Low oxygen pre-storage treatment is effective in reducing chilling injuries of deciduous fruit

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Abstract: Apple and pear fruits stored at low temperatures may suffer from chilling injury symptoms, caused by oxidative stress. Application of a low-oxygen (LO_2) atmosphere (0.5%) for 10 d at 20°C or 500 ppb 1-methylcyclopropene (1-MCP) at 20°C for 24 h, prior to cold storage at 0°C, were equally effective in reducing superficial scald on 'Granny Smith' apples, after six months of cold storage at 0°C plus seven days at 20°C. Compared to untreated control fruit, the LO_2 and 1-MCP-treated fruit produced less ethylene, α-farnesene and its oxidation product, 6-methyl-5-hepten-2-one (MHO), as determined by SPME/GC-MS technique. In addition, LO_2 pretreatment applied to Californian 'Bartlett' or Israeli 'Spadona' pears, was effective in reducing superficial scald, senescent scald and internal breakdown, after 4–4.5 months of cold storage at –1°C or 0°C, respectively, plus five to seven days at 20°C. We assume that LO_2 and 1-MCP pretreated fruit remained free of physiological disorders, due to the reduced production of ethylene and the oxidation product MHO during cold storage.

Keywords: *Malus domestica*; *Pyrus communis* L. ethylene; α-farnesene; 1-MCP; low-oxygen; superficial scald.

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1 Introduction

In apple and pear fruits, low-temperature storage results in oxidative stress and development of chilling injury symptoms, which are expressed as superficial scald (Watkins et al., 1995). It has been hypothesised that oxidation products of α-farnesene in the fruit peel, identified as several reactive oxygen species (ROS) of conjugated trienes (CT), are the main cause of superficial scald development (Anet, 1972; Ghahramani and Scott, 1998; Mir et al., 1999; Rowan et al., 2001; Whitaker, 2004). 1-MCP has already been shown to be very effective in preventing superficial scald development by reducing ethylene, α-farnesene, CTs and the end product 6-methyl-5-hepten-2-one (MHO) (Lurie and Watkins 2012; Whitaker, 2004). Also, application of a 0.5% low-oxygen (LO_2) atmosphere for ten days at 20°C to 'Granny Smith' apples prior to cold storage, was quite effective in reducing these metabolites, leading to less superficial scald appearance (Pesis et al., 2010; Sabban-Amin et al., 2011). Using confocal laser-scanning microscopy and fluorometer measurements of apple peel, we succeeded in determining ROS accumulation in control fruit, while nil amounts were found in LO_2 and 1-MCP treated fruit (Sabban-Amin et al., 2011). The effectiveness of LO_2 pretreatment in reducing other physiological diseases such as bitter pit, which is also induced during cold storage, was shown in 'Granny Smith' and 'Golden Reinders' apples (Pesis et al., 2010; Val et al., 2010).

Pear fruit are also susceptible to many physiological disorders during cold storage, including superficial scald, senescent scald and internal breakdown, which are symptoms of chilling injury (Drake et al., 2006; Whitaker et al., 2009). The development of superficial scald in 'Bartlett' pears was found to be associated with fast accumulation of α-farnesene and CTs (Whitaker et al., 2009). It is well known also in pear that 1-MCP can prevent or minimise these symptoms by reduction in ethylene, α-farnesene and CTs (DeEll and Ehsani-Moghaddam, 2011; Isidoro and Almeida, 2006). It was suggested in 'Anjou' pears that 1-MCP controlled scald by inhibiting α-farnesene synthesis and oxidation, whereas the anti-scald chemical ethoxyquin only inhibited α-farnesene oxidation (Bai et al., 2009). It is well known that pear, like apple fruit, can maintain its quality with reduced scald development in cold storage under low oxygen (Mattheis and Ruddell, 2011). However, the effect of LO_2 pre-storage treatment in reducing physiological diseases in pear has not been studied till now.

In this study, we examined the effectiveness of LO_2 pre-storage treatment and its role in preventing chilling injury symptoms in 'Granny Smith' apple and in 'Bartlett' and 'Spadona' pears.

