1-Methylcyclopropene and extreme ULO inhibit superficial scald in a different way highlighting the physiological basis of this disorder in pear

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ABSTRACT

Despite years of research on the biochemical basis of superficial scald in apples, relatively little is known about the specific regulatory processes involved in pears. To gain further knowledge on these processes, different storage scenarios, controlled atmosphere (CA), 1-methylcyclopropene treatment (1-MCP) and storage under very low O_2 concentration (xULO) were used in the scald susceptible 'Blanquilla' pears. Ethylene production, α-farnesene (AF), conjugated trienols (CTols) content and changes in ethanol levels were evaluated during storage and further related to superficial scald development and changes in fruit quality upon removal.

While 1-MCP completely inhibited ethylene production and fruit softening, only a partial and transient inhibition of these parameters was found for xULO-treated fruit. Both 1-MCP and xULO treatments completely controlled scald disorder, yet in different ways. The reduction in disorder incidence in 1-MCP treated fruit was the result of ethylene inhibition and reduced levels of α-farnesene and CTols. In contrast, xULO treatment only partially inhibited ethylene production and the levels of α-farnesene metabolites but led to increased ethanol levels that were directly related to the scald incidence inhibition. Collectively, these results highlight that superficial scald in pear is not strictly related to ethylene and α-farnesene metabolism and that other compounds, such as the weak antioxidant ethanol, play a determining role in 'Blanquilla' pear.

Keywords: α-farnesene, Blanquilla pear, ethanol, superficial scald

1. INTRODUCTION

Superficial scald is a physiological disorder that occurs after a prolonged cold storage period and affects a large part of pears marketed worldwide. This disorder, which manifests as brown or dark patches on the fruit skin, affects the fruit appearance and makes the fruit unsuitable for commercialisation as a fresh commodity (Emongor et al., 1994).

In apples, superficial scald has been clearly related to three main key factors that interact between them: ethylene production (Liu, 1977; Knee and Hatfield, 1981), the accumulation of high levels of α-farnesene before and during the first month of cold storage and the oxidation of this last compound in hydroperoxides or CTols (Lurie and Watkins, 2012). It is widely accepted that superficial scald is the result of an oxidative process induced during cold stress (Ju et al., 1996; Rao et al., 1998) and that the disorder should not appear during storage if fruit maintain sufficient antioxidants to prevent or limit α-farnesene oxidation (Zubini et al., 2007; Silva et al., 2010). Accordingly, the postharvest antioxidant diphenylamine (DPA), recently banned across EU producing countries, was extremely efficient in controlling superficial scald without significantly affecting ethylene metabolism (Lurie and Watkins, 2012).

For decades, the above-mentioned model has been widely accepted to explain superficial scald disorder in apple but also in pear. However, contradictive information have recently been published highlighting that the regulatory mechanisms involved in superficial scald development in pears are different than in apples. Indeed, the strict relationship observed in apples between ethylene production and scald incidence has not always been found in pears. For instance, 'Beurré d'Anjou' pears although producing significantly lower amounts of ethylene than 'Packham Triumph', were found to be more sensitive to superficial scald (Larrigaudière et al., 2016). As α-farnesene accumulated at the same levels in both cultivars, it was concluded that α-farnesene biosynthesis in 'Beurré d'Anjou' was not strictly ethylene-dependent and that other factors, such as low temperature, were directly involved in α-

farnesene biosynthesis (Larrigaudière et al., 2016). Similar results were also found by Calvo et al. (2015) in the same cultivar picked at different harvest dates, but also by Pesis et al. (2009) in apple lines suppressed for ACC metabolism.

Given that superficial scald is considered the result of an oxidative process, endogenous antioxidants may certainly play a determining role in the control of superficial scald. In pears, the high sensitivity of some cultivars to scald appears to be determined quantitatively (pear skin contains lower amount of antioxidants and showed scald symptoms with lower CTols levels than apples), but also qualitatively by specific antioxidants such as ascorbate that was found to play an important role in 'Packham Triumph' pear susceptibility to superficial scald (Larrigaudière et al., 2016). Although ethanol is also a weak antioxidant, only few studies have focused on defining the role that this antioxidant may have in scald control. An interesting correlation between ethanol levels and superficial scald incidence was found by Wang and Dilley (2000) in ILO (initial low oxygen) treated apples suggesting a potential role of this antioxidant in scald control. Similar results were also found by Scott et al. (1995) with ethanol vapours in 'Granny smith' apples but also by Chervin et al. (2001) when ethanol was applied in combination with controlled atmosphere (CA) storage of apples.

