Ascorbic Acid Content in ‘Passe-Crassane’ Winter Pear as Affected by 1-Methylcyclopropene during Cold Storage and Shelf Life

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Abstract. ‘Passe-Crassane’ is a winter pear which requires a cold storage period to produce ethylene and properly ripen. In this study, the effects of 1-methylcyclopropene (1-MCP), an ethylene perception inhibitor, were studied during cold storage (30, 60, 90, and 135 days) and shelf life at 20 °C (30 days) of ‘Passe-Crassane’ pears. Ethylene accumulation was monitored and quality parameters were measured. Oxidative stress of fruit was estimated by measuring lipid peroxidation. The cell antioxidant status was assayed determining ascorbic acid (AsA) content and the activities of the enzymes ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) involved in its oxidation and recycling. AsA content was positively affected by higher temperature (20 °C) and by 1-MCP after 90 days of storage. This effect was more evident after shelf life. Thiobarbituric acid reactive substances (TBARS) increased in pears kept at 20 °C and in treated pears, starting from 60 days of cold storage and remained elevated after shelf life. Although during storage 1-MCP enhanced the activities of APX and DHAR only at 90 days, after shelf life the effect on APX, MDHAR, and DHAR activities was more pronounced and enzyme activities were higher in treated pears sampled after 60 and 90 days of storage. The results indicate that 1-MCP has a beneficial effect on the antioxidant potential of winter pears: it maintained high AsA levels throughout storage and shelf life and improved the enzymatic mechanisms of AsA recycling, especially after shelf life. The effect of 1-MCP on pear ripening may not be solely due to its action on ethylene but also to an increase in antioxidant defense. A stress response linked to lipid peroxidation is triggered by the interaction of cold temperatures and treatment as ‘Passe-Crassane’ pears acquires ripening competence. However, it may be compensated by the high AsA content.

Most European pears (*Pyrus communis* L.) require a period of chilling exposure after harvest to produce autocatalytic ethylene, ripen properly, and synchronize the ripening (Sugar and Basile, 2013, 2014; Villalobos-Acuña and Mitcham, 2008). Winter pears need a long storage period at 0–1 °C to reach the right juiciness and to produce their typical aroma. However, physiological disorders can affect fruit quality during cold storage. Many of these disorders are due to a stressful condition that can induce the production of reactive oxygen species (ROS). ROS production is a typical feature of aerobic organisms and in normal conditions ROS concentration is kept under control by antioxidant systems present in all cell compartments. Nevertheless, certain conditions can determine an unbalance in the redox status of the cells. Plants and fruits have developed different strategies to contrast the accumulation of ROS and oxidative stress. These systems can be both enzymatic or not

and often imply the action of AsA. AsA is involved in several biological processes in plants (Arrigoni and De Tullio, 2002). In the ascorbate-glutathione cycle, H₂O₂ is converted to water by the reaction catalyzed by APX with the production of monodehydroascorbate (MDHA) and dehydroascorbate (DHA). Monodehydroascorbate reductase reduces MDHA to AsA and DHA is converted to AsA by DHAR. Glutathione reductase catalyzes the last step of the cycle reducing the oxidized form of glutathione (GSSG), produced by the action of DHAR, to the reduced form (GSH) with consumption of nicotinamide adenine dinucleotide phosphate (NADPH) (Potters et al., 2002). AsA is regarded as a biochemical marker to produce freshness and quality and its decrease is often considered a symptom of tissues senescence and is directly associated with the incidence of some physiological disorders of fruit during storage (Veltman et al., 1999).

Pear is a climacteric fruit showing a drastic increment in ethylene production during ripening (El-Sharkawy et al., 2003). The discovery of the physiological effects of 1-MCP (Blankenship and Dole, 2003) opened new scenarios in postharvest managing. 1-MCP is a gas that binds to ethylene receptors on cell

membranes, causing inhibition of exogenous ethylene perception and production of endogenous ethylene. As a result, all the physiological processes depending on ethylene are inhibited (Trinchero et al., 2004). 1-MCP is commonly applied to prevent the superficial scald in pears and to prolong shelf life. Some late pear cultivars, such as Passe-Crassane, require a long chilling treatment (60–100 d) before they gain the competence for climacteric rise and are capable of properly ripening (El-Sharkawy et al., 2003; Lelièvre et al., 1997). Analyses were conducted during storage at two different temperatures (0 °C and 20 °C), as well as after a period of shelf life at 20 °C.

