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‘Conference’ and ‘Abbé Fétel’ pears treated with 1-methylcyclopropene: physiological and quality implications of initial low oxygen stress and controlled atmosphere storage

M. Vanoli, M. Grassi, G. Bianchi, M. Buccheri, A. Rizzolo*
Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria, Unità di ricerca per i processi dell’industria agroalimentare (CREA-IAA), via G. Venezian 26, 20133 Milano, Italy.

Key words: α-farnesene, ethylene production, fermentative metabolites, pulp mechanical properties, sensory profiles, storage disorders.

Abstract: Superficial scald is a disorder developed in cold storage by ‘Conference’ and ‘Abbé Fétel’ pears and it has been related to the presence of oxidation products, mainly conjugated trienols (CTols), of which α-farnesene is primary, acting on epidermal cells. Among tested postharvest methods to control scald, there is treatment at harvest with 1-methylcyclopropene (1-MCP) and initial low oxygen stress (ILOS). The investigation presented here studied, in ‘Conference’ and ‘Abbé Fétel’ pears treated with 1-MCP (300 µL L^{-1}), the physiolological and quality implications of storage in controlled atmosphere (CA, 2 kPa O_2 + 0.7 kPa CO_2, -0.5°C) after two 2-weeks ILOS (0.3-0.5 kPa O_2) periods at three-week intervals after 13 and 21 weeks of storage and shelf life at 20°C up to seven days. Results showed that 1-MCP treatment severely reduced α-farnesene, $CTol_{269}$, $CTol_{281}$ and ethanol after ILOS treatment in both cultivars, and ethyl acetate in ‘Abbé Fétel’ pears. Furthermore, it impaired fruit softening, delayed skin yellowing and reduced ethylene production in shelf life. At sensory analyses, 1-MCP treated ‘Conference’ and ‘Abbé Fétel’ pears were described as being firmer and less juicy, sweet and aromatic than untreated fruit. 1-MCP treated pears did not develop superficial scald and soft scald in ‘Abbé Fétel’, nor superficial scald and black speck after 21 weeks of storage in ‘Conference’.

1. Introduction

‘Abbé Fétel’ is the most important pear cultivar in Italy in terms of production (CONERPO, 2010) and it can be stored in normal air (NA) for three to four months and in controlled atmosphere (CA) for up to six months (Bai *et al.*, 2009). However, when stored in NA for more than four months ‘Abbé Fétel’ pears became sensitive to superficial scald (Vanoli *et al.*, 2008). ‘Conference’ pears in Italy are often subjected to superficial scald in cold storage, and it has been reported that under predisposing climatic conditions, up to 70% of fruit developed scald, thus impairing their marketability (Folchi and Bertolini, 2008).

Scald is manifested as brown or black patches on the skin; it can take several forms and, along with superficial scald, it is an expression of damage and/or death within the surface layers of cells (Lurie and Watkins, 2012; Whitaker, 2013). Scald has been related to the presence of oxidation products [conjugated trienols (CTols), primarily α-farnesene], acting on epidermal cells (Whitaker, 2007) and could be prevented or controlled by storage in CA (Bertolini *et al.*, 1997; Lurie and Watkins, 2012). However, the low levels of oxygen used in CA for ‘Abbé Fétel’ pears can induce soft scald (Bertolini *et al.*, 2002; Rizzolo *et al.*, 2010; Vanoli *et al.*, 2010 a).

Up to now, the traditional strategy to prevent superficial scald in pears is a pre-storage treatment with ethoxyquin, which recently has been excluded from the list of active ingredients of chemicals used in food production (EC Council Directive 91/414) (Calvo and Kupferman, 2012). The most effective alternatives to ethoxyquin are treatment at harvest with 1-methylcyclopropene (1-MCP) or storage under controlled atmosphere with low levels of O_2 such as ultra-low oxygen, initial low oxygen stress, and dynamic controlled atmosphere (Calvo and Kupferman, 2012; Lurie and Watkins, 2012).

It was found that 1-MCP inhibited superficial scald and prevented or controlled soft scald and internal breakdown in ‘Bartlett’ pears (Villabolos-Acuña *et al.*, 2011 a, b). In ‘Conference’ doses ranging from 50 to 1000 µL L^{-1} did not prevent the formation of superficial scald, both in CA and in NA, but either controlled it, keeping the incidence of scald within commercially acceptable rates, or reduced symptom severity (Eccher Zerbini *et al.*, 2003; Rizzolo

* Corresponding author: anna.rizzolo@entecra.it

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et al., 2005; Folchi and Bertolini, 2008). The reduction of symptom severity in 'Conference' fruit was related to lower amounts of α-farnesene and CTols (Rizzolo *et al.*, 2005; Folchi and Bertolini, 2008). In 'Abbé Fétel' pears, after shelf life, CTols and α-farnesene were significantly higher in fruit affected by superficial scald and lower in those affected by senescent scald (Vanoli *et al.*, 2010 b).

Storage under controlled atmosphere with a low level of O_2 was suggested as an alternative to ethoxyquin and to 1-MCP treatment not only in controlling scald development but also in improving overall fruit quality (Prange *et al.*, 2011; Lurie and Watkins, 2012). Widespread adoption of low O_2 regimes has not taken place due to concerns of anaerobic damage when fruit is held below the lower oxygen limit (LOL), that is the environmental O_2 level at which cell metabolism changes from being predominantly aerobic to fermentative (Wright *et al.*, 2012). Zerbini and Grassi (2010) and Rizzolo *et al.* (2008, 2010) found that LOL for 'Conference' pears was 0.4 kPa O_2 and for 'Abbé Fétel' pears 0.6 kPa O_2. Vanoli *et al.* (2008, 2010 a) and Rizzolo *et al.* (2010) found that storage of 'Abbé Fétel' pears at 0.7 kPa O_2 at -0.5°C completely prevented superficial scald development and reduced soft scald incidence compared to 2 kPa O_2, but increased internal browning and internal breakdown. As for the initial low oxygen stress, Wang and Dilley (2000) found that an ILOS with 0.25 kPa O_2 for two weeks, carried out one or two times at two-month intervals, strongly inhibited α-farnesene and its volatile oxidation product (6-methyl-5-hepten-2-one), increased ethanol, and was effective in controlling scald development in several apple cultivars. As for pears, Calvo *et al.* (2002) found that ILOS (0.5 kPa O_2) followed by low oxygen CA (1.5 kPa O_2) significantly inhibited the development of superficial scald after nine months of storage in 'Beurré d'Anjou' cultivar, while Rizzolo *et al.* (2015) reported that in 'Conference' pears after an ILOS (0.2-0.5 kPa O_2) period followed by low oxygen CA (2 kPa O_2) there were lower amounts of α-farnesene, $CTol_{258}$ and acetaldehyde, and higher quantities of ethanol than after CA and NA storage, developing less scald than the other atmospheres.

The objective of the present research was to evaluate in 'Conference' and 'Abbé Fétel' pears the effect of 1-MCP application on fruit stored in a low oxygen CA after two two-week ILOS periods at three-week intervals. Physiological aspects (fermentative metabolites, conjugated trienols) in storage, and ethylene production, quality and sensory characteristic changes with post-storage shelf life and storage disorders are discussed.