Since the recent prohibition of the chemical anti-scald products by the European community, an important effort has been made to develop new alternatives to control superficial scald, especially in pears. Among the best alternatives, 1-methylcyclopropene (1-MCP) and the use of very low O_2 concentration during storage (extreme ULO or xULO) are undoubtedly among the best strategies to avoid superficial scald development. 1-MCP is a specific ethylene inhibitor acting at the receptor level (Sisler and Serek, 1997; 2003) inhibiting completely ethylene biosynthesis. By this way, 1-MCP inhibits the transcription processes and enzyme activities promoted by ethylene, and especially the ethylene-induced up-regulation of alpha-farnesene-synthase AFS1 (Lurie et al., 2005; Pechous et al., 2005; Gapper et al., 2006) that is

considered a key enzyme for scald development. xULO, on the other hand, likely controls superficial scald by limiting the levels of O_2 in the storage atmosphere and thus the oxidation processes involved in α-farnesene oxidation and final skin peroxidation. Thus said, very little is known on the mode of action of xULO. For instance, no information exist on the way by which xULO may affect α-farnesene synthesis and oxidation but also on the effects that this storage scenario may have on the generation or degradation of endogenous antioxidants in pears.

Accordingly, our work aimed to clarify the specific mode of action of these two strategies, bringing more information on the way by which xULO controls scald in Blanquilla pears but also on the specific regulatory mechanisms involved in the development of this physiological disorder in pears.

2. MATERIAL AND METHODS

2.1 Plant material and sampling

'Blanquilla' pears (Pyrus communis L.) were harvested from a commercial orchard located in Lleida (Catalonia, Spain). Fruit were picked of uniform size and free from defects at optimum harvest date and then immediately transferred to the laboratory to form the three following samples, each containing 350 fruit:

- CA: Control fruit stored for 5 and 8 months in controlled atmosphere at -0.5 ºC, 90 % RH and 2.5 % O_2 + 1.5 % CO_2.
- 1-MCP: Fruit kept overnight at -0.5 ºC and treated with 300 $nL.L^{-1}$ 1-MCP during 18 hours at -0.5 ºC using the product Smartfresh™ (Agrofresh Inc.) and as described in Chiriboga et al. (2011). Immediately after treatment, fruit were stored in CA in the same conditions than control.
- xULO: Fruit were stored during 5 and 8 months in controlled atmosphere at -0.5 ºC, 90 % RH with low O_2 levels (0.7 % O_2 + 0.5 % CO_2).

2.2 Determination of fruit quality

Fruit quality indexes were determined after 5 and 8 months of storage and after 1, 3 and 7 days of shelf life at 20 ºC.

Flesh firmness was measured on 30 fruit per sample with a penetrometer (T.R.Turoni srl., Italy) equipped with an 8 mm probe as described by Chiriboga et al. (2011). Total soluble solids (TSS; %) were measured on pear juice (blend of 5 fruit per replicate and 6 replicates per sampling) using a digital hand-held refractometer (Atago, Tokyo, Japan) whereas acid content (TTA) was measured on the same juice samples by titration using NaOH 0.1N and the results expressed as g malic acid L^{-1}.

136 Fruit surface colour was determined on 30 fruit with a colorimeter (CR-400, Minolta, Japan)
137 using the CIE L*a* b* colour space coordinates and the results expressed using the a*+b*
138 index for which an increase in index means an increase in yellowing.

139 **2.3 Determination of superficial scald incidence**

140 Scald incidence was estimated visually after 5 and 8 months of storage in the different
141 conditions immediately after removal (time 0) and after 3 and 7 additional days of
142 commercial life at 20 ºC. At each time the number of damaged fruit (% fruit with scald
143 symptoms) was determined on 3 replicates of 20 fruit each as described elsewhere (Calvo et
144 al., 2015).

145 **2.4 Determination of ethylene production**

146 Ethylene production was determined at 20 °C using a flow-through system. The ethylene
147 production rate was determined on three replicates of two pears each after 5 and 8 months of
148 storage in the different conditions and at different times during shelf life at 20ºC depending of
149 the storage removal.