Aim

The aim of this work was to evaluate the effects of factors affecting ethylene perception/production (temperature and postharvest treatment with 1-MCP) on the antioxidant potential and quality of ‘Passe-Crassane’ winter pears during storage and shelf life. Moreover, there is little information about the physiological effects of 1-MCP other than its ability to prevent ethylene action and its impact on the nutritional quality of fruit, in particular on the content of ascorbic acid in winter pear tissues during storage.

Materials and Methods

Fruit material and experimental setup. Pears (*P. communis* L., cv. Passe-Crassane) of uniform size and color were harvested at commercial maturity, before the climacteric rise in Campogalliano (Modena) in Italy (44°41′33″00 N, 10°50′34″80 E). The day after harvest, one set of samples (75 fruit) was treated with 1-MCP (0.4 µL·L⁻¹) at 20 °C for 15 h and stored at 0 °C and 95% relative humidity up to 135 d. Nontreated fruit (control, 75 fruit) were stored at the same conditions. Samples were taken after 30, 60, 90, and 135 d of storage and kept at –80 °C. Other fruit from each time point were subjected to a shelf life period of 30 d at 20 °C to evaluate the progression of ripening after storage. Another set of fruit (15) was kept at 20 °C for 30 d without 1-MCP treatment and without cold storage to simulate a period of shelf life.

Ethylene determination. Gas samples (1 mL) were taken by a syringe from the eye cavity of four pears per treatment (Saltveit, 1982) and injected in a Dani 3800 gas chromatograph (DANI Instruments S.p.A., Milan, Italy), equipped with a flame ionization detector (FID) and alumina column. The carrier gas was nitrogen, the column was set at 100 °C and both the injector and FID temperatures at 210 °C (Benedetti et al., 2008).

Quality attributes. Flesh firmness was measured on two opposite peeled sides of the pears, using a penetrometer (Facchini s.r.l., Italy) fitted with an 8-mm-diameter plunger.

Total soluble solids (%) were determined by a hand refractometer (Atago mod. N1, Tokyo, Japan) on juice obtained from squeezing 10 g of mesocarp.

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AsA determination. AsA was extracted by homogenizing 7 g of tissue in 10 mL of 6% (w/v) cold metaphosphoric acid and centrifuged at $10,000 \times g_n$ at 4 °C. The pellet was washed with 7 mL of 6% cold metaphosphoric acid and centrifuged at $10,000 \times g_n$ at 4 °C. The supernatants were combined and cold 6% metaphosphoric acid was added to a final volume of 25 mL. The extraction procedure was performed in quadruplicate. The extracts were filtered through a 0.45- μ m syringe filter before injection to the high-performance liquid chromatography. AsA content was determined using an Inertsil ODS-3 (5 μ m, 4.6×250 mm) column (GL Science, Tokyo, Japan) (Sinelli et al., 2008). The column was eluted with 0.02 M orthophosphoric acid at a flow rate of 0.7 mL·min⁻¹. AsA was monitored at 254 nm.

Enzyme extraction and assay. The extraction of APX (EC 1.11.1.11), MDHAR (EC 1.6.5.4.), DHAR (EC 1.8.5.1), and GR (EC 1.8.1.7) was performed on 5 g of mesocarp. Tissues were ground in prechilled mortars with 10 mL of 100 mM K-phosphate buffer (pH 7.8), 1 mM EDTA, 25% (w/v) glycerol, 0.25% (w/v) Triton X-100, and 0.5 g polyvinylpyrrolidone 2 mm β -mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (86 mM in dimethyl sulfoxide), and 1 mM Na-ascorbate were added to the buffer before use. The homogenate was centrifuged at $15,000 g_n$ at 4 °C for 30 min and supernatant was stored at -80 °C for determinations. The enzymes extraction was performed in quadruplicate.

Total protein content was quantified in the crude extract according to Bradford (1976) using bovine serum albumin (BSA; Sigma, Italy) as a standard.

Assays of enzyme specific activities were determined as earlier described (Cocetta et al., 2012).

