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Unravelling the physiological basis of superficial scald in pears based on cultivar differences

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ABSTRACT

Superficial scald is an important physiological disorder affecting both apple and pear fruit during postharvest storage. To date, superficial scald has been associated to many different preharvest and postharvest factors which are ultimately affected by the genetic characteristics of each cultivar. Accordingly, this work investigated differences in scald susceptibility during cold storage in two different pear cultivars 'Beurré d'Anjou' and 'Packham Triumph' and its relation to the changes in ethylene production, accumulation of α-farnesene and in its oxidation products (CTols), and finally changes in the fruit antioxidant potential and ascorbate levels.

Collectively the results from this study indicate that superficial scald in pear develops differently than in apples. The highest sensitivity observed in 'Beurré d'Anjou' pears was not related to ethylene and/or to the capacity of the fruit to accumulate α-farnesene, but rather to its capacity to prevent the accumulation CTols. Although presenting similar values in global antioxidant potential, the higher resistance of 'Packham Triumph' pears to superficial scald was positively associated to higher ascorbate levels. The potential involvement of ascorbate in preventing superficial scald development is further discussed.

1. Introduction

Despite many years of research, the biochemical or physiological mechanisms underlying superficial scald disorder in pome fruit are still debatable. It is generally accepted that scald is the result of an oxidative process (Lurie et al., 2005) in which α-farnesene (AF) and its oxidation products, the conjugated trienols (CTols), play a predominant role (Huelin and Murray, 1966; Anet, 1972; Whitaker, 2007; Whitaker et al., 2009). For decades, this model has been supported by different observations such as: 1. the beneficial effects of ventilation and oiled wraps on AF content and scald (Huelin and Coggiola, 1968); 2. the effects that the inhibitors of isoprenoid biosynthesis or ethylene (1-MCP) have on α-farnesene production and scald development (Ju and Curry, 2000a, 2000b); and 3. the induction *in vivo* of scald disorder through the application of exogenous CTols (Rowan et al., 2001). This model was recently confirmed by Pechous et al. (2005) and Gapper et al. (2006), which show in both apples and pears that the inhibition of α-farnesene synthesis by 1-MCP was closely correlated with the suppression of the alpha farnesene synthase (AFS1) gene activity.

Despite of this body of evidence, there are also different reports that contradict this model. The most notable was cited in the work of Rao et al. (1998) carried out in 'White Angel' × 'Rome Beauty' hybrid apple lines in which scald disorder was not related to AF and CTols but rather to peroxidation, peroxidase and catalase activities. Whitaker et al. (2000) also supported this finding on the same experimental model and found that oxidation products of α-farnesene are not required for induction of scald but rather in worsening the symptoms in fruit already compromised by oxidative stress. Finally in our recent work carried out in 'Beurré d'Anjou' pears we also hypothesized that AF and CTols are less responsible for scald development than antioxidants in pears (Calvo et al., 2015).

Given that superficial scald is considered the result of an oxidative process, it is accepted that this disorder depends on the balance between the content of oxidative species and antioxidants within the fruit skin (Ju et al., 1996; Rao et al., 1998; Diamantidis et al., 2002; Zubini et al., 2007; Whitaker et al., 2009; Silva et al., 2010).

Accordingly, several authors have studied the influence that some specific antioxidants (i.e. p-coumaryl fatty-acid esters, also

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referred as CT_{258} substances) have on scald development in apples (Du and Bramlage, 1993; Whitaker, 1998) but with diverse results depending on the cultivar. Rudell et al. (2009) established some correlations between α-tocopherol degradation and an increase of superficial scald incidence during storage. Although these results may indicate an action of α-tocopherol in scald prevention, this hypothesis was contradicted when this substance was applied exogenously to fruit (Anet and Coggiola, 1974; Ju et al., 2000). The activity of other water-soluble antioxidants has also been investigated. In this context, water-soluble antioxidant concentrations declined during storage and no individual antioxidant was associated consistently with scald or CTols accumulation (Barden and Bramlage, 1994).