150 Ethylene production was determined as previously described (Giné-Bordonaba et al., 2014),
151 placing the fruit in 1500 mL flasks continuously ventilated with humidified air at a flow rate
152 of approximately 1.5 $L.h^{-1}$. Ethylene production was measured by taking gas samples of
153 effluent air from respiration jars and injecting this sample into a gas chromatograph (Hewlett-
154 Packard 5890 Series II, Barcelona, Spain) fitted with a FID detector (Agilent Technologies
155 6890, Wilmington, Germany) and an alumina column 80/100 (2 m x 3 mm) (Teknokroma,
156 Barcelona, Spain).

157 **2.5 Determination of α-farnesene (AF) and conjugated trienols (CTols)**

158 AF and CTols were analysed on 5 replicates of one fruit each immediately after removal and
159 following the method described by Anet (1972), with some modifications (Calvo et al., 2015).
160 Briefly, at each removal time, a 2 mm thick strip of peel was removed from the equatorial

zone of each fruit and 5 discs (10 mm diameter) prepared using a cork borer. The discs were then immersed in 10 mL of HPLC grade hexane for 10 min with constant stirring and 1 mL of this solution was diluted in 4 mL of hexane. Measurements were performed calibrating first the equipment with HPLC grade hexane. Absorbance at 232 nm (α-farnesene) and 281–290 nm (conjugated trienols) were recorded using a UV-spectrophotometer (1001 Plus, Milton Roy, USA). Concentrations of α-farnasene and conjugated trienols were calculated using the molar extinction coefficients E_{232nm} = 27,700 for α-farnasene and $E_{281-290nm}$ = 25,000 for conjugated trienols (Anet, 1972) and the results expressed as nmol.cm^{-2}.

2.6 Extraction and analysis of ethanol

Ethanol was determined according to the protocol of Ke et al. (1994) with slight modifications. Briefly, ethanol was extracted from the flesh of 5 different fruit immediately after removal from the storage room. Juice samples (5 ml) were put in a 10 mL test tube with screw cap and stored at -25 ºC until analysis. For analysis the tubes were incubated in a water bath at 60 ºC during one hour and 1 ml of the headspace sample was injected onto a gas chromatograph (HP5890II, Hewlett Packard). The chromatograph was equipped with a flame ionization detector (at 200 ºC) and a column (2 mm x 2 m at 85 ºC) containing 5% Carbowax on 60/80 Carbopack (Supelco, Bellefonte, Pa, USA).

Ethanol concentrations were calculated using a standard curve, generated by injecting standard solutions of known concentration.

2.7 Statistical analysis

All data were evaluated through analysis of variance (ANOVA) using JMP 13.1.0 (SAS Institute Inc., Cary, NC, USA) software. Significant differences among treatments were calculated based on Tukey's HSD test ($p < 0.05$) or LSD test ($p < 0.05$) for ethylene measurements.

3. RESULTS AND DISCUSSION

3.1. 1-MCP and xULO efficiently control superficial scald but with noticeable difference in fruit ripening during commercial life

Control fruit stored in CA (2.5% O_2 + 1.5% CO_2) exhibited high superficial scald incidence both after 5 (Figure 1A) and 8 months (Figure 1B) of storage. Disorder incidence increased with storage duration but also and especially with the time of commercial life at 20 ºC. In contrast, no or very low disorder incidence were found in 1-MCP- and xULO-treated fruit (Figure 1) both after 5 or 8 months of cold storage. For both treatments superficial scald incidence was low even after 7 days of commercial life, a result that indicate that the inhibitory processes involved in scald control remained still active after removal from cold storage and transfer of the fruit to 20ºC. Although this result was expected for 1-MCP treated fruit and may be associated to limited turn-over of ethylene receptors (Sisler and Serek, 2003), such a result for xULO is remarkable. Indeed, it may indicate that scald control in xULO treated fruit was not exclusively associated to low O_2 concentrations during storage but also to low O_2-triggered regulatory processes that are maintained during shelf life.