The kinetics were measured using a spectrophotometer VARIAN Cary 50 Bio (ultraviolet visible) (Agilent Technologies Italia S.p.A., Cernusco sul Naviglio, MI, Italy). APX assay was performed using a buffer 100 mM K-phosphate (pH 7.0), 0.2 mM EDTA, 25 mM Na-ascorbate. The reduction of AsA ($\epsilon = 2.8 \text{ mm}^{-1} \cdot \text{cm}^{-1}$) was monitored at 25 °C at 290 nm.

The assay of MDHAR was performed using a buffer 50 mM Tris-HCl (pH 7.6), 2.5 mM Na-ascorbate, and 0.1 mM NADH. The activity of MDHAR was determined at 25 °C following the oxidation of NADH ($\epsilon = 6.22 \text{ mm}^{-1} \cdot \text{cm}^{-1}$) at 340 nm. The activity of DHAR was assayed at 25 °C through the absorbance at 265 nm due to the AsA production ($\epsilon = 14 \text{ mm}^{-1} \cdot \text{cm}^{-1}$). The assay was performed in a 50 mM K-phosphate buffer (pH 7), 0.1 mM EDTA, and 0.2 mM DHA. The GR activity associated to NADPH oxidation at 25 °C, was determined at 340 nm ($\epsilon = 6.22 \text{ mm}^{-1} \cdot \text{cm}^{-1}$). The assay buffer was 100 mM Tris-HCl (pH 7.8), 2 mM EDTA, 0.5 mM glutathione in the oxidized form (GSSG).

Estimation of lipid peroxidation. Five grams of mesocarp were homogenized in 25 mL of 5% (w/v) trichloroacetic acid then centrifuged at 4 °C at $10,000 g_n$ for 30 min.

The extract was added to an aqueous solution of 15% (w/v) TCA and 0.5% (w/v) 2-thiobarbituric acid. Samples (four biological replicates) were mixed and heated at 95 °C for 15 min in a water bath, cooled and centrifuged at $4,000 \times g_n$ for 15 min. Samples were then analyzed in a spectrophotometer (Jasco, model 7800, Tokyo, Japan) at 532, 600, and 440 nm. The value of absorbance at 532 nm was purged from the absorbance at 440 nm and at 600 nm due to sucrose and tonon-specific turbidity. The concentration of TBARS expressed as malondialdehyde (MDA) equivalents (nmol·g⁻¹ FW) was calculated according to Du and Bramlage (1992).

Statistical analysis. Analysis of variance was performed by SPSS software (SPSS Inc., Chicago, IL), using general linear model univariate analysis. Significant differences were calculated by Tukey's mean test. Differences at $P \leq 0.05$ were considered as significant.

Results

Ethylene. Control fruit stored at 20 °C did not accumulate remarkable amounts of ethylene (Fig. 1A). Fruit stored at 0 °C showed

a progressive increase of the hormone concentration and after 135 d it reached the highest level, most likely corresponding to the climacteric peak. Instead, in fruit treated with 1-MCP, ethylene levels were close to zero up to 90 d at 0 °C. After 135 d at 0 °C storage, the hormone concentration was similar to the one in untreated fruit after 30 d, with values compatible with the onset of the climacteric ripening.

After a shelf life of 30 d at 20 °C, the concentrations of ethylene were progressively higher in untreated samples stored for longer periods (Fig. 1B). Conversely, it remained lower in fruit treated with 1-MCP. During shelf life, ethylene levels diminished in all considered samples compared with the end of storage, greatly in control fruit and to a lesser extent in 1-MCP-treated ones.

Quality attributes. Samples kept at 20 °C after 30 d of storage showed a significant loss of firmness (Table 1). In control samples stored at 0 °C, the pulp showed no softening up to 90 d. After 135 d, a pronounced loss of firmness was evident in control fruits (-57% compared with 0 d storage). In fruit treated with 1-MCP, firmness did not change during storage at 0 °C.

Fig. 1. Changes in ethylene concentration during storage (A) and after shelf life (B) of 'Passe-Crassane' pears. Values are means of four replicates. Asterisks indicate differences among different treatments/temperatures. Different letters indicate statistical differences among values after different storage periods and after shelf life (30 d at 20 °C) ($P \leq 0.05$).