All these findings warrant further consideration of the importance of genetic regulation of resistance to superficial scald. In this way, the differences that may exist between different cultivars may be considered a valuable source of information to better understand the physiological basis of superficial scald in pear. Accordingly, the present study was conducted on 'Beurré d'Anjou' and 'Packham Triumph' pears to evaluate metabolic differences that could explain the disparity in superficial scald susceptibility exhibited among these two cultivars. Emphasis was given on the rates of ethylene production, accumulation of α-farnesene (AF) and its oxidation products and finally to the levels of antioxidants and ascorbate during storage in cold air.

2. Material and methods

2.1. Plant material and storage conditions

'Beurré D'Anjou' and 'Packham Triumph' pears (*Pyrus communis* L.) were harvested from a commercial orchard located in Alto Valle, Rio Negro, Argentina from 10 years old trees, planted at 4 × 2 m, on seedling rootstock trained in a modified Trellis system.

Fruit were picked of uniform size and free from defects at optimum harvest date the 10th February and 18th February for 'Beurré D'Anjou' and 'Packham Triumph' pears respectively.

Immediately after harvest, fruit were transferred to the laboratory and packed in cardboard boxes with two trays (20 fruit each), that were stored in regular air at −0.5 °C and 95% RH for 240 days.

2.2. Fruit quality determinations at harvest

Quality parameters were determined on 5 replicates of 10 fruit each. Flesh firmness (N) was measured using a fruit texture analyzer (FTA-GS14, Güss, South Africa), with an 8 mm diameter plunger. Two measurements were carried out per fruit on opposite sides, after removal of 2 mm of peel. Soluble solid content (SSC) and titratable acidity (TA) were determined using freshly prepared juice from each individual fruit. SSC (%) was measured using a temperature-compensated digital refractometer (Pal1, Atago, Japan) and TA ($g\,L^{-1}$) expressed as malic acid content was measured by titrating 10 mL juice with 0.1 N NaOH to an endpoint of pH 8.2, using a calibrated pH meter (Bicasa, B.E 105, Italy). The percentage of starch degradation (%) was determined by comparison to specific tables (INTA editions 2008, Argentina) for each variety after inserting a fruit slice of 1–1.5 mm from the equatorial zone in a lugol solution.

2.3. Ethylene measurements

Ethylene production was measured in 5 replicates of one fruit each after 0, 15, 30, 45, 60, 90, 120, 150,180, 210 and 240 days at −0.5 °C and during 30 days shelf life at 20 °C. At each time of analysis, fruit were sealed in 1.5 L airtight jar for 30 min. Gas samples of 1 mL were extracted with a syringe from the air headspace. The sample was analysed with a gas chromatograph (GC14-A, Shimadzu, Japan) equipped with an FID detector, an activated alumina column and injector operating at 240 °C, 40 °C and 110 °C respectively. Helium was used as carrier gas. Ethylene production curves were drawn to determine the value of maximal ethylene production during the self-life (nL/g h).

2.4. Determination of superficial scald incidence

Scald incidence was estimated visually after 60, 90, 120, 150, 180, 210 days at −0.5 °C and 7 additional days of commercial life at 20 °C. At each time the number of damaged fruit (% fruit with scald symptoms) and the severity of the symptoms was determined on 5 replicates of 10 fruit as described elsewhere (Calvo et al., 2015).

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

AF and CTols were analysed in 10 replicates of one fruit each, after 0, 15, 30, 45, 60, 90, 120, 150,180, 210 and 240 days at −0.5 °C following the method described by Anet (1972), with some modifications (Calvo et al., 2015). At each removal time, a strip of peel of 2 mm thick was removed from the equatorial zone of each fruit and 5 discs (10 mm diameter) discs prepared using a cork borer. The discs were then immersed in 10 mL of HPLC grade hexane for 10 min with constant stirring. 1 mL of this solution was diluted in 4 mL of hexane and used for analysis.

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_{232} nm = 27,700 for α-farnasene and $E_{281-290}$ nm = 25,000 for conjugated trienols (Anet, 1972) and expressed as nmol cm^{-2} of fruit.