2. Materials and Methods

The experiment was carried out in 2012 on 'Conference' and 'Abbé Fétel' pears (*Pyrus communis* L.) (about 1000 fruit/cv) harvested from commercial orchards in the Modena province (Italy) on 20 August and 10 September, respectively, at a commercial degree of maturity [mean ± standard error: 'Abbé Fétel': firmness, 62.5±1.3 N; hue, 104±0.6°; starch hydrolysis, 4.0 ± 0.5 (EUROFRU 1-10 scale); 'Conference': firmness, 71.0±1.6 N; hue, 108.9±0 .5°; starch hydrolysis, 3.0 ± 0.1 (EUROFRU 1-10 scale)] and randomized in 14 boxes. For each cultivar, on the day after harvest, half of the fruits were treated with 300 μL L^{-1} 1-MCP (Smartfresh™, AgroFresh Inc., Rohm and Haas, Spring House, PA, USA) and seven boxes of untreated fruit were used as control. 'Conference' fruits were then put in NA at -0.5°C for four weeks before the beginning of ILOS periods and CA storage, while for 'Abbé Fétel' pears the ILOS experiment began two days after the 1-MCP treatment. For both control and 1-MCP treated 'Conference' and 'Abbé Fétel' pears, two ILOS periods at 0.3-0.5 kPa O_2 for about two weeks were applied with a three-week interval in CA at 2 kPa O_2 + 0.7 kPa CO_2 at -0.5°C. Four containers were used, each one dedicated to one sample (1-MCP dose and cultivar); the gas composition of each container was controlled and checked with centralized analyzers, supervised by a specific Fruit Control Equipment software; fluorescence FIRM™ sensors monitoring (HarvestWatch™, Satlantic, Canada) was carried out in each container from the beginning of the first ILOS period till the first storage time (13 weeks). The first ILOS period was applied from d0 to d17, and the second one from d40 to d53. Then pears were stored in CA at -0.5°C in 2 kPa O_2 + 0.7 kPa CO_2 up to 21 weeks.

Samplings

α-farnesene, CTols and fermentative metabolites were analyzed (6 fruits/1-MCP dose/cultivar) at the beginning and at the end of the second ILOS period, and at the first storage time (13 weeks); hereafter these samplings are referred as d0, d13 and d40.

After 13 and 21 weeks of storage, 3 boxes/1-MCP dose/cultivar were put in shelf life at 20 °C up to 7 days. At 1, 5 and 7 days of shelf life (d1, d5, d7) 20 fruit/1-MCP dose/cultivar were analyzed for background skin color and pulp mechanical properties (firmness, stiffness and energy-to-rupture). Ethylene production was measured at d1, d5 and d7 on ten fruits of sample d7, while sensory analyses were carried out on ten fruits at d5 and d7. After 7 days at 20 °C the incidence of storage disorders was evaluated on 3 boxes/1-MCP dose/cultivar.

α-Farnesene and CTols

α-Farnesene and CTols ($CTol_{258}$, $CTol_{269}$, $CTol_{281}$) were determined in the skin according to Zoffoli *et al.* (1998), by sampling eight skin disks of 0.8 cm^2 area from the equatorial region of two pears (three replications) and extracting overnight at 2°C with 6 mL of HPLC-grade hexane with 1 g of anhydrous Na_2SO_4. The absorbance of the extracts at 232, 258, 269, 281 and 290 nm was measured using a Jasco (model 7800) spectrophotometer. Concentrations of α-farnesene and CTols were calculated according to Huelin and Coggiola (1970) and Du and Bramlage (1993). Data were expressed as nmol cm^{-2}.

Fermentative metabolites

Fermentative metabolites (ethanol, acetaldehyde and ethyl acetate) were determined on the pulp of the same fruit analyzed for α-farnesene and CTols by means of HS-SPME-GC, by pooling the six fruits of each sample. Ten grams of homogenized pulp (three replications) were put into 25 mL vials tightly closed with an aluminum cap with a silicone-Teflon rubber septum; samples were then immediately frozen and kept at -20°C until analysis. After 60 min thawing at room temperature, the SPME headspace volatile sampling was carried out for 30 min at 40°C using a 50/30μm DVB-CAR-PDMS fiber (Supelco), which was desorbed for 5 min in the GC injector port at 250°C. Fermentative metabolites were separated on a Supelcowax-10 column (60 m ×0.25 mm I.D., 0.25 μm film thickness) using the following conditions: carrier gas, helium at a flow of 1.5 mL min^{-1}; temperature program, 40°C×13 min, 15°C min^{-1} to 185°C; FID temperature, 250°C. Fermentative metabolites were quantified by relating the peak area of each one to that of external standards.

Background skin color

Background skin color was measured on the greener side of fruit with a Spectrophotometer CM-2600d (Minolta Co, Japan) using the primary illuminant D65 and 10° observer in the L*, a*, b* color space. From a* and b* values, hue (H°) and chroma (C*) were computed according to $H°=\arctan(b^* a^{*-1})$ and $C^*= (a^{*2} + b^{*2})^{-2}$.

Pulp mechanical properties

The mechanical properties of pear tissue of each fruit were measured on two opposite peeled areas in the equatorial region of the pear using an 8 mm diameter plunger mounted on an Instron Universal Testing Machine (model 4301, Instron Ltd, Great Britain) with crosshead speed at 200 mm min^{-1}. From the force-displacement curve the following pulp mechanical properties were measured (Rizzolo *et al*., 2014): firmness (N), stiffness (N mm^{-1}) and energy-to-rupture (mJ). Firmness, stiffness and energy-to-rupture readings were averaged for each fruit.

Ethylene production rate

The ethylene production rate (EP) was measured by static HS/GC on fruit put in 1.7 L gas-tight glass jars (ten replications, one fruit per jar) for 2 h at 20 °C according to Rizzolo *et al*. (2005). One milliliter of the headspace gas was sampled and analyzed using a deactivated aluminum oxide F1 (80-100 mesh) column (1/8 in 200 cm) at a column temperature of 100°C and FID detection. Quantitative data were obtained by relating the ethylene peak area to that of a 10 μL L^{-1} standard and were expressed as pmol kg^{-1} s^{-1}.

Sensory analysis

Sensory analyses were carried out in a sensory lab using a panel of ten short-term trained judges at d5 and d7 of shelf life at 20°C. For both the cultivars in each session, one peeled slice/1-MCP dose was presented to each panelist. At the beginning of the session, a slice of a fruit not included in the experimental plan was tasted to eliminate the first tasting effect. Drinking water was provided as a palate cleaner between samples. Each sample was evaluated for the intensity of attributes related to fruit structure (firm, juicy, grainy) and taste and flavor (sweet, sour, aromatic, bitter, astringent) using 120 mm unstructured line scales with anchors at 12 mm from the extremes (low, high). In addition, in order to have a rough idea of sample pleasantness, at the end of the tasting session, panelists were also asked to score samples for overall acceptability using a 120 mm unstructured line scale with "low" and "high" anchors near the extremes. Details on panel training and attributes are reported by Rizzolo *et al.* (2014).

Storage disorders

Storage disorders were evaluated on three boxes/1−MCP dose/cultivar. For each box the percentages of healthy fruit and of fruit affected by superficial scald, rots (both cultivars), soft scald ('Abbé Fétel'), early blackening, black speck and black spot ('Conference') were computed.

Statistical analysis

Data were submitted to analysis of variance (Statgraphics ver.7, Manugistic Inc., Rockville, MD, USA). Prior to statistical analysis the rating scores of each sensory attribute were standardized by panelists in order to remove the variability due to their using different parts of the scale (Bianchi *et al*., 2009). Percentage data were submitted to angular transformation before ANOVA.

3. Results

Oxygen levels and chlorophyll fluorescence

Figure 1 shows the oxygen partial pressure and the corresponding response of chlorophyll fluorescence (Fα) for control and 1-MCP treated 'Abbé Fétel' and 'Conference' pears from the beginning of the first ILOS period till the first storage time (13 weeks). By comparing the graphs of O_2 partial pressure of containers of control and 1-MCP treated 'Conference' pears, it is evident that during the first ILOS period the O_2 values ranged from about 0.2 to 0.6 kPa and during the second ILOS from 0.15 to 0.55 kPa. The two ILOS periods induced a remarkable rise in chlorophyll fluorescence (Fα) in untreated fruit and much smaller ones in 1-MCP treated pears, when O_2 concentration decreased below 0.4 kPa. In control 'Abbé Fétel' pears the O_2 concentration decreased below 0.6 kPa only for a short time during the first ILOS period, while during the second ILOS period O_2 values ranged from 0.3 to 0.6 kPa, causing a very slight rise in Fα at the end of the second ILOS period. On the other hand, in the container with 1-MCP treated 'Abbé Fétel' pears O_2 values ranged from 0.1-0.4 kPa during the two ILOS periods, causing a slight rise in Fα in coincidence with the two ILOS periods.