Table 1. Changes in firmness, TSS, AsA, and lipid peroxidation (TBARS) during storage of 'Passe-Crassane' pears.

Time	Firmness (N)		TSS (%)		AsA (mg·100 g ⁻¹ FW)		TBARS (μmol·100 g ⁻¹ FW)	
	20 °C, -MCP	0 °C, -MCP	20 °C, -MCP	0 °C, -MCP	20 °C, -MCP	0 °C, -MCP	20 °C, -MCP	0 °C, -MCP
0 d	68.72 a	68.72 a	13.32 a	13.32 a	1.215 a	1.215 a	0.350 a	0.350 a
30 d	55.98 b*	66.21 a	14.50 a	15.12 a	1.397 ab	1.188 a	2.176 b*	0.768 a
60 d	n.d.	63.88 a	16.17 a	14.72 a	1.757 b*	1.164 a	n.d.	1.011 ab*
90 d	n.d.	58.43 a*	13.60 a	14.55 a	0.888 a*	1.512 a	n.d.	1.827 b*
135 d	n.d.	29.77 b*	15.42 a	14.10 a	0.751 a*	1.215 a	n.d.	0.673 a*

MCP = methylcyclopentene; TSS = total soluble solids; AsA = ascorbic acid; TBARS = thiobarbituric acid reactive substances; n.d. = not determined.

Values are means of four replicates. Asterisks indicate differences among different temperature/treatments. Different letters indicate statistical differences among different days of storage ($P \leq 0.05$).

After shelf life (Table 2), untreated fruit showed a marked softening, whereas in pears treated with 1-MCP, firmness did not change significantly. The only exception was observed in fruit stored for 135 d which showed a 52% decrease after the shelf life period and around half the firmness of the ones stored for 30 d and then kept at 20 °C.

In samples maintained at 20 °C, the soluble solids content increased markedly (+22% in 30 d) (Table 1), whereas in pears stored at 0 °C, it remained stable up to 135 d. Treatment with 1-MCP had no effect on the content of sugars and the values were similar in both treated and untreated pears until the end of storage.

Soluble solids content did not change after the shelf life period in control fruits. Conversely, in treated pears, sugar levels were progressively enhanced as the cold storage period protracted. Shelf life affected positively total soluble solids in treated pears previously stored for 90 and 135 d.

Ascorbic acid. In fruit stored at 20 °C, AsA content almost doubled after 30 d of storage (Table 1). During cold storage, AsA levels in control pears reached a maximum at 60 d, then markedly declined by 49% after 90 d and a further 15% at the end of the trial. Fruit treated with 1-MCP maintained the same AsA levels throughout the cold storage. Treated pears stored at 0 °C for 90 and 135 d showed significantly higher AsA contents compared with control fruits.

At the end of the shelf life, AsA was higher in control samples previously cold stored for 30 d compared with treated fruits (+29%). In 1-MCP treated samples stored for 90 and 135 d, after shelf life a greater AsA content was recorded (+162%) (Table 2).

Enzyme specific activities. The activity of APX in pears stored at 20 °C significantly decreased after 30 d by 43% (Fig. 2A). Regarding the nontreated fruit stored at 0 °C, the enzyme activity showed a progressive decrease until 60 d of storage, and then values were fairly stable until the end of storage. In pears treated with 1-MCP, APX maintained constant levels of activity up to 90 d of cold storage, then, in the last phase, underwent a sharp decline of activity. 1-MCP positively affected APX activity at 90 d of storage, then negatively influenced this parameter at the end of the cold period.

After shelf life (Fig. 3A), APX activity was similar in control samples, regardless of storage. Moreover, in all treated fruits, APX activity showed higher levels than controls except for pears stored for 135 d. The shelf life period negatively affected the enzyme activity of untreated pears stored for 30 and 60 d, whereas the trend was increasing in treated fruit at the same time points.

MDHAR activity slightly diminished in pears stored at 20 °C and after 30 d it was significantly lower compared with all samples at 0 °C (Fig. 2B). Instead, in all fruit stored at 0 °C the enzyme activity increased, reaching a peak after 60 d. In untreated fruit, this level was maintained up to 90 d, whereas in the treated ones, there was a rapid decline.

In fact, a significantly higher MDHAR activity was determined at 90 d of storage in controls compared with treated fruit. From 90 d until the end of the storage, activity declined in control pears, reaching levels similar to those found at harvest (0 d).