2.6. Quantification of total antioxidant capacity

Total antioxidant capacity was determined using the DPPH test. Measurements were performed on 5 replicates of 10 fruit each, after 0, 15, 45, 60, 90, 120, 150, 180, 210 and 240 days at −0.5 °C. The entire peel of the fruit was removed, frozen with liquid nitrogen, lyophilized and ground to a fine powder that was homogenized with 10 mL of 80:20 methanol:water (v/v). Samples were left for 2 h at room temperature in a constant shaking bath, and then centrifuged at 20 °C (24,000*g*) for 15 min. The supernatant obtained was then filtered and diluted with milli-Q water (1:4; v/v). A 20 μL aliquot of the diluted extract was then mixed and stirred with 980 μL 1-diphenyl-2- picrylhydrazyl (DPPH; Sigma Aldrich, Steinheim, Germany) in the dark for 30 min at 4 °C.

Initial absorbance (Ai) was measured at 517 nm on the blank and final absorbance (Af) was measured after the incubation period using a UV-spectrophotometer (1001 Plus, Milton Roy, USA) following calibration with double distilled water. Inhibition (%) was calculated as follow: (Ai) − (Af)/(Ai) * 100. Under these conditions, an increase in value corresponded to increase in the total antioxidant activity.

2.7. Determination of ascorbate levels

Total ascorbic acid was extracted following the protocol of Misra and Seshadri (1967) on 6 replicates of 5 fruit each. Samples were obtained mixing the skin of each replicate with 300 mL 1% (p/v) H_3PO_4 and homogenizing the resulting solution with a homogenizer. 0.15 mL of sample were added to 0.5 mL of 30 mg L^{-1} 2,6-diclorofenol-indofenol and incubated during 30 s. All

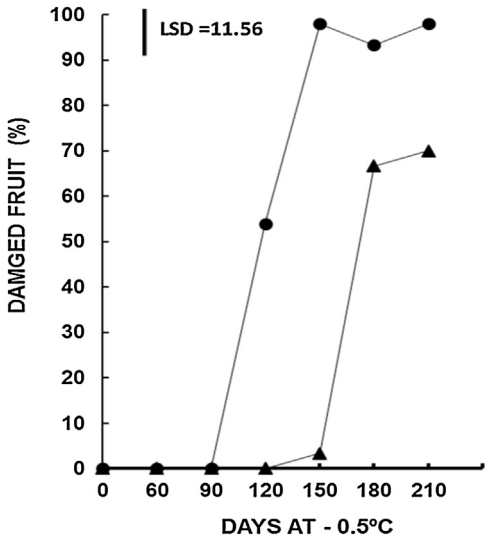

Fig. 1. Superficial scald incidence (% of damaged fruit) in 'Beurré d'Anjou' (●) and 'Packham Triumph' pears (▲) pears after different periods of storage at −0.5 °C and 7 days at 20 °C. Error bar shows LSD value for the interaction cultivar*storage time ($p < 0.01$).

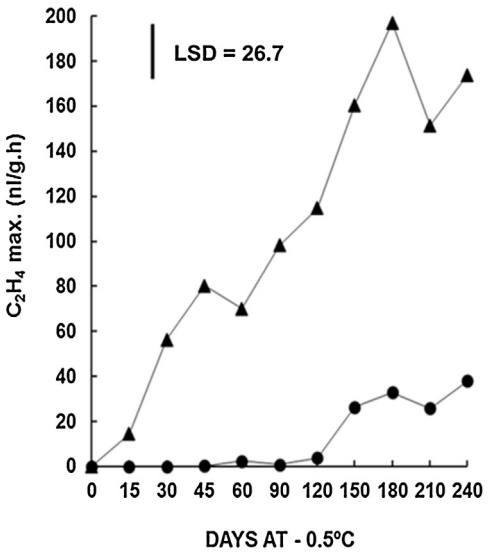

Fig. 2. Changes in the maximum ethylene production value upon removal at 20 °C after different periods of storage at −0.5 °C in 'Beurré d'Anjou' (●) and 'Packham Triumph' (▲) pears. Each point represents mean values of 5 replicates of one fruit each. Error bar shows LSD value for the interaction cultivar*storage time ($p < 0.01$).