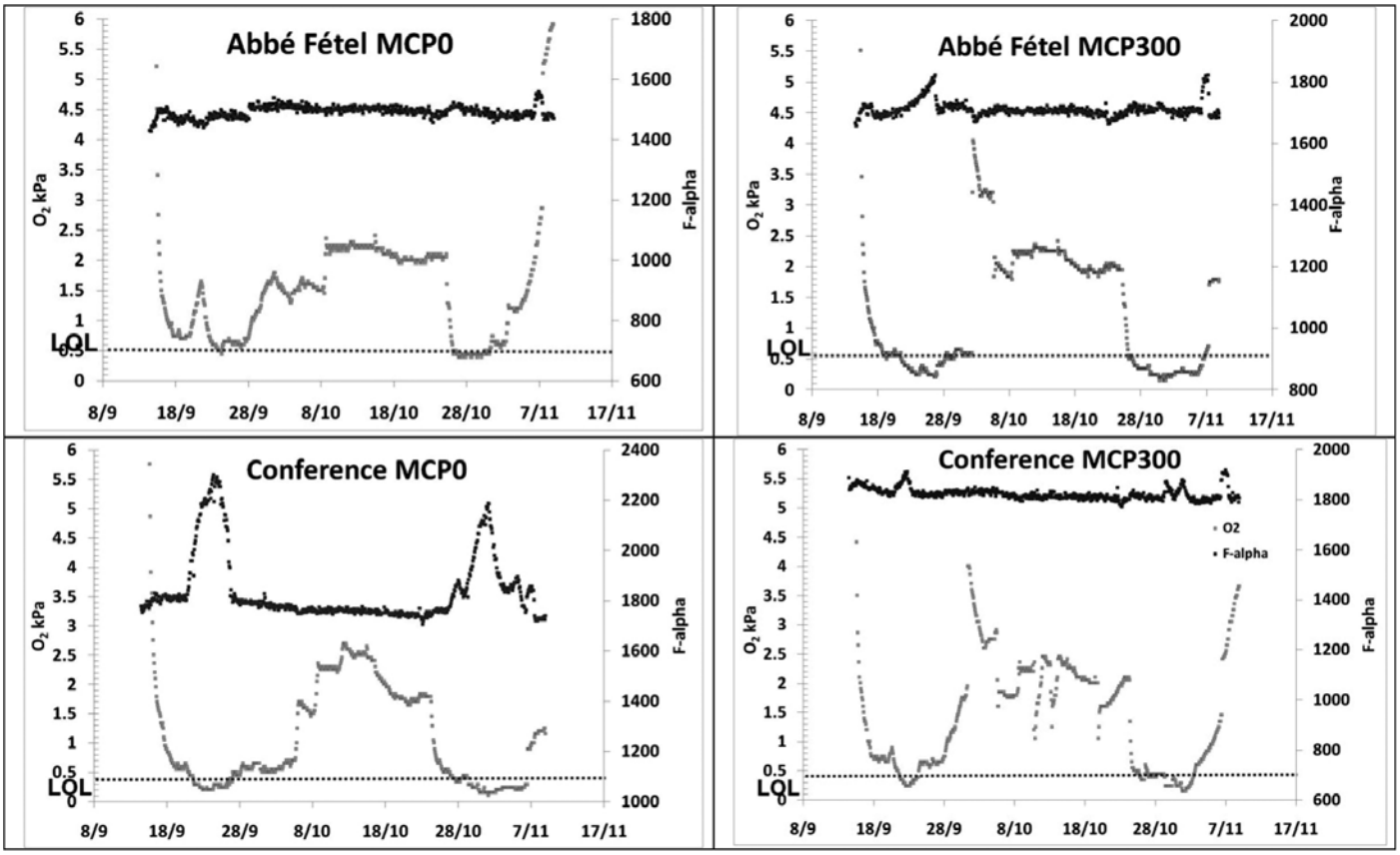

Fig. 1 - Oxygen partial pressure (gray) and corresponding response of chlorophyll fluorescence Fα (black) for control and 1-MCP treated containers of 'Abbé Fétel' and 'Conference' fruit. The dotted lines indicate the O_2 partial pressure inducing stress evidenced by Fα increase ('Abbé Fétel' 0.6 kPa; 'Conference' 0.4 kPa)

α-Farnesene and conjugated trienols

On average 1-MCP treated 'Conference' pears had lower amounts of α-farnesene, $CTol_{269}$ and $CTol_{281}$ than control fruit, and $CTol_{281}$ at the end of the ILOS period (d13) was not detectable in 1-MCP treated fruit (Table 1). In control fruit α-farnesene, $CTol_{269}$ and $CTol_{281}$ significantly increased at d40, in correspondence wth the first storage time. Likewise, 1-MCP treated 'Abbé Fétel' pears

Table 1 - Amounts (mean ± standard error) of α-farnesene (α-FARN), CTols ($CTol_{258}$, $CTol_{269}$, $CTol_{281}$) and fermentative metabolites (ACE, acetaldehyde; EtOH, ethanol; EtAc, ethyl acetate) in control (MCP0) and 1-MCP treated (MCP300) 'Conference' pears at the beginning (d0) and at the end (d13) of the second ILOS period and in correspondence with the first storage time (d40) and ANOVA results

	α-FARN nmol cm^{-2}	$CTol_{258}$ nmol cm^{-2}	$CTol_{269}$ nmol cm^{-2}	$CTol_{281}$ nmol cm^{-2}	ACE µg kg^{-1}	EtOH µg kg^{-1}	Et Ac µg kg^{-1}
	MCP0						
d0	11.32±2.97	0.97±0.24	1.16±0.18	0.21±0.08	27.33±5.38	38.77±13.85	0.10±0.05
d13	9.26±2.81	0.80±0.27	0.90±0.22	0.60±0.33	29.15±3.23	69.45±3.71	0.14±0.09
d40	23.04±8.69	1.80±0.60	2.32±0.58	0.91±0.33	35.34±0.29	24.71±1.13	1.77±1.52
	MCP300						
d0	3.77±0.28	1.01±0.15	1.08±0.12	0.14±0.04	40.26±4.42	55.81±7.39	0.06±0.03
d13	2.96±0.31	0.62±0.15	0.68±0.16	nd	17.31±8.67	21.30±14.83	0.05±0.04
d40	3.90±0.37	0.77±0.15	0.93±0.14	0.04±0.02	33.89±3.82	23.01±2.66	0.87±0.45
	ANOVA [z]						
A: day	NS	NS	*	*	NS	*	NS
B: 1-MCP	**	NS	*	**	NS	NS	NS
A × B	NS	NS	NS	*	NS	**	NS

[z] ***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant.