After shelf life (Fig. 3B), an increment in MDHAR activity was registered in both treated and untreated fruit stored at 0 °C for 60 and 90 d. At these sampling points, the increase in enzyme activity was higher for 1-MCP-treated pears (+26% and +88%, respectively).

The activity of DHAR did not change during the 30 d of storage at 20 °C (Fig. 2C). Treated fruit slightly increased the DHAR activity within the first 30 d, having at this time point significantly higher level (+23%) compared with control. In both treated and untreated fruit a peak was observed at 60 d of storage, when enzymatic activity was more than six times higher than the previous sampling. Following this peak, DHAR activity was further enhanced until 90 d in treated pears. Conversely, in control fruits, it decreased by 17%. At 135 d, enzyme activity in treated pears rapidly diminished to values similar to those recorded at the beginning of the trial, but remained significantly higher compared with untreated ones.

After shelf life (Fig. 3C), DHAR activity showed a trend similar to MDHAR in all samples. During shelf life, pears previously stored for 60 and 90 d showed higher values than those stored for 30 and 135 d. 1-MCP treatment positively affected DHAR activity at 60 and 90 d.

In samples stored at 20 °C, the activity of GR did not change and was lower than the values registered at 0 °C (Fig. 2D). No differences were observed between treated and untreated fruit during storage, yet there was a progressive increment over the entire period. The only exception was recorded at the end of cold storage, when GR activity levels of untreated fruits were higher than those of treated ones.

After shelf life (Fig. 3D), the activity of GR did not undergo significant changes in treated samples. On the other hand, in control pears, the enzyme activity underwent a progressive enhancement until the end of the trial.

Lipid peroxidation. TBARS levels increased dramatically during storage at 20 °C, reaching values 6-fold higher after 30 d (Table 1). After 30 d of cold storage, a marked increase in TBARS values was observed with a maximum at 90 d. At the end of storage, the levels decreased to values not statistically different from those at the beginning of the trial. A significant higher content in TBARS was observed in 1-MCP treated pears starting from 60 d until the end of cold storage.

During the shelf life period, TBARS increased in 1-MCP treated samples stored previously at 0 °C for 30 d and remained stable in the other treated samples (Table 2). In contrast, nontreated fruit showed an opposite pattern and pears stored for 90 and 135 d

Table 2. Changes in firmness, TSS, AsA, and lipid peroxidation (TBARS) after different storage periods followed by shelf life (30 d at 20 °C) of 'Passe-Crassane' pears.

Time	Firmness (N)		TSS (%)		AsA (mg·100 g ⁻¹ FW)		TBARS (μmol·100 g ⁻¹ FW)	
	0 °C, -MCP	0 °C, +MCP	0 °C, -MCP	0 °C, +MCP	0 °C, -MCP	0 °C, +MCP	0 °C, -MCP	0 °C, +MCP
30 d + S.L.	23.46 a*	60.39 a	16.80 a	15.12 a	2.419 a*	1.192 a	0.521 ab*	2.711 a
60 d + S.L.	2.327 b*	56.59 a	16.20 a	16.80 ab	1.885 a	1.532 a	0.743 b*	2.847 a
90 d + S.L.	5.451 b*	51.27 a	16.12 a	17.05 ab	0.747 b*	1.953 ab	0.556 b*	4.260 a
135 d + S.L.	3.185 b*	33.75 b	15.52 a	18.60 b	1.020 b*	3.184 b	0.288 a*	3.043 a

MCP = methylcyclopropene; TSS = total soluble solids; AsA = ascorbic acid; TBARS = thiobarbituric acid reactive substances.

Values are means of four replicates. Asterisks indicate differences between treatments. Different letters indicate statistical differences among values after different storage periods followed by shelf life ($P \leq 0.05$).

Fig. 2. Changes in specific activity of ascorbate peroxidase (A), monodehydroascorbate reductase (B), dehydroascorbate reductase (C), and glutathione reductase (D) during storage of 'Passe-Crassane' pears. Values are means of four replicates. Asterisks indicate differences among different treatments/temperatures. Different letters indicate statistical differences among different days of storage ($P \leq 0.05$).

showed lower levels after shelf life. 1-MCP-treated samples always showed higher levels compared with control.