Table 1
Initial maturity indexes at harvest for 'Beurré d'Anjou' and 'Packham Triumph' pears. Data represent means ± s.d (n = 50).

Cultivars	BEURRÉ D'ANJOU	PACKHAM TRIUMPH
Firmness (N)	64.6 ± 1.8	59.1 ± 2.1
SSC (%)	11.5 ± 0.8	11.7 ± 1.8
Acidity (g L^{-1})	4.42 ± 0.3	3.20 ± 0.8
Starch index (%)	47.1 ± 9.2	44.6 ± 8.5

the extraction process was carried out at 4 °C and in absence of light. Total ascorbic acid was analysed spectrophotometrically at 524 nm correcting the values obtained from the value of a blank in which the extract was replaced by TCA. Data are expressed in mg/100 g.

2.8. Statistical analysis

All data were evaluated through analysis of variance (ANOVA) using the Statistical Analysis System (SAS version 9.1, SAS Institute, Inc., Cary, NC, USA) software. Least significant difference (LSD; $p < 0.01$) were calculated for mean separation using critical values of t for two-tailed test.

3. Results

3.1. Scald incidence

Significant differences in scald incidence were observed for the different cultivars during shelf life period at 20 °C (Fig. 1). In 'Beurré d'Anjou' pears, the first symptoms of scald were observed after 90 days of cold storage and reach a maximum value (100%) after 150 days of storage. In 'Packham Triumph' pears very slight incidence was noted after 150 days of storage and the incidence was maximum after 180 days of storage and thereafter reaching an incidence of 70%.

3.2. Initial maturity at harvest

Few differences in quality parameters were found for the two cultivars at harvest. Both cultivars where harvested at optimal maturity and presented similar firmness, SSC values and starch index (Table 1). Nonetheless, slight but significant differences in acidity were observed among cultivars with 'Packham's Triumph' pears presenting the lowest acidity values (Table 1).

3.3. Induction of ethylene capacity during the chilling period

As expected for 'winter' pears ethylene production was not detectable at harvest (result not shown). Later, significant differences were found for the two cultivars.

In 'Packham Triumph' pears, 15 days of cold storage were enough to induce significant amounts of ethylene production upon removal (Fig. 2).

Maximal ethylene production increased steadily with time in cold storage to reach a maximal value after 180 days. A very different behaviour was found for 'Beurré d'Anjou' pears. In this cultivar a 150 days of chilling were needed to induce ethylene production upon removal. After this time, the levels in ethylene remained at a similar value. Maximum ethylene production was a 5 to 6-fold lower than that of 'Packham Triumph' pears (Fig. 2).

3.4. Changes in α-farnesene metabolism during cold storage

In 'Packham Triumph' pears, the levels of α-farnesene (AF) increased steadily with time up to 90 days of cold storage (Fig. 3A). Later, AF remained constant (≈80 nmol cm^{-2}) and then declined up to the end of the storage period. 'Beurré d'Anjou' pears showed a similar increase in AF levels up to 90 days of storage, yet in this cultivar AF levels were lower at 90 days and immediately decreased thereafter (Fig. 3A).

In both cultivars, CTols levels remained very low during the first 60 days of storage (Fig. 3B). Then, a different behaviour was found among cultivars. In 'Beurré d'Anjou' pears a sharp increase in CTols was found during the 30 following days of storage. CTols values were maximum at time 120 days and remained at this maximum value during all the remaining storage period. In contrast, in 'Packham Triumph' pears CTols levels continuously increased to reach at the end of the storage a maximum value similar to those observed in 'Beurré d'Anjou' pears (≈20 nmol cm^{-2}).