Table 2 - Amounts (mean ± standard error) of α-farnesene (α-FARN), CTols ($CTol_{258}$, $CTol_{269}$, $CTol_{281}$) and fermentative metabolites (ACE, acetaldehyde; EtOH, ethanol; EtAc, ethyl acetate) in control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' pears at the beginning (d0) and at the end (d13) of the second ILOS period and in correspondence with the first storage time (d40) and ANOVA results

	α-FARN nmol cm^{-2}	$CTol_{258}$ nmol cm^{-2}	$CTol_{269}$ nmol cm^{-2}	$CTol_{281}$ nmol cm^{-2}	ACE μg kg^{-1}	EtOH μg kg^{-1}	Et Ac μg kg^{-1}
	MCP0						
d0	14.80±4.65	0.83±0.14	0.70±0.14	0.42±0.10	10.38±0.31	14.86±5.18	0.92±0.07
d13	15.10±1.41	0.70±0.05	0.63±0.02	0.38±0.01	16.40±3.56	31.10±6.65	0.08±0.06
d40	55.14±9.81	3.83±0.53	6.41±0.91	5.14±0.79	26.78±6.56	39.62±15.98	0.06±0.01
	MCP300						
d0	2.51±0.08	0.43±0.05	0.34±0.06	0.18±0.03	14.03±2.83	17.89±5.11	0.51±0.03
d13	2.69±0.06	0.47±0.01	0.34±0.01	0.18±0.01	16.21±2.10	34.94±5.34	0.09±0.05
d40	10.91±1.94	1.01±0.30	1.01±0.32	0.62±0.17	27.33±3.04	48.99±11.30	0.04±0.02
	ANOVA [z]						
A: day	***	***	***	***	***	*	***
B: 1-MCP	***	***	***	***	NS	NS	**
AxB	**	***	***	NS	NS	NS	***

[z] ***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant.

had lower amounts of α-farnesene, $CTol_{258}$, $CTol_{269}$ and $CTol_{281}$ than control fruit (Table 2). The α-farnesene and CTols concentrations did not significantly change from the beginning (t0) to the end of the second ILOS period (d13) both in control and 1-MCP treated 'Abbé Fétel' fruit, and then they increased at d40. The concentration of α-farnesene in pear skin was three to five times higher in control than in 1-MCP treated fruit, those of $CTol_{258}$ and $CTol_{269}$ two ('Conference') to four-six times ('Abbé Fétel'), and that of $CTol_{281}$ two to eight times in 'Abbé Fétel' and fifteen to twenty-three times in 'Conference'.

In both cultivars the ratios $CTol_{258}/CTol_{281}$ and $CTol_{269}/CTol_{281}$ (Fig. 2) were higher in 1-MCP treated fruit than in control ones, being, on average, three times higher in 'Conference' pears, and about 50% higher in 'Abbé Fétel' treated fruit. In 'Conference' the highest values for both ratios were observed in 1–MCP treated fruit at d 40, while in 'Abbé Fétel' in 1–MCP treated fruit at d0 and d13. Control 'Abbé Fétel' and 'Conference' pears at d40 showed the lowest values for both the ratios.

Fermentative metabolites

In 'Conference' pears the 1-MCP treatment at harvest did not influence the amounts of fermentative metabolites (Table 1). In control 'Conference' pears ethanol increased with the second ILOS period, then it significantly decreased

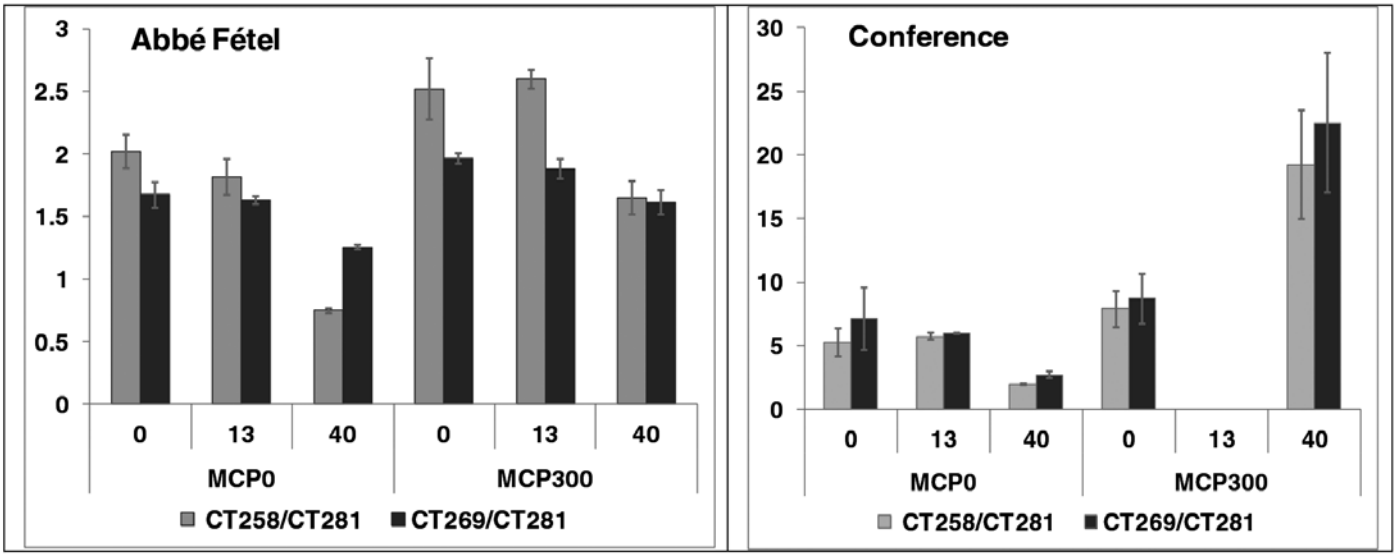

Fig. 2 - $CTol_{258}/CTol_{281}$ and $CTol_{269}/CTol_{281}$ ratios of control (MCP0) and 1–MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears at the beginning (d0) and the end (d13) of the second ILOS period and in correspondence with the first storage time (d40). Bars refer to standard error of the mean.

at d40 (i.e. 13 weeks storage time), while in 1-MCP treated fruit it decreased with the second ILOS period and did not change further at d40. The sampling time had no significant influence on acetaldehyde and ethyl acetate amounts both in control and 1-MCP treated fruit, probably due to the high standard errors of data, especially for ethyl acetate.

The 1-MCP treated 'Abbé Fétel' pears (Table 2) had, on average, lower ethyl acetate amounts at d0 than control fruit. Ethyl acetate both in control and 1-MCP treated pears decreased steeply with the ILOS period, and afterwards did not change at d 40. Both in control and 1-MCP treated 'Abbé Fétel' fruit acetaldehyde concentration did not change from the beginning (d0) to the end of the second ILOS period (d13), and then increased at d40, while ethanol increased throughout the sampling time.

Ethylene production

Ethylene production in control 'Conference' pears was lower than in 'Abbè Fétel' (Fig. 3). In 'Conference' pears EP was significantly affected only by the 1-MCP treatment (Table 3), whereas in 'Abbé Fétel' fruit both 1-MCP treatment and post storage shelf life significantly influenced EP (Table 3). In both cultivars the 1-MCP treatment reduced EP to values lower than 10 pmol kg^{-1} s^{-1}. In 'Abbé Fétel' control and 1-MCP treated fruit after 13 weeks storage showed a decreasing EP with shelf life, and after 21 weeks a minimum EP at d5.

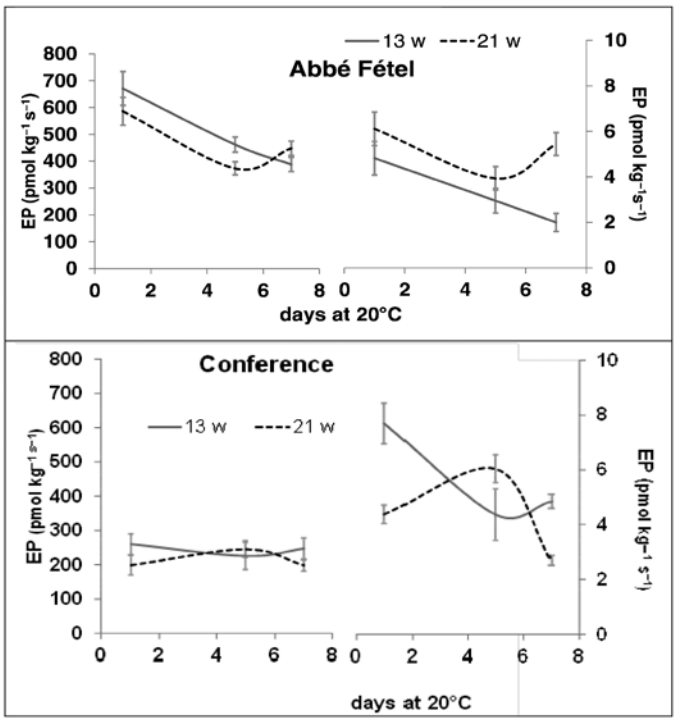

Fig. 3 - Ethylene production rate (EP) of control (left) and 1–MCP treated (right) 'Abbé Fétel' and 'Conference' pears during shelf life at 20°C after 13 and 21 weeks of storage. Bars refer to standard error of the mean. Results of ANOVA analysis are reported in Table 3.