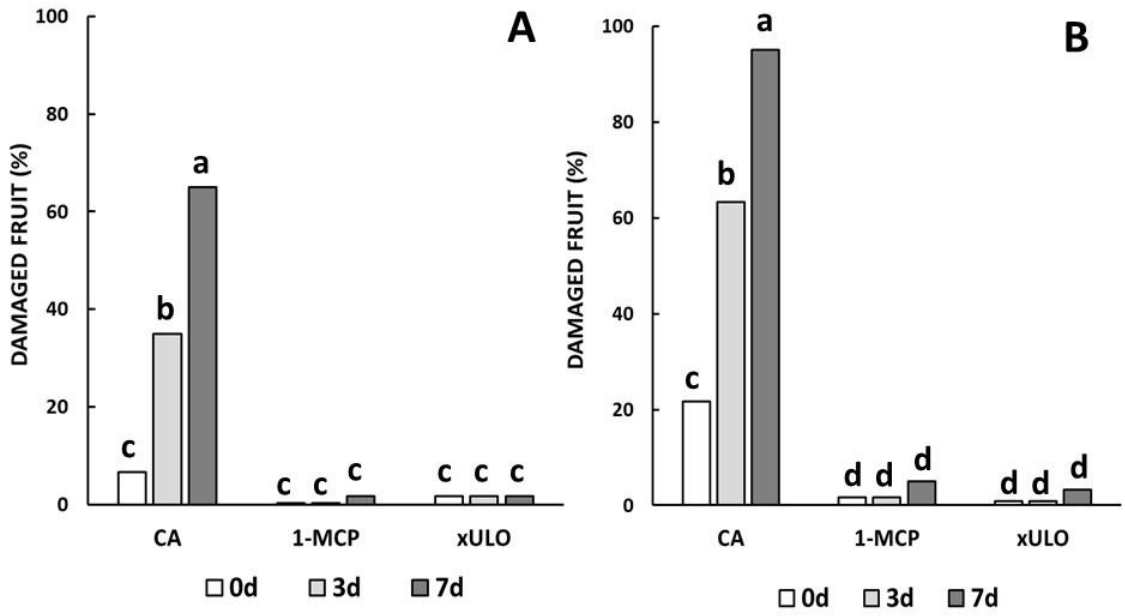


Figure 1: Changes in superficial scald incidence during shelf-life at 20 ºC (0d - white bars, 3d - grey bars, and 7d - dark grey bars) in 'Blanquilla' pears after 5 (A) and 8 (B) months of storage. CA: Control fruit stored in controlled atmosphere; 1-MCP: Fruit initially treated with 1-MCP (300 nL L^{-1}) and stored in CA; xULO: Fruit stored at very low O_2 levels (0.7% O_2 and 0.5% CO_2). Mean values with the same letter are not significantly different according to ANOVA and Tukey's HSD test ($p < 0.05$).

The 'evergreen' behaviour is undoubtedly the main problem associated to the application of 1-MCP treatment in pears. 'Evergreen' pears lose their ability to ripen adequately and remain firm and green even after shelf life (Chiriboga et al., 2013).

Storage scenario	Time (days at 20ºc)	Quality parameters			
		Firmness (N)	SSC (%)	Acidity (g L^{-1})	Colour (a*+b*)
CA	1	48.1 +/- 4.9 **b**	15.0 +/- 0.3 **ab**	2.6 +/- 0.1 **ab**	28.2 +/- 1.6 **d**
	3	24.5 +/- 3.9 **c**	14.6 +/- 0.2 **b**	2.5 +/- 0.3 **ab**	30.2 +/- 2.0 **a**
	7	14.7 +/- 2.9 **d**	14.9 +/- 0.4 **ab**	1.6 +/- 0.1 **c**	29.8 +/- 2.1 **ab**
xULO	1	55.9 +/- 4.9 **a**	15.5 +/- 0.4 **a**	2.7 +/- 0.2 **a**	28.0 +/- 1.5 **d**
	3	46.1 +/- 6.8 **b**	15.0 +/- 0.2 **ab**	2.3 +/- 0.2 **ab**	29.2 +/- 1.7 **bc**
	7	15.6 +/- 3.9 **d**	15.2 +/- 0.1 **ab**	2.2 +/- 0.3 **b**	30.0 +/- 1.9 **ab**
1-MCP	1	54.9 +/- 4.9 **a**	15.1 +/- 0.5 **ab**	2.7 +/- 0.3 **a**	28.2 +/- 1.2 **d**
	3	55.9 +/- 4.9 **a**	14.8 +/- 0.2 **b**	2.3 +/- 0.2 **ab**	28.3 +/- 2.0 **d**
	7	54.9 +/- 3.9 **a**	14.7 +/- 0.3 **b**	2.4 +/- 0.3 **ab**	28.6 +/- 1.5 **cd**

Table 1: Changes in the main quality attributes in 'Blanquilla' pears stored for 5 months in different storage scenarios and after a 1, 3 and 7 days shelf-life period at 20ºC. CA: Control fruit stored in controlled atmosphere; 1-MCP: Fruit initially treated with 1-MCP (300 nL L^{-1}) and stored in CA; xULO: Fruit stored at very low O_2 levels (0.7% O_2 and 0.5% CO_2). Mean values with the same letter are not significantly different according to ANOVA and Tukey's HSD test ($p < 0.05$).