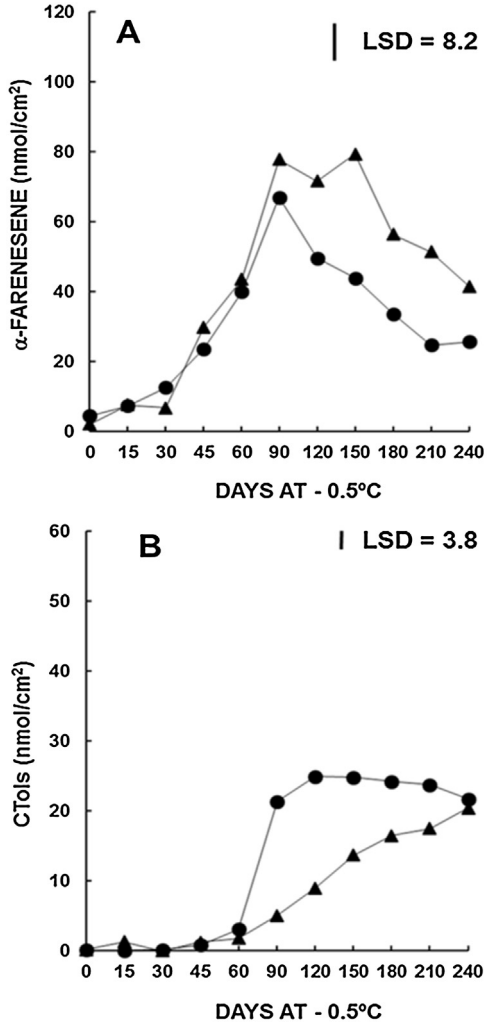

Fig. 3. Changes in α-farnesene (A) and in its oxidation products CTols (B) in 'Beurré d'Anjou' (●) and 'Packham Triumph' (▲) pears after different periods of storage at −0.5 °C. Each point represents mean values of 10 replicates of one fruit each. Error bar shows LSD value for the interaction cultivar*storage time ($p < 0.01$).

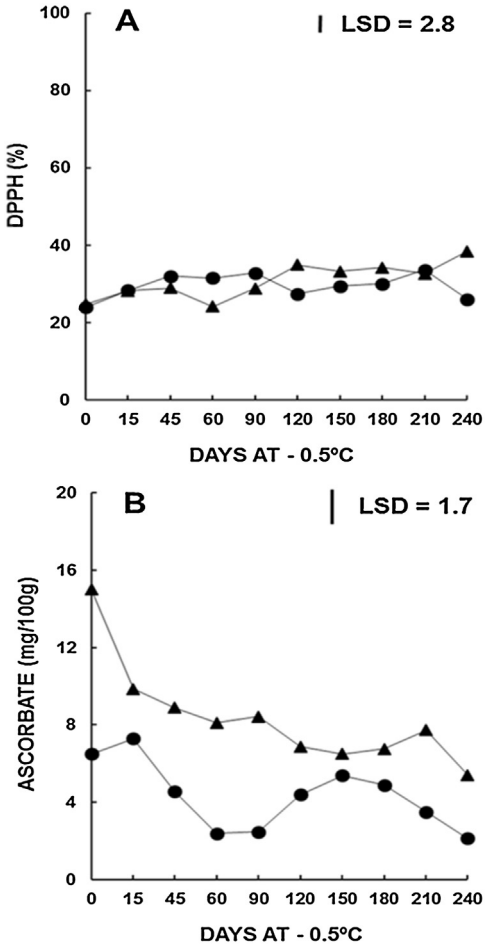

Fig. 4. Changes in the skin antioxidant potential (% of inhibition) (A) and in ascorbate levels (B) in 'Beurré d'Anjou' (●) and 'Packham Triumph' (▲) pears after different periods of storage at −0.5 °C. Each point represents mean values of 10 replicates of one fruit each. Error bar shows LSD value for the interaction cultivar*storage time ($p < 0.01$).

3.5. Changes in global antioxidant potential and ascorbate levels during cold storage

In both cultivars, the antioxidant potential (DPPH) remained relatively constant during all the storage period (Fig. 4A). For most of the analysed time points, the differences between cultivars were not significant and no clear patterns in the changes of the antioxidant potential were found.