Pulp mechanical characteristics

Upon removal, 1-MCP treated 'Conference' and 'Abbé Fétel' pears maintained firmness similar to that at harvest ('Conference': 71.0±1.6 N; 'Abbé Fétel': 62.5±1.3 N), but lower stiffness and higher energy-to-rupture than at harvest (values at harvest: stiffness: 'Conference', 21.0±0.5 N mm^{-1}; 'Abbé Fétel', 24.5±0.6 N mm^{-1}; energy-to-rupture: 'Conference', 0.106±0.04 J; 'Abbé Fétel', 0.074±0.003 J). The same scenario was found for control 'Conference' pears, whereas control 'Abbé Fétel' fruit had lower firmness and energy-to-rupture than at harvest.

On average, 1-MCP treated 'Conference' pears had higher firmness, stiffness and energy-to-rupture than control fruit, without any difference between storage times; firmness and energy-to-rupture did not change with shelf life, whereas stiffness decreased, but to a lesser extent than control fruit (Fig. 4). In control 'Conference' pears the values of all the mechanical properties decreased with shelf life with the main changes at d5.

As for 'Abbé Fétel' pears (Fig. 4), on average, 1-MCP treated fruit showed higher firmness, stiffness and energy-to-rupture than control fruits, without any difference between the storage times, except for a higher energy-to-rupture of 1-MCP treated fruit after 21 weeks of storage.

Table 3 - Multifactor ANOVA results for pulp mechanical characteristics (firmness, *F*, stiffness, *St* and energy-to-rupture, E_f), color parameters (lightness, L*; chroma, C* and hue) and ethylene production rate (EP) for 'Abbé Fétel' and 'Conference' pears

	Abbé Fétel							Conference						
	EP	*F*	*St*	E_f	L*	C*	Hue	EP	*F*	*St*	E_f	L*	C*	Hue
A: storage time	NS	NS	NS	***	NS	**	***	NS	NS	NS	NS	***	***	***
B: 1-MCP	***	***	***	***	***	***	***	***	***	***	***	***	***	***
C: shelf life	***	***	***	***	***	***	***	NS	***	***	***	***	***	***
A×B	NS	NS	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*
A×C	NS	*	NS	*	NS	NS	NS	NS	*	NS	**	NS	NS	NS
B×C	***	***	***	***	NS	NS	*	NS	***	***	***	**	*	*
A×B×C	NS	**	**	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant)

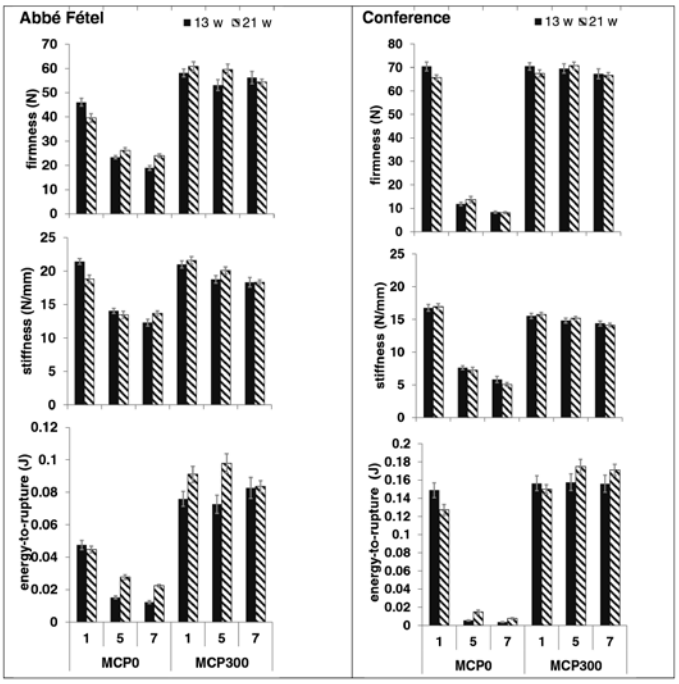

Fig. 4 - Firmness, stiffness and energy-to-rupture of control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears after 1, 5 and 7 days of shelf life at 20°C after 13 and 21 weeks of storage. Bars refer to standard error of the mean. Results of ANOVA analysis are reported in Table 3.

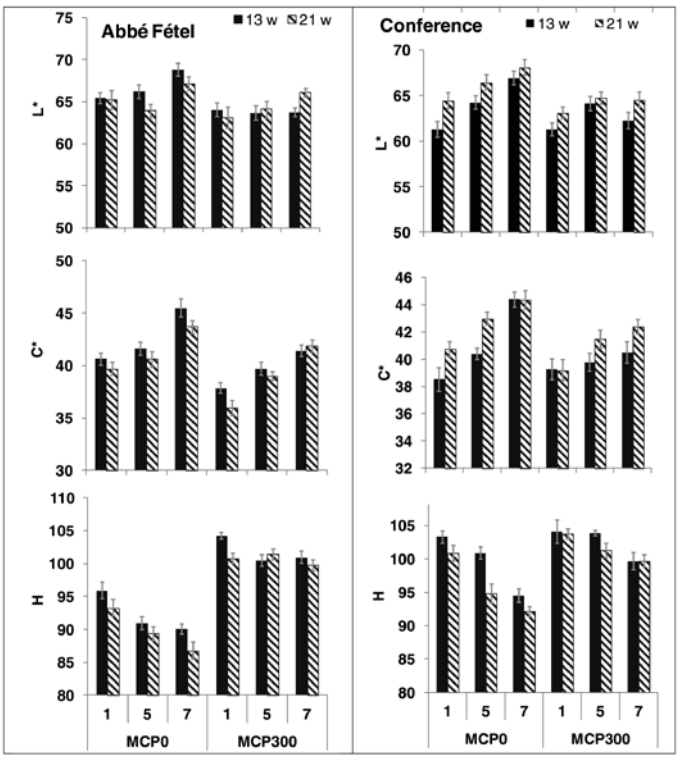

Fig. 5 - Lightness (L*), chroma (C*) and hue angle (degree) of control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears after 1, 5 and 7 days of shelf life at 20°C after 13 and 21 weeks of storage. Bars refer to standard error of the mean. Results of ANOVA analysis are reported in Table 3.

At d1 of shelf life, control 'Abbé Fétel' pears had lower firmness and energy-to-rupture but similar stiffness than d1 1-MCP treated fruit. Firmness, stiffness and energy-to-rupture of control 'Abbé Fétel' pears decreased after 5 days and then they did not change further. In 1-MCP treated 'Abbé Fétel' pears, firmness and energy-to-rupture did not change with shelf life, whereas stiffness decreased after 5 days, but to a lesser extent than in control fruit, without any further change with the increase of shelf life time.

Skin color

On average in 'Conference' pears L* and C* were higher in control fruit after 21 weeks storage (Fig. 5) than in 1-MCP treated fruit at both storage times and in control fruits after 13 weeks storage, while the highest H° was found for 1-MCP treated fruit at both storage times and the lowest for control fruit after 21 weeks. Hue decreased with storage time only in control fruit. At d1 of shelf life control 'Conference' pears had L*, C* and H° values not different from those of 1-MCP treated fruit. With shelf life, L* and C* increased and H° decreased both in control and 1-MCP treated fruit, with control fruit at d7 showing the highest values of L* and C* and the least of H°.

