus refrigerated air on the incidence of senescent scald and internal breakdown in pears of two genotypes, 'Swiss Bartlett' and 'Cold Snap', as a function of storage period at 0 °C. The near ambient O_2 partial pressure of 18 kPa was chosen as a control for the low O_2 treatment and its corresponding impact on disorder development and oxidative stress metabolism. The temporal development of senescence disorders in these pear fruit was compared to changes in whole fruit concentrations of GABA, AA, dehydroascorbate (DHA), GSH and glutathione disulphide (GSSG).

2. Materials and methods

2.1. Plant material and postharvest treatments

In 2012, pear fruit were harvested during the commercial harvest period. 'Swiss Bartlett' pears (*Pyrus communis* L.) were harvested on August 22 from a commercial orchard near Port Burwell, ON, Canada, whereas 'Cold Snap' pears were harvested on September 11 from a commercial orchard near Simcoe, ON, Canada. For each cultivar, the harvested material was randomly divided into 24 boxes (~17 kg of fruit per box) for postharvest treatments.

Pears were transported to the storage research facility at the University of Guelph (Guelph, ON, Canada) within 4 h of their harvest. For each cultivar, 12 boxes containing pears were equally distributed into three separate air-tight 6 mil polybags, and exposed to 300 nL L^{-1} 1-MCP for 24 h at the ambient temperature of 23 °C. The 1-MCP gas concentration used for each polybag treatment is equivalent to the registered label rate for SmartFresh™ in Canada, and was calculated as described previously (Lum et al., 2016a). A similar quantity of boxes not exposed to 1-MCP remained at 23 °C for 24 h.

Following 1-MCP exposure, polybags were opened and ventilated. A complete randomized block design was used for the storage experiment to eliminate the possibility of room and chamber effects. Four separate temperature-controlled rooms were set to 0 °C, each containing two lab-style CA chambers (0.5 m^3 volume; Storage Control Systems, Sparta, MI, United States). Each CA chamber contained one box each of 'Cold Snap' pears treated with 1-MCP (1-MCP fruit) and one box of non-1-MCP fruit (non-1-MCP fruit). In addition, each CA chamber contained one box each of 'Swiss Bartlett' fruit treated with 1-MCP and one box of non-1-MCP fruit. All CA chambers were sealed with a plexiglass lid and flushed with 98 kPa N_2 until the target O_2 concentration (18 kPa or 2.5 kPa O_2) was realized. Thereafter, CO_2 was added until a partial pressure of 2 kPa was achieved; this CO_2 concentration was chosen as an intermediate partial pressure to the range (0.5–5 kPa CO_2) that is typically used for prolonged storage of pear fruit in other jurisdictions (Kader, 2007). Also, one box of 1-MCP fruit and one box of non-1-MCP fruit from each cultivar were stored within each cold room without CA (refrigerated air). For each cultivar and treatment, fruit were sampled periodically during the storage period. 'Swiss Bartlett' fruit were sampled at 6, 13, 34, 55, 111, and 167 d, whereas 'Cold Snap' fruit were sampled at 7, 21, 28, 56, 119 and 180 d. For each sampling, 14 pears were chosen randomly for assessment of quality, including physiological disorders (10 pears) and metabolite analysis (four pears) as described below. Fruit sampled for metabolite analysis were immediately frozen in liquid N_2 and stored at −80 °C.

2.2. Evaluation of fruit quality

Immediately after harvest, fruit maturity was assessed on each of three biological replicates (replicate represented fruit from one of three distinct locations within the orchard) of 10 pears. For each replicate, three representative pears were transferred to a 9 L plastic vessel and sealed air-tight for 60 min. At the end of the