In contrast, significant differences in ascorbate values were found between cultivars at harvest (Fig. 4B, time 0; 15 mg 100 g^{-1} and 6.5 mg 100 g^{-1} in 'Packham Triumph' and 'Beurré d'Anjou' pears, respectively). In 'Packham's Triumph' pears ascorbate levels sharply decreased during the first 15 days of storage and then decreased gradually to reach a final value of 5.4 mg/100 g (nearly 3-fold lower than at harvest) at the end of storage period. In 'Beurré d'Anjou' pears ascorbate levels remained constant during the first 15 days of storage but later decreased up to 90 days of storage. A further increase up to day 150 followed by a final decrease of the ascorbate levels were observed in this pear variety. Overall, ascorbate levels significantly differed between cultivars during all the initial storage period and mainly up to 90 days (Fig. 4B).

4. Discussion

4.1. Sensitivity to scald in pear is cultivar dependent

Fruit of different apple and pear cultivars vary widely in their susceptibility to superficial scald. Albeit decades of research, the reason of such variability is not clearly defined. In apples, Pechous and Whitaker (2004) related scald resistance to various rate of expression of α-farnesene synthase gene (AFS1) and to the resulting differences in accumulation rate of α-farnesene and CTols during cold storage. For others authors the difference in sensitivity between cultivars in apple is related to the amount of antioxidants in the peel and the influence that certain preharvest factors may have on α-farnesene synthesis (Emongor et al., 1994). Thus said, information on differences in scald susceptibility among different pear varieties is poorly documented. In analogy with apples, it is generally assumed that differences between pear cultivars is mainly related to the ability of each cultivar to produce ethylene and/or to regulate ethylene metabolism during cold stress (Lurie and Watkins, 2012). The content of antioxidants at harvest and/or the endogenous potential of each cultivar to regulate the levels of enzymatic and non-enzymatic antioxidants during cold acclimation are also considered as key factors (Lurie and Watkins, 2012). Our results clearly showed that scald susceptibility in pears is cultivar dependent with 'Packham Triumph' pears being somehow less susceptible than 'Beurré d'Anjou' pears. Nonetheless, comparative

studies using a wide range of cultivars with different superficial scald susceptibility are still needed to better understand the physiological basis of this disorder in pears.

4.2. Cultivar differences are not related to initial fruit maturity (standard indexes)

The results presented herein shows that the standard parameters used to evaluate fruit maturity at harvest may not be employed to predict the differences in scald susceptibility between cultivars. These results are in accordance with our previous work carried out in 'Beurré d'Anjou' pears from different maturity stage (Calvo et al., 2015). They also confirm that standard ripening indexes are not useful to predict scald in pear and that other indexes more representative of the real physiological maturity at harvest are needed to predict this disorder.

4.3. Scald sensitivity in pear is not directly related to the rate of ethylene production

'Beurré d'Anjou' and 'Packham Triumph' pears belongs to the class of pears called 'winter pears' that produced only very few levels of ethylene at harvest. For these cultivars, a chilling period is required to induce ethylene production and normal fruit ripening during the commercial life. During this process, low temperature promotes ethylene synthesis (Knee et al., 1983; Blankenship and Richardson, 1985) and the following increase in ethylene production upon removal generally proceeds via the activation of the transcription ACC synthase and ACC oxidase in cold (Blankenship and Richardson, 1985; Jobling et al., 1991) and a further increase in activity at room temperature (Larrigaudière and Vendrell, 1993) leading to a burst of ethylene production.

Although this behaviour has been also observed in some apple cultivar such as 'Granny Smith' (Larrigaudière at al., 1997), chilling requirement is basically a characteristics of 'winter' pears. It is interesting to note that both the 'Granny Smith' and most of the 'winter' pear cultivars are very sensitive to superficial scald. This may indicate that the metabolic processes that take place during chilling requirement are of paramount importance for the induction of this physiological disorder.

In apples, ethylene is known to play a key role in the development of superficial scald since this hormone is involved in the synthesis of α-farnesene (Ju and Curry, 2000b). Accordingly, it is generally accepted that pear follows the same scheme and that ethylene is a key factor involved in scald development (Gapper et al., 2006). Our results do not support this general idea. Indeed, 'Packham's Triumph' pears although producing higher levels of ethylene upon removal (Fig. 2) were less sensitive to superficial scald (Fig. 1).