In 'Abbé Fétel' the highest L* value was found in control fruit after 13 weeks of storage and the lowest in 1-MCP treated fruit at both storage times (Fig. 5). Chroma on average was higher in control fruit than in 1-MCP treatment without any influence of storage time, whereas H° was higher in 1-MCP treated fruit than in control pears, without any difference between storage times, with control fruits having the lowest H° value after 21 weeks storage. At d1 of shelf life control 'Abbé Fétel' pears had higher L* and lower H° than 1-MCP treated fruit. L* increased with shelf life in control fruit at both storage times and in 1-MCP treated ones after 21 weeks of storage. H° decreased with shelf life only in control fruit, while C* increased both in control and 1-MCP treated fruit, with control fruit having the highest values at d7.

Sensory analysis

With regard to sensory analysis, 'Conference' 1-MCP treated pears were on average firmer, more grainy, less juicy, sweet, sour and aromatic than control ones (Fig. 6), with the average scores being (1-MCP and control, respectively): 84 and 45 for sensory firmness, 52 and 38 for graininess, 25 and 71 for juiciness, 47 and 62 for sweetness, 19 and 24 for sourness, and 33 and 59 for aromatic. No changes in sensory profile with storage time and shelf life were observed for 1-MCP treated 'Conference' pears. In contrast, in control 'Conference' pears sensory firmness decreased during shelf life, without any differences between the storage times, while sweetness decreased with shelf life after 13 weeks storage and increased after 21 weeks storage, with d5 pears after 13 weeks being sweeter than fruit after 21 weeks. Juiciness in control fruit increased with shelf life only after 13 weeks storage and it

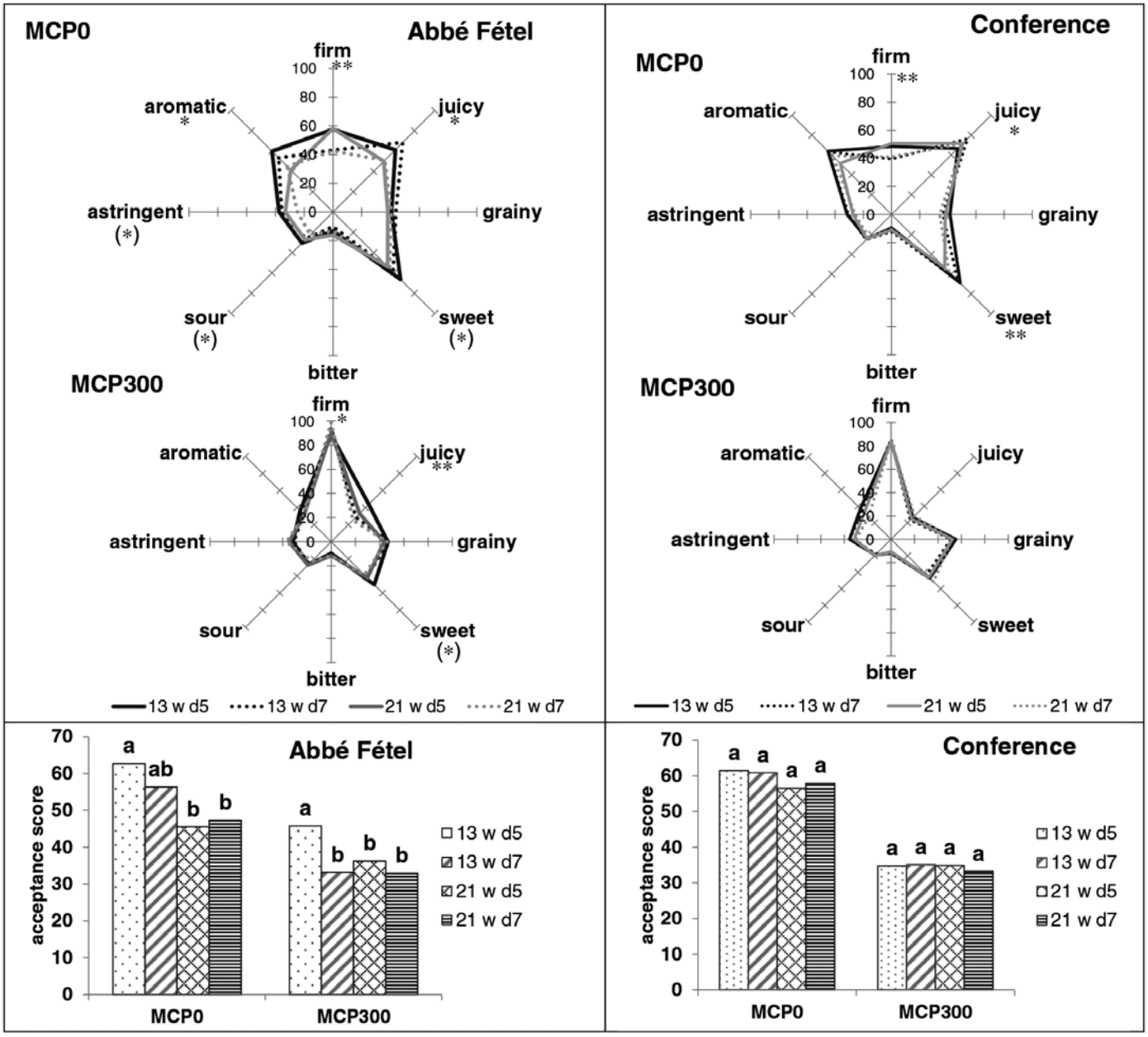

Fig. 6 - Sensory profiles and overall acceptability of control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears after 13 and 21 weeks of storage and 5 (d5) and 7 (d7) days of shelf life at 20°C. Within each 1-MCP dose, for each attribute the significance of F-ratio (**, P<0.01; *, P<0.05; (*), P<0.10; no symbol, not significant) is reported. Within each 1-MCP dose, bars with different letters refer to statistically different means (Tukey's test, P < 0.05%).

was not significantly affected by the storage time. Overall acceptability was higher in control fruit and it was not influenced by storage time and shelf life both in control and 1-MCP treated 'Conference' fruit.

On average control 'Abbé Fétel' pears were less firm and more juicy, sweet and aromatic than 1-MCP treated fruit, the average scores being (1-MCP and control, respectivley): 93 and 50 for sensory firmness, 33 and 58 for juiciness, 43 and 59 for sweetness, and 33 and 50 for aromatic. Similarly to that found for 'Conference' pears, control 'Abbé Fétel' fruit at d5 of shelf life (Fig. 6) were firmer than at d7, without any influence of storage time. Juiciness was higher in d7 fruit after 13 weeks storage than in those after 21 weeks. Sweetness and aromatic scores were lower in d5 fruit after 21 weeks than in d5 ones after 13 weeks, the latter showing also higher sourness and astringency than fruit stored for 21 weeks at the end of shelf life. As for 1-MCP treated 'Abbé Fétel' pears, sensory firmness did not change with shelf life in fruit stored for 13 weeks, whereas it increased in those stored for 21 weeks, with the d7 fruit after 21 weeks being firmer than fruit at d5 of both storage times. 1-MCP treated 'Abbè Fétel' pears at d5 after 13 weeks of storage were less firm and juicier and sweeter than those of the same storage time at d7 and than those stored for 21 weeks. Overall acceptability decreased with storage time and shelf life: control fruit after 13 weeks storage and 5 days of shelf life had the highest overall acceptability, while 1-MCP treated fruit at

Table 4 - Storage disorders in control (MCP0) and 1-MCP treated (MCP300) 'Conference' pears after 13 and 21 weeks of storage and 7 days of shelf life at 20°C and ANOVA results (n=3)

	healthy	Early blackening	Black spot	Superficial scald	black speck	rot
			MCP0			
13 w	33.2 ±0.3	59.4±0.7	0	0	4.6 ±0.4	0.3±0.1
21 w	22.2±0.4	0	71.3±0.3	4.7±0.4	0	0.3±0.3
			MCP300			
13 w	29.0±0.01	64.3±0.01	0	0	5.3±0.01	1.3±0.01
21 w	10.8±0.2	0	88.4±0.2	0	0	0.5±0.1
			ANOVA [z]			
A: storage time	**	***	***	**	***	NS
B: 1-MCP	NS	NS	*	**	NS	NS
A × B	NS	NS	*	**	NS	NS

[z] ***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant.

d7 after 13 weeks and at d5 and d7 after 21 weeks storage had the least overall acceptability. In addition, 1-MCP treated 'Abbé Fétel' pears stored for 13 weeks at 5 days of shelf life had an overall acceptability score that was not different from that of control fruit stored for 21 weeks.