In this work, 1-MCP treated Blanquilla pears, as observed for others pears cultivars (Argenta et al., 2003; Trinchero et al., 2004; Chiriboga et al., 2011), exhibited a typical 'evergreen' behaviour maintaining their initial firmness values even after 7 days of commercial life (Table 1) and regardless of the storage duration. After 5 months of cold storage, xULO-stored fruit remained firm during the first 3 days but soften adequately later (Table 1). After 8 months of cold storage (results not shown), xULO-stored fruit although presenting very high firmness values immediately after removal, exhibited a significant firmness loss reaching values similar to those of CA-stored fruit after 3 and 7 days of shelf-life at 20ºC.

That said, and although different regulatory mechanisms are likely involved, it is interesting to note that the specific behaviour in firmness loss for both treatments was clearly related to the fruit capacity to produce ethylene (Figure 2). In 1-MCP-treated fruit, the impairment of fruit softening during storage and shelf-life was undoubtedly associated to the action that this compound has on ethylene signalling pathway (Sisler and Serek, 2003), whereas other kind of inhibitory mechanisms may account for xULO-treated fruit.

Notwithstanding the differences in firmness loss observed between treatments, stored fruit exhibited similar values in sugar content and acidity upon removal (Table 1), two quality parameters known to be ethylene independent (Pech et al., 2008). In contrast, 1-MCP-treated fruit remained slightly greener during the entire shelf life period (Table 1), complying with a typical evergreen behaviour and with the idea that the main quality differences observed between treatments were ethylene dependent.

3.2 The two different storage scenario differently regulate ethylene production and α-farnesene metabolism highlighting key differences in their mode of action

3.2.1 Specific effects on ethylene metabolism

Results describing the kinetics of ethylene production (Figure 2) confirm that ethylene plays a differential role in scald control for the different storage strategies. Control fruit produced

maximal ethylene amounts immediately upon removal and ethylene production steadily decreased later indicating that fruit were removed at a post-climacteric stage (Figure 2).

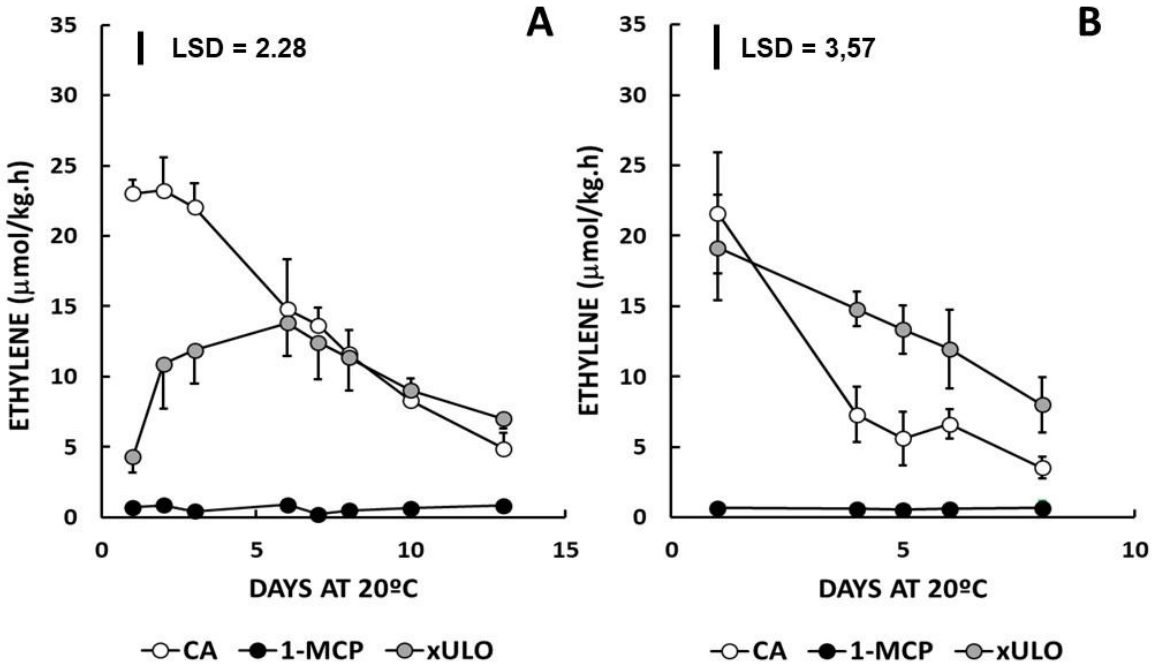


Figure 2: Changes in ethylene production rates during shelf life at 20ºC in 'Blanquilla' pears after 5 (A) and 8 (B) months of storage. CA: Control fruit stored in controlled atmosphere; 1-MCP: Fruit initially treated with 1-MCP (300 nL L^{-1}) and stored in CA; xULO: Fruit stored at very low O_2 levels (0.7% O_2 and 0.5% CO_2). Each point represents the mean of 3 replicates of two different fruit +/- s.d. LSD (<0.05) are shown for the interaction shelf time x treatments.