This activation of ethylene production in Packham's Triumph' pears is the reflection of important regulation of the ACC metabolic pathway in cold. In contrast to apples in which such activation is generally associated with increased sensitivity to scald (Larrigaudière et al., 1997), lower scald incidence was found in the earlier activated pear cultivar. Such results indicate that ethylene likely play a different role in the induction of scald disorder in apples compared to pears. Although ethylene plays a predominant role on α-farnesene synthesis in apples (Ju and Curry, 2000b) this role in pear remains unclear.

4.4. Understanding the roles of α-farnesene and its oxidation products in scald incidence in pear

In this experiment, the pattern of α-farnesene (AF) accumulation observed for both cultivars was similar to that previously described for apples (Whitaker et al., 1997; Giné-Bordonaba et al., 2013) and pears (Isidoro and Almeida, 2006; Whitaker et al., 2009).

In general, changes in AF metabolism were in accordance with the model that associated scald incidence to AF accumulation and oxidation. However, some discrepancies need to be highlighted. Despite producing very low levels of ethylene, 'Beurré d'Anjou' pears accumulated significant amounts of AF in cold. Such results show that AF synthesis in 'Beurré d'Anjou' pears does not exclusively depend on ethylene and that other factors, such as low temperature, might be directly involved. These results are in accordance with the results of Calvo et al. (2015) in 'Beurré d'Anjou' pears but also with the results of Pesis et al. (2009) in apple line suppressed for ACC synthase or ACC oxidase. They also indicate that the regulation of AF metabolism in pear is cultivar dependent and need to be better clarify determining for each cultivar the specific effects of cold temperature on AF metabolism and on the activity AF synthase gene (AFS1).

Another important point that may explain the difference in scald sensitivity between cultivar is the specific potential that each cultivar have to prevent AF oxidation. Although presenting lower levels of AF, 'Beurré d'Anjou' pears exhibited the highest levels of CTols during storage. Furthermore, in this cultivar CTols reached a maximum level during a relatively short period (90d) whereas the increase in the amounts of these compounds occurs much progressively in 'Packham's Triumph' pears. All these results indicate that the specific sensitivity to scald of each cultivar is not determined by the initial levels of AF but rather by its capacity to prevent the oxidation of AF into damaging compounds (CTols) likely by its antioxidant potential or specific antioxidants.

4.5. Understanding the role of antioxidant potential of the fruit and specific antioxidants in the prevention of superficial scald in pear

It is widely recognized that the fruit sensitivity to scald is directly determined by its endogenous antioxidant potential (Lurie and Watkins, 2012). In general fruit that exhibited higher antioxidant potential at harvest are less prone to develop scald. Although there is a large amount of information describing the effects of different strategies aiming to control the fruit antioxidant potential (Shaham et al., 2003; Rudell and Mattheis, 2009; Lurie and Watkins, 2012), only little is known on the effect of such strategies between different cultivars. Anet (1974) analysed the specific antioxidant content of sixteen apple cultivars and identified up to eleven different cuticular antioxidants. α-tocopherol was of general occurrence in all the cultivars, whilst the presence of the others depended not only on the cultivar but also on the maturity stage of the sample. Although the relationship between the presence of antioxidants and scald sensitivity has been well established in apples (Gallerani et al., 1990), the specific role that each antioxidant may play in pear remains to be also elucidated.

The analysis of total antioxidant potential as carried out in this work did not allow to explain the difference in scald sensitivity observed between cultivars. In general, DPPH values remained constant during all the storage period in a similar way than total phenolic compounds (results not shown). In contrast, significant differences were observed in ascorbate levels. Ascorbate levels in 'Packham's Triumph' pears remained significantly higher during all the storage period and especially during the first months of storage which seem to be determining in the induction of superficial scald.