2 Material and methods

'Granny Smith' apples from northern Israel were brought to the laboratory and treated on the day of harvest. Control fruits were immediately stored at 0°C. For 1-MCP treatment, 40 kg of fruit were placed in a sealed 250-L chamber and treated with 0.5 μL/L 1-MCP released from SmartFresh^{SM} powder (0.14% w/w active ingredient) for 24 h at 20°C. The LO_2 treatment was applied by flushing two 250-L chambers (40 kg per chamber in four plastic boxes) with N_2 gas from a cylinder until the O_2 concentration was around 2%, then sealing them and holding the temperature at 20°C for ten days, during which the O_2 concentration decreased to 0.5% (Pesis et al., 2010). After the LO_2 treatment, the chambers were opened and the fruit were transferred to cardboard boxes and stored in air at 0°C for up to six months. Three trays (ten fruit/each tray) from each treatment were transferred to 20°C for seven days of shelf life after two, four and six months of cold storage, for superficial scald assessment. In addition fruit were enclosed in jars for ethylene measurements by gas chromatograph (GC) and peel samples were taken for α-farnesene and MHO volatile measurements, using gas chromatograph mass spectrometry (GCMS) and solid phase microextraction (SPME) techniques, according the method of Pesis et al. (2010).

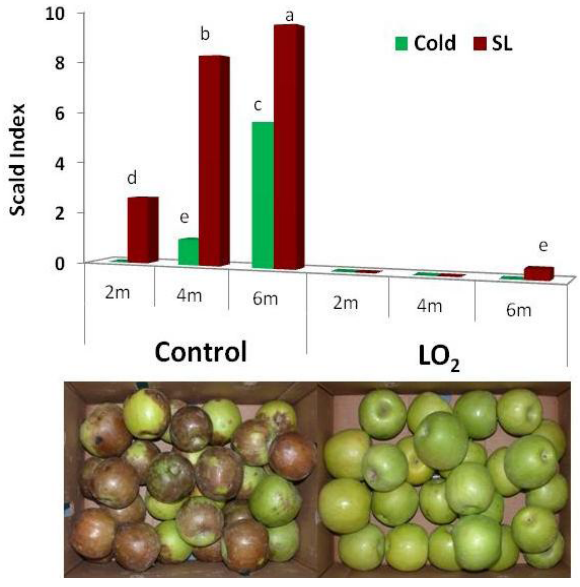
'Bartlett' pears from northern California, USA, were brought to the lab at the University of Davis and treated the next day. Control fruit were stored immediately at –1°C. For the anti-scald diphenyl amine (DPA) treatment, fruit were dipped for 5 min in a 1,750 ppm DPA solution (Pace International, Seattle, WA, USA). The LO_2 treatments were applied in 300 L stainless steel chambers. Fruit were placed in the chambers in four plastic totes, each containing 60 fruits, i.e., 240 fruit per chamber. N_2 gas was flushed from a liquid N_2 cylinder until the oxygen levels in the chamber reached less than 1% and then the chambers were sealed for 4 and seven days at 20°C. During treatment, CO_2, O_2, acetaldehyde and ethanol levels were measured daily in the chamber headspace, as described by Pesis et al. (2010). After treatment, the fruit were dried and transferred to cardboard boxes for four months' storage at –1°C plus seven days' shelf life at 20°C. Peel samples were taken for α-farnesene measurements using GCMS/ SPME technique, according to the method of Pesis et al. (2010).

'Spadona' pears from northern Israel, were treated on the day of harvest with an antifungal dip (scholar 0.1%) prior to all other treatments. Low O_2 (LO_2) atmosphere for seven days at 20°C was applied in a 700 L chamber, to 12 plastic totes containing *ca*. 120 kg fruit. During LO_2 treatment the gases CO_2, O_2, ethylene. acetaldehyde and ethanol levels were measured daily to avoid accumulation of excessive anaerobic respiration products (Pesis et al., 2010). Fruit removed from treatment were transferred to air storage at 0°C for 4.5 months plus five days' shelf life. The LO_2 treatment was compared with the commercial treatment, which is a dip in an anti-scald agent for 0.5 min (Ethoxyquin-DeccoScald 0.15%).

