Ethylene Production and Quality in 1-Methylcyclopropene Treated ‘Abbé Fétel’ Pears after Storage in Dynamically Controlled Atmosphere

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Abstract
This research studies the ethylene production rate (EP) and quality in 1-MCP treated ‘Abbé Fétel’ pears after storage in DCA compared to NA and CA. 1-MCP treated (300 ppb) and control fruit were stored at -0.5°C in NA, CA (2 kPa O2 + 0.7 kPa CO2) and DCA (0.7 kPa O2 + 0.3 kPa CO2). After 4 and 6 months storage, fruit were held up to 7 d at 20°C. Skin colour, firmness and EP were measured during shelf life and the incidence of disorders after 7 d. 1-MCP treatment drastically reduced EP, which began to recover after 7 d at 20°C, except for DCA stored pears. In control fruit, NA stored pears showed the highest EP. 1-MCP treated fruit were the greenest at the end of shelf-life, especially after CA and DCA. Control fruit stored in DCA and in CA were greener than NA both at 1 d and 7 d of shelf life. Pears treated with 1-MCP did not soften during shelf life, while in control fruit firmness decreased from about 40 N to about 15-20 N, whatever the storage atmosphere. 1-MCP treatment prevented soft and superficial scald and internal breakdown, independently of storage atmosphere. DCA prevented superficial scald in control fruit, while it increased internal browning and breakdown in control and 1-MCP treated pears. No differences were found for soft scald incidence between control DCA and CA stored fruit. The highest percentage of sound fruit was found in NA stored 1-MCP treated pears, and the lowest in control fruit stored in DCA.

INTRODUCTION
Dynamically Controlled Atmosphere (DCA) is an innovative storage technique based on the non destructive chlorophyll fluorescence measurement to dynamically set O2 levels slightly above the tolerance level of the fruit (Prange et al., 2003; DeLong et al., 2004; Zanella et al., 2008).

HarvestWatch™ (Satlantic Inc., Halifax. N.S., Canada), a chlorophyll fluorescence sensor system, is able to detect the presence of low-O2 stress in chlorophyll containing fruit and vegetables by using a fluorescence interactive response monitor (FIRM) that produces a theoretical estimate of F0 at zero irradiance, called Fα (Prange et al., 2003): when O2 levels decrease below an O2 threshold, Fα immediately increases; if the O2 level increases above the threshold level, Fα returns to approximately pre-stressed values. The spike of Fα at the O2 threshold indicates the transition of the fruit from aerobic to anaerobic metabolism, as found by Eccher Zerbini et al. (2006) and Gasser et al. (2008). These authors studied the respiration quotient (RQ) and the fluorescence signal Fα in pears and apples, finding that both methods yielded the identical O2 threshold. The critical limit for the O2 level was 0.4 kPa for ‘Conference’ pears and 0.6 kPa for ‘Abbé Fétel’ pears, when RQ was >> 1. The O2 limit for ‘Abbé Fétel’ pears was confirmed by Rizzolo et al. (2008). Usually in DCA storage the O2 level can be set 0.1-0.2 kPa above the O2 threshold. Therefore, with DCA it has been possible to reduce O2 levels in CA storage (2.0 kPa) to 0.5 kPa for ‘Conference’ pears and to 0.7 kPa for ‘Abbé Fétel’, without fruit damage.

DCA can be applied to any chlorophyll-containing fruit or vegetable (Prange, 2002, 2003) but has been mainly studied in apples and, on a commercial scale, it has been applied in packaged salad operations (Prange, pers. commun.) and in apple storage.
Very few studies exist on DCA storage of pears (Eccher Zerbini, 2006; Vanoli et al., 2007; Rizzolo et al., 2008). The use of the lowest O\(_2\) level tolerated by the fruit could optimize the CA benefits, as oxidative reactions and senescence processes should slow down, preventing the development of physiological disorders and quality degradation. After 5-9 months storage, DCA stored apples were generally firmer and more acidic than those stored in ULO-CA; furthermore, DCA apples did not accumulate fermentation products and had no off-flavours (Zanella et al., 2005; DeLong et al., 2004; Prange et al., 2003; Gasser et al., 2008). In the same manner, DCA pears were greener, firmer, sweeter, and more acidic compared to CA stored pears up to 5 months storage (Vanoli et al., 2007). DCA can exert a positive effect on the control of storage disorders. Superficial scald was completely controlled by DCA in ‘Granny Smith’, ‘Cortland’ and ‘Delicious’ apples (DeLong et al., 2004; Zanella et al., 2005). On the other hand, O\(_2\) injury could be aggravated by the very low O\(_2\) levels in DCA storage, as found by DeLong et al. (2007) in ‘Delicious’ apples compared to CA stored apples. Also the incidence of external CO\(_2\) injury was slightly higher in DCA than in ULO-stored fruit, perhaps because of the high CO\(_2\) level relative to the very low O\(_2\) levels (Zanella et al., 2005). DCA stored ‘Abbé Fétel’ were less sensitive to superficial scald and soft scald, although more sensitive to internal browning with respect to NA or CA stored pears (Vanoli et al., 2007).