Storage disorders

In 'Conference' pears at the end of storage and after 7 days at 20°C the percentage of healthy fruit was very low and decreased with storage time both in control and 1-MCP treated fruit (Table 4). Three types of peel disorders were detected: blackening, superficial scald and black speck. Two forms of blackening were distinguishable, differing for the color and severity of the disorder: early blackening, characterized by a grey net covering part of the peel, without any specific localization, and black spot, characterized by a very tight black net which, in the most severe forms, covered almost all the fruit surface. The percentages of fruit affected by the two forms of blackening were very high (Table 4). Early blackening was found only after 13 weeks of storage and its incidence was not influenced by 1-MCP treatment; black spot was found only after 21 weeks of storage, with higher incidence in 1-MCP treated fruit than in control ones. Superficial scald incidence was low (less than 5%) and developed only in control fruit after 21 weeks of storage. Black speck incidence was low (about 5%) and was found both in control and 1-MCP treated pears after 13 weeks of storage. Rot incidence was very low and was influenced neither by storage time nor by the 1-MCP treatment.

In 'Abbé Fétel' pears the percentage of healthy fruit on average was 93.3% in 1-MCP treated fruit and 77.4% in control ones and slightly decreased with storage time (Table 5). Two types of peel disorders were detected: soft scald and superficial scald, both of them developed only in control fruit. The incidence of soft scald increased somewhat with the increase of storage time, while superficial scald was detected at percentages lower than 1.5% only after 21 weeks of storage. Rot incidence was low, ranging from 4.2% (1-MCP treated after 21 weeks) to 8.6% (control after 21 weeks), and was not significantly influenced by storage time nor by the 1-MCP treatment.

Table 5 - Storage disorders in control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' pears after 13 and 21 weeks of storage and 7 days of shelf life at 20°C and ANOVA results (n=3)

	Healthy	Soft scald	Superficial scald	rot
		MCP0		
13 w	82.5±0.3	10.6±0.1	0	5.6±0.2
21 w	72.8±0.1	15.1±0.2	1.4±0.4	8.6±0.2
		MCP300		
13 w	95.6±0.03	0	0	4.4±0.03
21 w	91.5±0.2	0	0	4.2±1.2
		ANOVA [z]		
A: storage time	*	NS	NS	NS
B: 1-MCP	***	***	NS	NS
A × B	NS	NS	NS	NS

[z] ***, P<0.001; *, P<0.05; NS, not significant.

4. Discussion

In control and 1-MCP treated 'Conference' pears, the two ILOS periods induced a remarkable rise in chlorophyll fluorescence (Fα) in untreated fruit and much smaller ones in 1-MCP treated pears, when O_2 concentration decreased below 0.4 kPa, which is the lower O_2 limit (LOL) at which metabolism of 'Conference' pears changes from aerobic to fermentative (Zerbini and Grassi, 2010). The lesser Fα increase found in 1-MCP treated 'Conference' pears could be due to respiration reduction induced by 1-MCP, which reduces LOL, as observed in other pear cultivars (Watkins, 2006) and in 'Abbé Fétel' fruit by Rizzolo *et al.* (2008, 2010). In control 'Abbé Fétel' pears the O_2 concentration decreased below 0.6 kPa, which is its LOL, only for a short

period during the first ILOS period, while during the second ILOS period O_2 values ranged from 0.3 to 0.6 kPa. In contrast, in the container with 1-MCP treated 'Abbé Fétel' pears during the two ILOS periods O_2 values ranged from 0.1-0.4 kPa, causing a slight rise in Fα in 1-MCP treated 'Abbé Fétel' pears probably due to the fact that in this container the O_2 values were much lower than the LOL value.

It has been reported that fruit stored in ultralow oxygen pressure, below 2 kPa inducing fermentation, when compared to fruit stored in air develop lower quantities of straight-carbon chain compounds, esters, aldehydes and ketones (Mattheis *et al*., 1991, Fellman *et al.,* 1993) and increased amounts of ethanol, acetaldehyde and ethanol-derived ethyl esters, mainly ethyl acetate (Argenta *et al*., 2004). Furthermore, Lumpkin *et al.* (2014) found for apples that acetaldehyde, ethanol and ethyl esters amounts increased with pO_2, decreasing from 1.5 kPa to 0.3 kPa, mainly during the first weeks of storage, while Mattheis *et al.* (2013) found in 'd'Anjou' pears a significant correlation between pithy brown core incidence and ethanol, suggesting a relationship between disorder development and abnormal oxidative metabolism due to an hypoxic storage environment. According to these findings we can infer that, in control 'Conference' pears and in control and 1-MCP treated 'Abbé Fétel' fruit, the stress due to the low pO_2 applied during the ILOS period, as highlighted by the fluorescence monitoring, impacted on fermentative metabolites. The reduction of respiration induced by 1-MCP significantly influenced fermentative metabolite development, as in 'Conference' no more ethanol was produced during the low pO_2 ILOS period and in 'Abbè Fètel' pears it lowered the production of ethyl acetate.

As for the relationships between ILOS period and α-farnesene and CTols content in the peel, for both cultivars no significant changes in their concentrations following the ILOS period were found, with the exception of $CTol_{281}$ in 1-MCP treated 'Conference' pears, which became not detectable at the end of the ILOS period. Then, after 13 weeks of storage α-farnesene and CTols concentration in the peel increased in control fruit of both cultivars and in 1-MCP treated 'Abbé Fétel' pears. On the other hand, in both cultivars 1-MCP treatment significantly inhibited the production of α-farnesene and CTols not only during the ILOS period but also after the CA storage period till 13 weeks storage time, with 'Abbé Fétel' pears showing a more marked reduction in α-farnesene, $CTol_{258}$ and $CTol_{269}$ concentrations and 'Conference' fruit in $CTol_{281}$ concentration.