As expected, 1-MCP treatment completely inhibited ethylene production regardless of the storage duration, whereas xULO treatment only partially inhibited this production (Figure 2). After 5 months of storage, xULO-treated fruit exhibited a typical climacteric behaviour with a peak in ethylene production at 6 days (figure 2A) and a typical post climacteric behaviour similar to control after longer storage duration (Figure 2B).

Collectively these results indicate that inversely to 1-MCP that irreversibly inhibited ethylene metabolism and fruit ripening, xULO treatment only delayed this process. The delay in fruit

ripening in this sample is the result of the action of both cold temperature and low O_2 concentrations. Low temperature likely acted as a stress factor (Larrigaudière et al., 2001) increasing ACC metabolism as observed in others pears cultivars (Knee et al., 1983; Blankenship and Richardson, 1985; Lelièvre et al., 1997). This activation process generally proceeds via the activation of ACC synthase and ACC oxidase transcription (Blankenship and Richardson, 1985; Jobling et al., 1991), but without the corresponding increase in enzyme activities mainly because enzymes are inhibited by cold storage in accordance to the Arrhenius law. In consequence, the fruit physiological maturity is increased but without induction of the ethylene-triggered processes during cold storage. This may explain why xULO-treated fruit did not softened during cold storage but also why these fruit exhibited an important increase in ethylene production upon removal from cold storage. In this sense, we should keep in mind the role played by low O_2 concentration. A decrease in the O_2 concentration within the storage atmosphere led to the inhibition of the oxidase enzymes activities, and especially ACC oxidase, that most likely are key factors determining the action of the xULO treatment on superficial scald inhibition.

3.2.2 Specific effects on α-farnesene metabolism

The pattern of α-farnasene (AF) accumulation observed in control fruit was similar to that previously described in other pear cultivar (Chen et al., 1990; Isidoro and Almeida, 2006; Whitaker et al., 2009), with low initial values at harvest that increased during the first 5 months of cold storage and then decreased later due to *in vivo* oxidation (Figure 3A).

A clear different pattern was found for the 1-MCP treated fruit for which AF accumulation was drastically but not completely inhibited (Figure 3A). Despite producing very low levels of ethylene (Figure 2), 1-MCP treated fruit accumulated significant amounts of AF in cold. Therefore, it may be that AF synthesis as observed for other pear cultivar (Calvo et al., 2015;

Larrigaudière et al., 2016), does not exclusively depend on ethylene, and that other factors, such as low temperature storage for instance may modulate AF synthesis in 'Blanquilla' pear.