Quality preservation and control of physiological disorders obtained with DCA in apples are similar to those obtained by 1-MCP treatment. However, Zanella et al. (2005, 2008) found that 1-MCP delayed the degradation of organic acids resulting in higher levels of titratable acidity especially after shelf life and was more effective in maintaining higher firmness for late picked apples. The combination of 1-MCP action with the extremely low level of O\(_2\) in DCA did not appreciably improve firmness in ‘Gala’ apples (Zanella et al., 2008). In ‘Abbé Fétel’ pears stored in DCA, Vanoli et al. (2008) found that 1-MCP treatment allowed the O\(_2\) level to be reduced to 0.3 kPa, compared to 0.6 kPa in untreated fruit, without causing any increase in the fluorescence signal. Furthermore, 1-MCP treatment markedly reduced O\(_2\) and CO\(_2\) exchange rates in CA and NA stored pears, while fruit in DCA did not show any difference in respiration rate between 1-MCP treated and control fruit (Rizzolo et al., 2008).

In the present work the effect of DCA storage on ethylene production, quality characteristics and physiological disorders of ‘Abbé Fétel’ pears during shelf life at 20°C was compared to that of NA and CA storage and of 1-MCP treatment.

**MATERIALS AND METHODS**

‘Abbé Fétel’ pears were picked on 19 September 2005, from a commercial orchard in Modena province, Italy, and directly randomized in 48 boxes (about 50 fruit per box).

After 48h at -0.5°C in air, 24 boxes were treated with 300 ppb 1-MCP in gastight containers for 24h and the remaining boxes were used as control (0 ppb 1-MCP). After 2 weeks at -0.5°C, treated and untreated fruit were put in 3 storage rooms at -0.5°C: NA, CA (2 kPa O\(_2\) + 0.7 kPa CO\(_2\)) and DCA.

Two FIRM sensors were put in both CA and DCA over the control fruit, as, in a previous study, 1-MCP treated pears had a lower O\(_2\) limit (Vanoli et al., 2007), choosing 4 of the greenest and 4 of the most yellow pears for each storage atmosphere. In DCA, after reaching 2 kPa O\(_2\), fruit were left without oxygen supplementation, with oxygen levels decreasing due to respiration; when an increase in fluorescence signalled a too low O\(_2\) level, then O\(_2\) was set to a level immediately above the stress level, while keeping CO\(_2\) as low as possible; this resulted in DCA with 0.8 kPa O\(_2\) + 0.3 kPa CO\(_2\).

The day after harvest, a sample of 30 fruit was analysed for fruit mass, skin colour on the greenest side of the fruit (Minolta Spectrophotometer), flesh firmness (8 mm diameter plunger mounted on an Instron UTM, crosshead speed 200 mm/min), starch hydrolysis (EUROFRU scale, where 1=minimum, 10=maximum starch hydrolysis), soluble solids and titratable acidity.
After 4 and 6 months storage, 3 boxes per 1-MCP dose per atmosphere were put in shelf life at 20°C. A sample of 20 fruit was analysed for ethylene production (EP) and skin colour after 1, 3 and 7 d of shelf life and for flesh firmness at 1 and 7 d.

EP was measured on single fruit put into a 1.7 L gastight glass jar for 1h at 20°C, sampling 1 ml of the headspace gas and analysing it by GC according to Rizzolo et al. (2005). EP was expressed as pmol/kg/s.

Storage disorders were examined at the end of the shelf life period (7 d).

Data were processed by analysis of variance, considering storage time, 1-MCP dose, storage atmosphere and day of shelf life as factors (PROC GLM, SAS/STAT, SAS Institute Inc., Cary, NC, 1999). Means were compared by Tukey’s test at p<0.05.