The α-farnesene and CTols trends observed are in agreement with previous findings on 'Conference' and 'Abbé Fétel' pears (Lo Scalzo *et al.*, 2002; Folchi and Bertolini, 2008; Eccher Zerbini *et al.*, 2005; Vanoli *et al.*, 2010 b) and could be due to the fact that low temperature storage induces, in scald-susceptible cultivars, a high rate of α-farnesene synthesis, which causes its marked accumulation in the skin during the first two to three months of storage. Then, the concentration of α-farnesene declines as a consequence of its *in vivo* oxidation to the highly reactive conjugated trienols (Gapper *et al*., 2006; Isidoro and Almeida, 2006; Whitaker, 2007), which disrupt cell membranes and lead to polyphenoloxidase-mediated browning of the skin (Bain and Mercer, 1963) and necrosis of the hypodermal cell layers. Moreover, it was found that both in apples and pears inhibition of α-farnesene synthesis by 1-MCP was closely correlated with suppression of the α-farnesene synthase gene Pc*AFS1*, which encodes the last enzyme in the α-farnesene biosynthetic pathway (Lurie *et al.*, 2005; Pechous *et al.*, 2005; Gapper *et al*., 2006). In highly scald-susceptible apple and pear cultivars, inhibition of ethylene production and α-farnesene synthesis by pre-storage 1-MCP treatment is often lost after several months in cold storage, and this coincides with loss of scald control (Gapper *et al.*, 2006; Tsantili *et al.*, 2007). For 'd'Anjou' pears Zoffoli *et al.* (1998) reported that $CTol_{269}$ was the main peak for conjugated trienols and increased during cold storage, as did the other two CT peaks; also for 'Packham's Triumph' pears the three CTols increased during storage, but $CTol_{258}$ was proportionally higher than $CTol_{281}$ and almost the same as $CTol_{269}$, while in 'Bartlett' pears the main CTol was $CTol_{258}$, which increased with storage, with very low amounts for the other two CTols. Whitaker *et al.* (2001) associated the absorbance measured at 258 nm in the skin hexane extracts to a family of p-cumaryl fatty esters which act as antioxidant rather than to an oxidation product of α-farnesene. In view of these findings, our results suggest that the capacity of 'Conference' pears stored in CA after ILOS periods to generate scald-related antioxidants ($CTol_{258}$) is higher than the fruit's ability to produce scald-related α-farnesene oxidation products ($CTol_{281}$), while the opposite scenario was found for control 'Abbè Fétel' fruit. Considering the ratio $CTol_{258}/CTol_{281}$, a potential marker of superficial scald, Du and Bramlage (1993) found that values lower than 1.0 were generally associated to high scald susceptibility while values greater than 2.0 were associated to lower scald susceptibility. In this work, higher values of $CTol_{258}/CTol_{281}$ were found in 1-MCP treated fruit of both cultivars, whereas values below 1.0 and below 2.0 were found in control fruits of 'Abbé Fétel' and 'Conference', respectively, both developing some superficial scald at the end of storage. Du and Bramlage (1993) reported that $CTol_{269}/CTol_{281}$ ratio values generally reflected those of the $CTol_{258}/CTol_{281}$ ratio, but differences were less distinct when comparing lots of apple fruit with different scald potential. On the other hand, Zoffoli (1994), when considering the trends of the $CTol_{258}/CTol_{281}$ and $CTol_{269}/CTol_{281}$ ratios with cold storage time in pears, suggested that $CTol_{258}$ could be the precursor to $CTol_{269}$, and $CTol_{269}$ the precursor to $CTol_{281}$. In the present study, data of the two ratios confirmed Zoffoli's (1994) hypothesis: indeed in 'Abbé Fétel' pears at the beginning and at the end of the second ILOS period $CTol_{258}$ was predominant, both in control and 1-MCP treated fruit, but in correspondence with the first storage time $CTol_{269}$ was predominant in control fruit, while it was almost the same in 1-MCP treated pears. Similarly, in control 'Conference' fruit at the first storage

time, $CTol_{269}$ was predominant. The low incidence of superficial scald found in this work could be due either to seasonal non predisposing conditions, as low superficial scald incidence was observed in 'Abbé Fétel' fruit stored in NA (Rizzolo, Grassi and Vanoli unpublished) or to the beneficial effect of CA in 'Conference' pears with respect to NA stored fruit (Rizzolo *et al.,* 2015).

Both in 'Conference' and 'Abbé Fétel' pears, 1-MCP treatment prevented the development of scald after storage and shelf life. In 'Conference' a high proportion of fruit developed blackening in a less severe form after 13 weeks storage, and with higher incidence and severity after 21 weeks storage. Blackening has been detected in 'Conference' pears for about twenty years, but its incidence and severity has been increasing over the last few years. According to previous observations (Bertolini, personal communication), blackening development is not related to α-farnesene and CTols development and it is neither controlled nor prevented by 1-MCP treatment, rather it induced higher severity than in control fruit. Black speck was developed in 'Conference' only after 13 weeks storage and, similarly to blackening, it was neither controlled nor prevented by 1-MCP treatment. Black speck has been reported for mature-green 'd'Anjou' pears (Lee *et al*., 1990) and it was suggested that it is provoked by fruit stress related to low temperature in conjunction with low oxygen CA storage, as confirmed by Mattheis and Rudell (2011), who found that the low O_2 partial pressure set points established by monitoring fruit chlorophyll fluorescence can prevent 'd'Anjou' scald but may result in black speck development.

The pre-storage 1-MCP treatment drastically reduced ethylene production during post-storage shelf life both in 'Conference' and 'Abbé Fétel' fruit, as found in previous studies on these cultivars (Eccher Zerbini *et al.*, 2003, 2005; Rizzolo *et al.*, 2005, 2008; Vanoli *et al.*, 2008, 2010 a) and on other pear cultivars (Watkins, 2006 and references herein). Control 'Abbé Fétel' pears produced more ethylene than control 'Conference' pears and were less sensitive to 1-MCP treatment, as shown mainly by the sensory analysis results and, secondly, by quality parameters, as found by Eccher Zerbini *et al.* (2003, 2005).

Control fruit from both cultivars with shelf life underwent skin yellowing and pulp softening. However, control 'Abbé Fétel' pears already upon removal after 21 weeks storage in CA soften to firmness values lower than 40 N, the threshold value corresponding to edible-firm texture for this cultivar (Predieri and Gatti, 2009). 'Conference' control fruit softened rapidly with shelf life, reaching the minimal level of acceptable eating quality of 10 N (Chiriboga *et al*., 2013) already after 5 days of shelf life at 20°C. Fruit softening in control pears was similar to that found in previous experiments on CA and DCA 'Abbé Fétel' fruit stored at -0.5°C (Rizzolo *et al.*, 2014; Vanoli *et al.*, 2015) and on 'Conference' fruit stored in NA and CA at -1°C (Folchi and Bertolini, 2008) and in air at -0.5°C (Chiriboga *et al.*, 2013). The treatment with 1-MCP at the concentration of 300 nL L^{-1} prevented ripening during the 7-day shelf life at 20°C, even if slight decreases in firmness, stiffness and energy-to rupture were found for 1-MCP treated 'Abbé Fétel' pears; these slight changes, indeed, influenced the sensory firmness and juiciness. In contrast, in 1-MCP treated 'Conference' pears, only stiffness slightly decreased with shelf life, and no changes in the sensory profile were found with storage time and shelf life. On the contrary, Folchi and Bertolini (2008) observed that in 'Conference' pears harvested at 64.7 N and treated with 300 nL L^{-1} 1-MCP, stored at -1°C up to four months there was a slight softening of 6 N, followed by a further softening to 40 N, prolonging storage up to seven months plus seven days of shelf life at 20°C. This difference could be due to the fact that 'Conference' pears had been harvested at a less advanced stage of maturity as assessed by the firmness value of 71 N and hue value of 108°, which are similar to the values reported by Chiriboga *et al.* (2013) for early and mid harvests. These authors reported that in two years 'Conference' pears harvested before or around the commercial harvest date remained firm after treatment with 1-MCP and lost their ability to soften even after several days at 20°C. In contrast 1-MCP applied at more advanced stages of maturity slowed down the softening process without completely blocking it.

5. Conclusions

The 1-MCP treatment at harvest influenced the physiological and quality changes of 'Conference' and 'Abbé Fétel' pears stored in CA after ILOS periods. 1-MCP dramatically decreased the concentrations of α-farnesene and CTols in the fruit skin at the end of the ILOS period both in 'Conference' and 'Abbé Fétel' pears, and affected the concentrations of fermentative metabolites in the fruit pulp, lowering, after the ILOS period, the ethanol concentration in both cultivars and the ethyl acetate amount in 'Abbé Fétel' pears. The 1-MCP treatment drastically reduced the ethylene production during shelf life, impaired fruit softening to edible texture, and delayed fruit yellowing both during storage and shelf life. Upon sensory tasting, 1-MCP treated fruit was firmer and less juicy, sweet and aromatic than control fruit, without any changes with shelf life in 'Conference' fruit and with a slight decrease in sensory firmness and juiciness in 'Abbé Fétel' pears. It was confirmed that 1-MCP prevents superficial scald in both cultivars, and soft scald in 'Abbé Fétel' pears, whereas in 'Conference' fruit it either has no effect or enhances the incidence and severity of blackening, a disorder which may not be dependent on oxidation products in the skin.

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