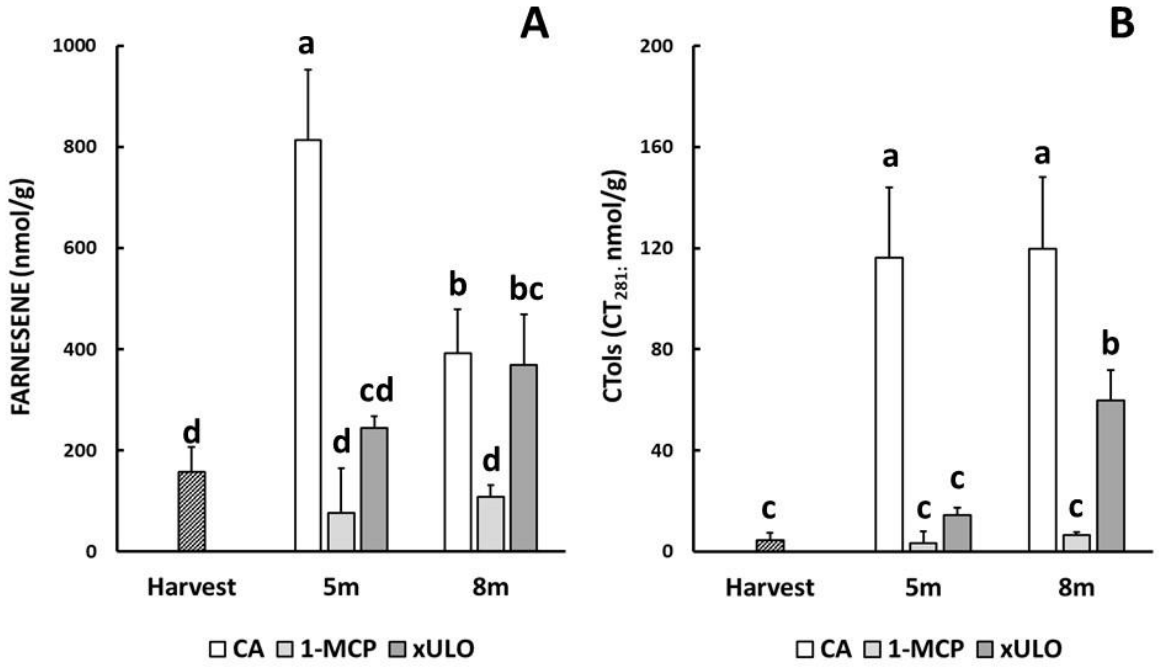


Figure 3: Changes in α-farnesene content (A) and in its oxidation products CTols (B) in 'Blanquilla' pears after different periods of storage. Dashed grey bars: initial levels at harvest; CA (white bars): Control fruit stored in controlled atmosphere; 1-MCP (light grey bars): Fruit initially treated with 1-MCP (300 nL L^{-1}) and stored in CA; xULO (dark grey bars): Fruit stored at very low O_2 levels (0.7% O_2 and 0.5% CO_2). Each point represents the mean of 5 replicates of one fruit each +/- s.d. Mean values with the same letter are not significantly different according to ANOVA and Tukey's HSD test ($p < 0.05$).

Although lower levels of α-farnesene were also found in xULO stored fruit, these levels steadily increased during storage reaching similar levels to control after 8 months of storage (Figure 3A). Compared to 1-MCP, xULO-stored fruit were less effective to prevent α-farnesene accumulation, a result that may be related to the increase in physiological maturity that was observed in fruit stored under this condition. In this context, and although in both

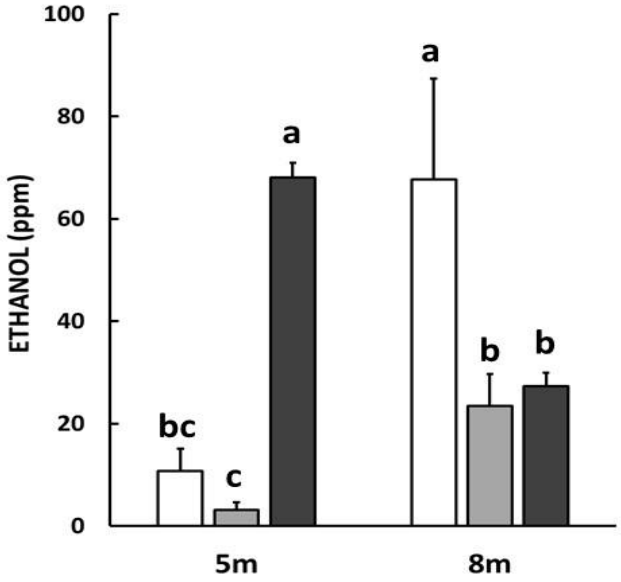
cases very low ethylene amounts are produced during cold storage, we may think that the observed increased in the fruit physiological maturity associated to the xULO treatment (Figure 2) may, in turn, lead to a different regulation of the AFS enzyme activity. Future studies are needed to clarify this aspect and especially the exact regulatory mechanisms involved in superficial scald control in fruit stored under xULO.