3 Results and discussion

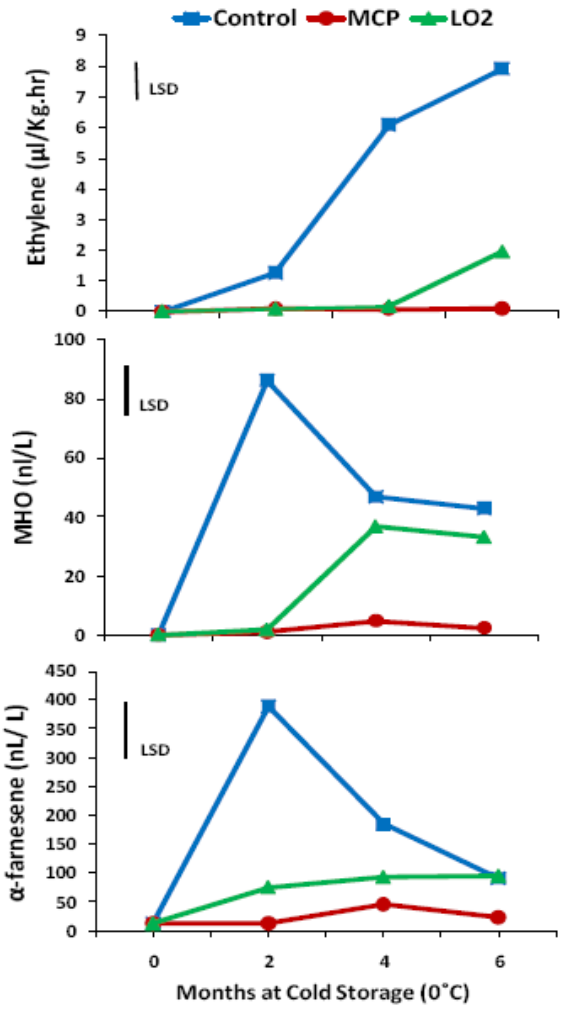
Superficial scald development in 'Granny Smith' apples was described in terms of ascending scald-severity index (0–10) (Figure 1). Unlike the control fruit, in which scald symptoms appeared at an early stage, i.e., after two months in cold storage plus seven days at 20°C, apples pretreated with LO_2 showed very minor scald symptoms only after six months' storage at 0°C (Figure 1). After six months at 0°C plus seven days at 20°C, the differences between control and LO_2-treated fruit was most obvious, all control fruit exhibiting severe superficial scald, whereas the LO_2-treated fruit were completely green (Figure 1). The reduction in scald appearance in LO_2 and 1-MCP-treated fruit was correlated to lower levels of ethylene, α-farnesene and its oxidation product MHO during six months of cold storage (Figure 1 vs. Figure 2). Many reports have shown that the effectiveness of 1-MCP in preventing scald appearance is correlated to ethylene reduction, leading to lower α-farnesene and MHO production (Mir et al., 1999; Rowan et al., 2001; Whitaker, 2004; Lurie and Watkins 2012). We assume that MHO, which was found in high amounts in the control fruit, but not in LO_2 or 1-MCP treated fruit, is one of the reasons for ROS accumulation, as we have shown previously, using confocal laser-scanning microscopy and fluorometer measurements (Sabban-Amin et al., 2011). 1-MCP reduced ethylene to minimal production during cold storage, however the LO_2 treatment, which is not a chemical treatment and may be used even for organic fruit, was also quite effective (Figure 2). The reduction in α-farnesene and its oxidation by LO_2 treatment has been shown already with 'Granny Smith' apples grown in Australia, USA and Israel (Ghahramani and Scott, 1998, Pesis et al., 2010; Sabban-Amin et al., 2011).

Figure 1 Effect of LO_2 pretreatment on superficial scald index (0–10) of 'Granny Smith' apples after two, four, and six months' storage at 0°C plus seven days' shelf life (SL) at 20°C (see online version for colours)



Note: Coloured pictures were taken after six months at 0°C + 7 days at 20°C.