RESULTS AND DISCUSSION

At harvest, ‘Abbé Fétel’ pears had (mean±standard error): fruit mass=333±13.3 g; starch hydrolysis=6.5±0.35, skin colour (hue°)=99±0.3°, flesh firmness=51±0.8 N, soluble solids=13.4±0.18°Brix and titratable acidity=3.6±0.12 meq/100 g juice.

Ethylene Production

Ethylene Production (EP) during shelf life was totally prevented by 1-MCP treatment at 300 ppb after 4 months storage and drastically reduced after 6 months storage, whatever the storage atmosphere (Fig. 2), as previously found by Vanoli et al. (2007). Rizzolo et al. (2008), studying ‘Abbé Fétel’ pears during storage in DCA, CA and NA at -0.5°C, found that already at this low temperature EP was prevented by 300 ppb 1-MCP without differences among the storage atmospheres. EP in 1-MCP treated pears did not change during shelf life at 20°C in DCA stored fruit, while in NA and in CA it showed a slight increase at 7 d.

In untreated fruit, EP was affected by storage time, atmosphere and shelf life. At 1 d of shelf life there was no difference in EP with storage atmosphere after 4 months, while after 6 months storage NA pears showed the highest EP, about twice as much as in the other fruit. During shelf life, in NA pears EP did not change after 4 months storage, while it dramatically decreased from 1 d to 3 d after 6 months storage, and then remained at a constant level. In CA pears, EP showed a peak at 3 d after 4 months storage and a decreasing trend after 6 months storage, while DCA pears developed about the same
amount of ethylene, whatever the storage time and the day of shelf life. At the end of shelf life, NA untreated pears showed the highest EP, both at 4 and 6 months storage, and DCA and CA pears developed similar amounts of ethylene. A previous experiment (data not shown) confirmed the highest EP in NA storage with respect to CA and DCA and the lack of differences in EP between CA and DCA pears at the end of shelf life at 20°C. EP of control fruit was not different from that found in 2001, 2002 and 2004 seasons but much lower than that found in 2003 (Eccher Zerbini et al., 2003, 2005; Vanoli et al., 2007).

Skin Colour

Skin colour changed from green to yellow during shelf life, showing a different trend according to the storage atmospheres and the 1-MCP treatment (Fig. 2). On average, 1-MCP treatment kept the fruit greener, especially in DCA and CA storage. After 4 months storage, at 1 d shelf life, there was no difference in skin colour among treated and untreated DCA-CA pears and NA treated pears, while after 6 months storage, 1-MCP DCA-CA stored fruit and DCA control fruit were greener than CA control fruit and NA treated and untreated pears. At 7 d of shelf life, whatever the storage time, DCA and CA 1-MCP treated pears were the greenest; NA treated fruit was slightly greener than control DCA-CA pears after 4 months storage, while after 6 months there was no difference among these fruit. NA untreated fruit were less green than the other treatments both at the beginning and at the end of shelf life, whatever the storage time, as already found by Vanoli et al. (2007) in 2003 and in 2004. In 2003 1-MCP at 300 and 100 ppb reduced yellowing in NA and CA stored pears, while in 2004 1-MCP (100 ppb) kept the fruit greener at the beginning of the shelf life, while at 7 d, especially after long storage, there was no difference in skin colour between treated and untreated pears. In 2004, on average, skin yellowing developed faster than in 2005 and DCA and CA treated pears were the greenest at the beginning of the shelf life period, while after 7 d at 20°C, even if 1-MCP was less effective in 2004 than in 2005, DCA storage kept the fruit the greenest until the end of storage.

Firmness

Softening was completely prevented by 1-MCP treatment, whatever the storage atmosphere and the storage time, as flesh firmness remained at about 45 N during all the shelf life period without differences among the storage atmospheres (Fig. 2). After 4 months’ storage, at 1 d of shelf life control fruit showed firmness values slightly lower than treated ones; at 7 d, untreated fruit softened to about 15 N in CA and NA and to 10 N in DCA. After 6 months storage, and 1 d of shelf life treated fruit were firmer than the untreated pears without differences between the storage atmospheres; among control pears, DCA fruit were firmer than NA, but all the fruit softened to the same firmness values.

A slight softening was found in 300 ppb 1-MCP treated pears by Vanoli et al. (2007) in 2003, while in 2004 1-MCP (100 ppb) kept the fruit firmer than the control pears after 3 months storage and only at the beginning of shelf life after 5 months but then treated pears softened normally to 25 N. In 2004, DCA treated pears had the highest firmness both after 3 and 5 months storage at 3 d of shelf life; and at 7 d after 3 months storage NA treated fruit showed the highest firmness and DCA control pears the lowest, while after 5 months there were no differences among storage atmospheres.