Another important point that may explain the difference in scald sensitivity between treatments is the way by which α-farnesene is oxidised to CTols during shelf life. CTols have been identified as the predominant *in vivo* oxidation products of AF both in apples (Whitaker et al., 1997) and pears (Whitaker, 2007) and it is generally assumed that scald incidence in pears is proportional to AF oxidation (Chen et al., 1990; Gapper et al., 2006). Accordingly, control fruit that exhibited the highest incidence of superficial scald during shelf life also showed the highest amounts of CTols (Figure 3B). Although AF levels increased between 5 and 8 months of storage, this increase was not followed by an increase in CTols likely because in the latest months of storage CTols are degraded to 6-methyl-5-hepten-2 one (MHO) that is considered the main oxidation product of CTols ((Whitaker and Saftner, 2000). Inversely to control fruit, 1-MCP treated fruit only exhibited very low levels of CTols (figure 3B) depicting a clear relationship between the levels of AF and the levels of CTols. The % of AF oxidized to CTols was very similar for the two removals (4 % and 6.5 % respectively) and proportional to the initial levels in AF. Although we cannot discard the involvement of other protective mechanisms induced by 1-MCP such as a higher resistance to oxidative damage (Larrigaudière et al., 2004), scald control in 1-MCP treated 'Blanquilla' pear seem to be directly related to a direct inhibition of AF synthesis and consequently to very low levels of CTols. Accordingly, inhibition of superficial scald in 1-MCP treated 'Blanquilla' pears is then directly related to the way by which this treatment delay fruit maturity and ethylene production

The clear relationship previously observed between AF and CTols for 1-MCP treated fruit was not found in xULO stored fruit. Even though both treatments controlled superficial scald to a similar extent, xULO treated fruit exhibited higher AF oxidation rates that increased along the storage duration (5.8 % and 16.3 % after 5 and 8 months respectively). Furthermore, control and xULO fruit although presenting similar CTols levels after 8 months of cold storage exhibited clear differences in scald incidence. Collectively these results indicate that, in contrast to 1-MCP treated fruit, for which scald disorder seem to be directly related to the levels and oxidation of AF, others processes are clearly involved in the regulation of superficial scald in xULO stored pears.

With this in mind, we further investigated the putative role that the accumulation of certain antioxidants and especially the weak antioxidant ethanol, which is known to accumulate during storage under low O_2 concentration (Nichols and Patterson, 1987; Patterson and Nichols, 1988), may have on the control of superficial scald.

3.3 Control of superficial scald in xULO stored 'Blanquilla' pears is determined by ethanol



348 **Figure 4**: Changes in the levels of ethanol in 'Blanquilla' pears after 5 months and 8 months
349 of storage. CA (white bars): Control fruit stored in controlled atmosphere; 1-MCP (light grey
350 bars): Fruit initially treated with 1-MCP (300 nL L^{-1}) and stored in CA; xULO (black bars):
351 Fruit stored at very low O_2 levels (0.7% O_2 and 0.5% CO_2). Each square represents the mean
352 of 5 replicates of one fruit each +/- s.d. Mean values with the same letter are not significantly
353 different according to ANOVA and Tukey's HSD test ($p < 0.05$).
354
355 Ethanol levels were analyzed immediately after removal to better appreciate the levels
356 corresponding to storage conditions. Within this context, clear differences in ethanol levels
357 were found between treatments (Figure 4). While 1-MCP treated fruit exhibited lower ethanol
358 levels during all the experimental period, control fruit exhibited a late increase in ethanol only
359 after 8 months of storage (Figure 4). This increase took place earlier in xULO than in CA-
360 stored fruit which led us to hypothesized that the difference in timing of ethanol accumulation
361 is likely
362 an important parameter that determine scald sensitivity in xULO treated pears.
363 Similar relationship between scald control and ethanol levels were previously found in apples
364 submitted to various initial low oxygen stress (Wang and Dilley, 2000), but also in 'Granny
365 Smith' apples (Pesis et al. 2007) for which the same relationship with time was found. Our
366 results are also in accordance with Scott et al. (1995) that treated Granny smith' apples with
367 ethanol vapours and with the work of Chervin et al. (2001) where ethanol vapours were
368 applied to apples in combination to controlled atmosphere (CA) storage. They are finally in
369 accordance with our previous results in which we have showed that other fruit antioxidants
370 (i.e. ascorbate) play a similar role to the one described herein for ethanol, in 'Packham
371 Triumph' pears (Larrigaudière et al., 2016), thus highlighting that endogenous antioxidants
372 neo-synthetized during cold storage play a determining role for scald control in pears.

Conclusion:

Although we can expect important variations between cultivars, the results presented here support our previous work (Larrigaudière et al., 2016) in which we hypothesized that specific antioxidants play a determining role for scald control in pears. They also support the theories in which scald development is not strictly related to AF and CTols in apples (Rao et al.; 1998) and pears (Calvo et al., 2015; Larrigaudière et al., 2016), but to others oxidative events. New studies are encouraged in this direction to further clarify the biochemical basis of scald disorder in pear and hence capable of bringing adequate control strategies for this disorder.

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