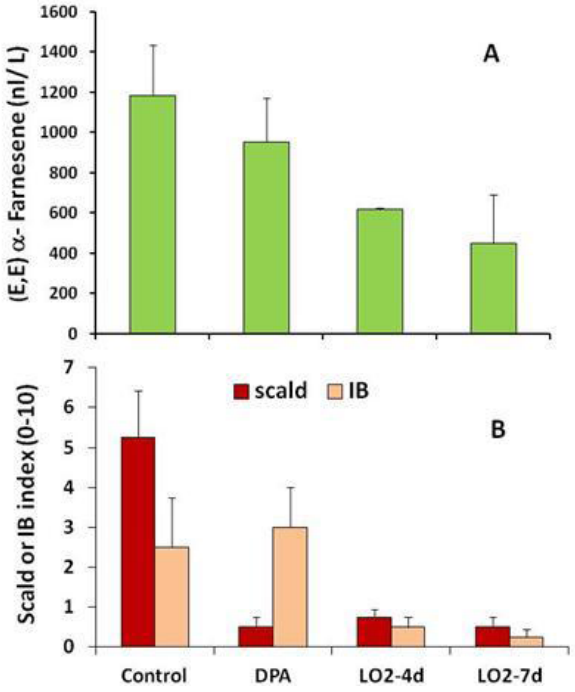
Figure 2 Effect of 1-MCP and LO_2 pre-treatment (LO_2) on ethylene, 6-methyl-5-hepten-2-one (MHO) and α-farnesene accumulation in 'Granny Smith' apples during six months at 0°C (see online version for colours)



With 'Bartlett' pears grown in California the LO_2 treatment was effective in reduction of (*E*, *E*) α-farnesene production, already after two months at –1°C (Figure 3). The reduction in (*E*, *E*) α-farnesene production was greater in the LO_2-7d-treated fruit than in the LO_2-4d-treated fruit (Figure 3). During seven days of LO_2 treatment at 20°C more anaerobic metabolites accumulated, leading to stronger inhibition of ripening and ethylene production, which increased the effectiveness in scald reduction later in storage (Figure 3). With apples also, we showed that the longer duration of LO_2 treatment (ten days) was more effective than the shorter (seven days) in controlling superficial scald (Pesis et al., 2007 vs. Pesis et al., 2010). In addition to reducing superficial scald incidence on 'Bartlett' pears, the LO_2 treatment was effective in reducing internal breakdown (IB), exhibited as brown core especially during shelf life. Here the LO_2

treatment succeeded in preventing this disorder, while the anti-scald agent DPA had no effect (Figure 3). It is known that anti-scald agents such as DPA or ethoxyquin are efficient in preventing superficial scald occurrence, but probably, as they do not inhibit ethylene and α-farnesene production, they are not effective against other disorders, including internal breakdown (Bai et al., 2009).

Figure 3 Effect of DPA dip or LO_2 pretreatment for 4 (LO_2-4d) or 7 (LO_2-7d) days on (*E,E*) α-farnesene production after two months at –1°C plus two days at 20°C (A), and on superficial scald and internal breakdown (IB) development in 'Bartlett' pears after four months at –1°C plus seven days at 20°C (B) (see online version for colours)



With 'Spadona' pears grown in Israel, the LO_2 treatment for seven days caused reductions in ethylene and CO_2 production, also in comparison with the ethoxyquin treatment, during cold storage and shelf life (Figure 4). In addition to low ethylene levels, the respiration levels were also the lowest in LO_2-treated fruit, indicating a slower metabolic rate in these fruit (Figure 4). During shelf life at 20°C the rates of ethylene and CO_2 production increased dramatically, but still the LO_2-treated pears exhibited the lowest levels (Figure 4). The low ethylene evolution during storage is probably the reason that these fruit suffered the least senescent scald and internal browning after 4.5 months in cold storage plus five days at 20°C (Figure 5). It is well known that low oxygen in cold storage can prevent scald appearance in pears, but some conditions (excessive anaerobic conditions) may cause damage, such as black speck (Mattheis and Rudell, 2011). In this work, we show that pretreatment for seven days at 20°C with low oxygen is sufficient to almost totally prevent superficial scald, senescent scald and internal browning in pear fruit.