Storage Disorders

Pears showed soft scald, superficial scald, internal browning and internal breakdown, whose incidence increased with storage time and was strongly affected by storage atmosphere and by 1-MCP treatment (Fig. 3). In control fruit, soft scald already appeared after 4 months storage in all three storage atmospheres, showing the highest incidence in CA pears. With storage time, its incidence dramatically increased in DCA and CA pears, the latter showing the highest incidence (45%), even if not significantly
different from DCA (36%). Already in 2004 Vanoli et al. (2007) found that in CA storage the incidence of soft scald was almost twice as much as in DCA.

Superficial scald was present in NA control pears after 4 and 6 months storage, and at a very low incidence in CA fruit after 6 months storage (3%), when the percentage for NA fruit was about 30%, confirming the data of 2004 (Vanoli et al., 2007).

1-MCP treatment totally prevented soft and superficial scald, whatever the storage atmosphere. In 2003, 1-MCP at 300 ppb prevented superficial scald after 4 months storage and reduced it after 6 months, while 1-MCP at 100 ppb reduced superficial scald and totally prevented soft scald which developed only in DCA storage; in 2004 1-MCP at 100 ppb had no effects on superficial scald but drastically reduced soft scald in CA and in DCA (Vanoli et al., 2007).

Internal browning was higher in DCA pears than in CA ones whatever the storage time and the 1-MCP treatment, and it was absent in NA storage.

Internal breakdown was present at a very low incidence in NA storage (1%) and showed the highest incidence in DCA untreated fruit. 1-MCP treatment totally prevented internal breakdown in CA stored pears, while it only decreased the percentage of this disorder in DCA stored fruit.

The percentage of fruit rots was about 20% after 4 months storage, without significant differences in storage atmospheres (data not shown), while, after 6 months storage it increased only in NA control fruit (43%).

The highest percentage of sound fruit was found in NA storage both in treated and untreated fruit after 4 months storage, even if there were no significant differences among the three atmospheres; after 6 months storage the highest percentage of healthy fruit was found in NA treated fruit and the lowest in the DCA untreated ones (Fig. 3).

CONCLUSIONS
Our results show that ‘Abbé Fétel’ pears stored in DCA maintained a good firmness and colour, even if there were no significant improvements with respect to CA and NA fruit quality. Unfortunately, the low O\(_2\) level used in DCA caused severe product losses, as the incidence of some storage disorders was higher. DCA pears showed more internal browning and internal breakdown than in CA and in NA storage, as occurred also in apples (DeLong et al., 2007). On the other hand, DCA pears were not affected by superficial scald and developed less soft scald than CA, confirming that the low concentration of O\(_2\) used in DCA prevented superficial scald and did not increase soft scald, as already found in pears and apples (Vanoli et al., 2007; DeLong et al., 2004; Zanella et al., 2005, 2008). Despite that, untreated DCA pears showed the lowest percentage of sound fruit. In 2004, DCA was more effective in controlling soft scald, probably because DCA pears showed a lower degree of senescence, being greener, firmer, and more acidic than the other treatments up to the end of the storage time.

1-MCP treatment at 300 ppb had a strong effect on pear quality: 1-MCP totally prevented ethylene production and softening whatever the storage time and the atmosphere, so fruit did not ripen to an eating quality. Only skin colour changed in treated fruit, as they showed a slight yellowing during shelf life at 20°C. 1-MCP also had a strong effect on storage disorders, as it totally prevented soft and superficial scald, in DCA-CA storage and in NA, respectively, while it did not control internal browning and internal breakdown.

Further studies are needed to better clarify the relationship between DCA storage, fruit maturity and factors inducing storage disorders in ‘Abbé Fétel’ pears.

Literature Cited
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**Figures**

Fig. 1. Time course of Fa and O₂ concentration during DCA and CA storage of ‘Abbé Fétel’ pears.

Fig. 2. Ethylene production, skin colour (hue) and firmness of 1-MCP treated (empty symbols) and control (filled symbols) ‘Abbé Fétel’ pears stored in DCA (circle), CA (square) and NA (triangle) for 4 and 6 months. Bars refer to standard errors.
Fig. 3. Storage disorders of 1-MCP treated and control ‘Abbé Fétel’ pears stored in DCA, CA and NA for 4 and 6 months. Bars refer to standard errors.