Discussion

In winter pears, low temperature is a trigger of induction and synchronization of ripening, leading to an increase in the synthesis of autocatalytic ethylene (Lelièvre et al., 1997). Temperature is confirmed to be a factor, which influences ethylene production and ripening process in winter pears, in fact no ethylene biosynthesis was detected in fruit stored at 20 °C. In this work, the first response to 1-MCP was the drastic inhibition in ethylene production during cold storage. In pears treated with 1-MCP and then subjected to shelf life, ethylene levels were lower compared with untreated pears.

During the ripening of pears, a gradual softening of the fruit is commonly observed. This occurs as a result of progressive modifications in the cell wall components (Giovannoni, 2001) and in degradation of the cell membranes

(Supapvanich and Tucker, 2013). In this work, samples stored at 0 °C showed a reduction in firmness at the end of storage, as the low temperature triggered the onset of ripening as previously shown in winter pears (Villalobos-Acuña and Mitcham, 2008). After shelf life, flesh softening was observed in all control samples. Softening is completely inhibited in 1-MCP-treated fruit, in which ripening was delayed. The effect of 1-MCP is also evident after shelf life. Only treated pears being stored up to 135 d showed a significant decrement after shelf life, although this trend was already evident in fruit stored for 90 d. Softening proceeded in a different way in fruit stored at 20 °C. They did not wholly develop a juicy and buttery texture and did not show changes in total and soluble pectins as we previously reported (Spinardi et al., 2007). Moreover, they underwent a marked decrement in firmness after 30 d, which did not depend on the action of ethylene, since the levels of the hormone were close to zero. In fact, cell wall disassembly related to climacteric fruit softening

is regulated not only by hormonal cues, but also by developmental and environmental factors. As in Charentais melon, 'Passe-Crassane' flesh softening may include both ethylene-dependent and independent components that are correlated with differential regulation of cell wall degrading enzymes (Pech et al., 2008). Furthermore, a different response of pears at 20 °C or after 1-MCP treatment may be related to different sensitivity of particular cell wall degrading enzymes to ethylene and temperature (Johnston et al., 2009). It is interesting to observe that the samples kept at 20 °C showed a significant increment in total soluble solids and in AsA concentration that could be due to a partial disassembly of cell wall polysaccharides (Gilbert et al., 2009). The increment in AsA at 20 °C could also be related to the significant decrease in APX activity, which consumes AsA as substrate, and the contemporary lack of changes in MDHA and DHAR activities in the same samples. The increase in AsA may as well be due to the activation of the AsA biosynthetic pathway.

Fig. 3. Comparison between specific activities of ascorbate peroxidase (A), monodehydroascorbate reductase (B), dehydroascorbate reductase (C), and glutathione reductase (D) after shelf life of 'Passe-Crassane' pears. Values are means of four replicates. Asterisks indicate differences between treatments. Different letters indicate statistical differences among values after different storage periods followed by shelf life (30 d at 20 °C) ($P \leq 0.05$).

AsA is synthesized mainly from glucose and L-galactono-1,4-lactone-dehydrogenase (GLDH, EC 1.3.2.3), the terminal enzyme in this synthesis, is regulated by the mitochondrial electron flux, i.e., respiration can control AsA synthesis (Millar et al., 2003). At 20 °C, fruit respiration is expected to be higher than at low temperatures during cold storage and AsA increment may be linked to an increased activity of GLDH coupled to the mitochondrial electron transport chain.

AsA content remained stable in treated pears during storage at 0 °C. In contrast, untreated fruit after a peak at 60 d, showed significantly lower concentrations until the end of storage. Our results do not correspond to those reported in the summer variety 'Blanquilla' where AsA content was lower in fruit treated with 1-MCP (Larrigaudière et al., 2004). Those discrepancies may be related to varietal differences, which results in a different mechanism in the regulation of AsA metabolism and to a different response to chilling especially between winter and summer pears. Our results are in agreement with those reported on 'Conference' autumn pears by Chiriboga et al. (2013), who observed higher AsA levels in 1-MCP-treated fruit throughout the storage period at 0.5 °C. In fact, in 'Passe-Crassane', higher AsA contents are associated with the absence of ethylene production (not only after 1-MCP treatment but also after 20 °C shelf life). Similar results with regard to an ethylene-independent

ascorbate pathway were reported by Vilaplana et al. (2006) in apples. Another hypothesis that may explain the higher AsA levels in 1-MCP-treated pears refers to a stress perception during cold storage, when pears become competent to ripening and capable of starting climacteric. Ascorbate concentrations generally increase in plants under stress conditions and correlate closely with enhanced stress tolerance (Allen, 1995). 1-MCP treatment at low temperatures may possibly trigger in winter pears a stress response linked to ROS accumulation, and consequently to an increase in AsA content, that favors stress compensation.