Figure 4 Effect of ethoxyquin dip or LO_2 pretreatment (LO_2) for seven days at 20°C on ethylene and CO_2 production by 'Spadona' pears during ten weeks at 0°C and after removal to 20°C for five days (see online version for colours)

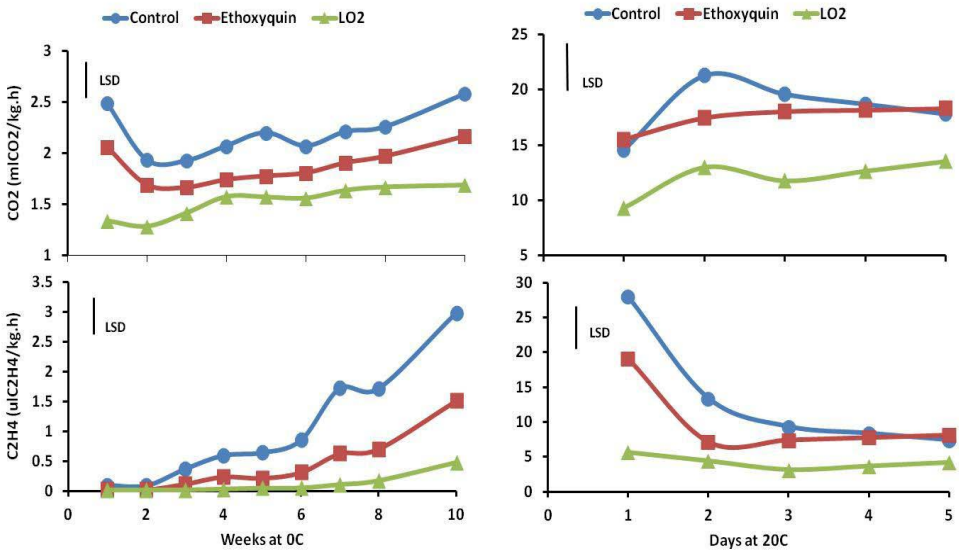
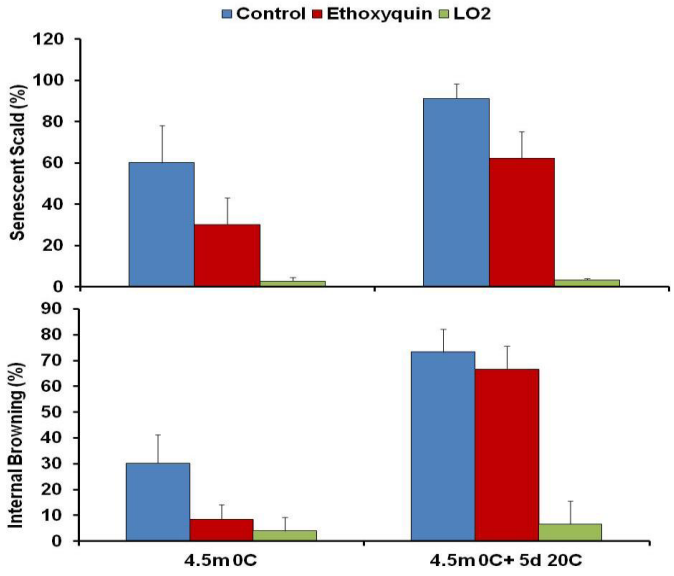


Figure 5 Effect of ethoxyquin dip or LO_2 pretreatment (LO_2) for seven days at 20°C, on senescent scald and internal browning percentage, in 'Spadona' pears after 4.5 months at 0°C plus five days at 20°C (see online version for colours)



4 Conclusions

Application of low-O_2 stress at 20°C (LO_2) for ten days to 'Granny Smith' apples and for seven days to 'Bartlett' and 'Spadona' pears prior to cold storage, were effective in reducing chilling injury symptoms exhibited as superficial scald on apples and superficial scald, senescent scald and internal breakdown in pear fruit. The effectiveness of LO_2 is probably due to the fact that these pretreatments reduced ethylene and α-farnesene production and the formation of α-farnesene oxidation products, leading to less MHO accumulation. The low-O_2 stress provides a simple non-chemical means to maintain apple and pear quality in storage that could be suitable for organic fruit markets.

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