With respect to the effect of 1-MCP on the antioxidant enzymes activities during cold storage, the results from this work are consistent with what was recently published in 'Empire' apples (Lee et al., 2012a). In fact, the changes in the specific activities of the enzymes were not always consistent with 1-MCP treatment. The activity of APX was higher in fruit treated with 1-MCP after 90 d of storage, in accordance with what was observed in 'Blanquilla' pears (Larrigaudière et al., 2004). At the same time point, the effect of 1-MCP on DHAR was opposite to that on MDHAR. AsA levels have been assured mainly by the high activity of DHAR, which guaranteed an efficient recycling. Moreover, APX activity showed opposite trends at different temperatures. Pears kept at 20 °C showed decreased levels after 30 d, whereas those stored at 0 °C maintained

higher values. A higher APX activity can be associated with chilling tolerance in several fruits stored at low temperatures (Singh and Singh, 2013).

GR showed a steady increment in activity levels as an adaptation to low temperatures in control samples and to a lesser extent in fruit treated with 1-MCP. In many plant species, acclimatization to low temperatures coincides with an increase in activity of GR. Zhao and Blumwald (1998) presume that there is a role of primary importance for GR and MDHAR in adaptation to low temperature. During shelf life, 1-MCP treatment showed the greatest effectiveness on enhancing antioxidant enzymatic potential, by increasing the activity of APX, DHAR, and MDHAR significantly. Besides the maintenance of AsA levels in response to 1-MCP, an increase in TBARS was recorded starting from 60 d. This is in accordance with data reported by Lee et al. (2012b) on apples. The differences in the responsiveness of winter pears to the combination of cold and 1-MCP, compared with early season varieties, could be due to different behavior regarding cold adaptation, as previously suggested (El-Sharkawy et al., 2003). Interestingly, TBARS in treated pears reached maximum values at 60 and 90 d of cold storage. At this time, 'Passe-Crassane' most likely become capable of starting climacteric, and 1-MCP treatment at low temperatures may possibly trigger a stress response linked to ROS accumulation.

An increment in oxidative stress is consistent with a previous work in which 1-MCP treatment was associated with increased levels of H₂O₂, along with increased activity of both enzymatic and nonenzymatic antioxidants during shelf life of cold-stored pears (Chiriboga et al., 2013). Moreover, lipid peroxidation can be due to the decrement of various antioxidant compounds. For example, a negative effect of 1-MCP on chlorogenic acid and procyanidin B2 has been reported in 'Cripps Pink' apple (Hoang et al., 2011) and also in juices of 'Shampion' and 'Idared' (Kolniak-Ostek et al., 2014).

In conclusion, fruit treated with 1-MCP have maintained their antioxidant systems. This is evident even after a period of shelf life, when 1-MCP enhanced such systems (see APX-, DHAR-, and MDHAR-specific activities). At the same time, AsA content was preserved during the whole postharvest of winter pears. The 1-MCP treatment therefore did positively affect the nutritional value of winter pears with respect to that vitamin and helps to maintain it during shelf life. Furthermore, AsA pathway was not ethylene dependent, in fact higher levels were recorded when ethylene perception/production was prevented. A stress response triggered by cold temperatures and 1-MCP treatment is however detectable as 'Passe-Crassane' acquire the ability to ripen and such behavior is kept also during shelf life. Nevertheless, high levels of ascorbate in fruit are expected to favor stress compensation. 1-MCP-treated fruits exhibited also higher enzymatic antioxidant potential. These results support the hypothesis that the beneficial effects of 1-MCP on 'Passe-Crassane' ripening were not exclusively due to its action on ethylene but also to an increase in antioxidant defense in pear.

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