To our knowledge, this is the first study establishing a specific relationship between scald occurrence and ascorbate. Although ascorbate has been mainly associated with the development of pulp disorder in pears (Veltman et al., 1999; Larrigaudière et al., 2001), we cannot discard its putative action also in skin tissue. Due to its hydrophilic properties, ascorbate will not act directly in the epi-

cuticular space but rather more externally scavenging the reactive oxygen species generated inside the cell. Ascorbate may also act as a specific inhibitor of polyphenol oxidase (PPO) enzyme activity (Martinez and Whitaker, 1995). Our results are in accordance with the recent results of Busatto et al. (2014) that showed that accumulation of chlorogenic acid and its further oxidation by PPO are key factors involved in the occurrence of superficial scald in apples.

4.6. Conclusions

Our findings suggest that specific sensitivity to scald is not mainly determined by ethylene nor the initial levels of AF but more directly to the specific capacity of each pear cultivar to prevent AF oxidation. In this context, specific antioxidants (i.e. ascorbate) rather than total antioxidant potential are of prime importance. New studies are encouraged in this direction to further clarify the compounds of interest in pears and bring adequate control strategies for this physiological disorder.

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References

Anet, E.F.L.J., Coggiola, I.M., 1974. Superficial scald, a functional disorder of stored apples. X. Control of α-farnesene autoxidation. J. Sci. Food Agric. 25, 293–298.
Anet, E.F.L.J., 1972. Superficial scald, a functional disorder of stored apples. IX. Effect of maturity and ventilation. J. Sci. Food Agric. 23, 763–769.
Anet, E.F.L.J., 1974. Superficial scald, a functional disorder of stored apples: XI. Apple antioxidants. J. Sci. Food Agric. 25, 299–304.
Barden, C.L., Bramlage, W.J., 1994. Accumulation of antioxidants in apple peel as related to preharvest factors and superficial scald susceptibility of the fruit. J. Am. Soc. Hortic. Sci. 119, 264–269.
Blankenship, S.M., Richardson, D.G., 1985. Development of ethylene biosynthesis and ethylene-induced ripening in d'Anjou pears during the cold requirement for ripening. J. Am. Soc. Hortic. Sci. 110, 520–523.
Busatto, N., Farneti, B., Tadiello, A., Vrhovsek, U., Cappellin, L., Biasioli, F., Velasco, R., Costa, G., Costa, F., 2014. Target metabolite and gene transcription profiling during the development of superficial scald in apple (Malus × domestica Borkh). BMC Plant Biol. 14, 193.
Calvo, G., Candan, A.P., Civello, M., Giné-Bordonaba, J., Larrigaudière, C., 2015. An insight into the role of fruit maturity at harvest on superficial scald development in 'Beurré d'Anjou' pear. Sci. Hortic. 192, 173–179.
Diamantidis, G.R., Thomai, T., Genitsariotis, M., Nanos, G., Bolla, N., Sfakiotakis, E., 2002. Scald susceptibility and biochemical/physiological changes in respect to low preharvest temperature in 'Starking Delicious' apple fruit. Sci. Hortic. 92, 361–366.
Du, Z.Y., Bramlage, W.J., 1993. A modified hypothesis on the role of conjugated trienes in superficial scald development on stored apples. J. Am. Soc. Hortic. Sci. 118, 807–813.
Emongor, V.E., Murr, D.P., Lougheed, E.C., 1994. Preharvest factors that predispose apples to superficial scald. Postharvest Biol. Technol. 4, 289–300.
Gallerani, G., Pratella, G., Budini, R., 1990. The distribution and role of natural antioxidant substances in apple fruit affected by superficial scald. Adv. Hortic. Sci. 4, 144–146.
Gapper, N.E., Bai, J., Whitaker, B.D., 2006. Inhibition of ethylene-induced α-farnesene synthase gene PcAFS1 expression in 'd'Anjou' pears with 1-MCP reduces synthesis and oxidation of α-farnesene and delays development of superficial scald. Postharvest Biol. Technol. 3, 225–233.
Giné-Bordonaba, J., Matthieu-Hurtiger, V.B., Westercamp, P.C., Coureau, C.D., Dupille, E., Larrigaudière, C., 2013. Dynamic changes in conjugated trienols during storage may be employed to predict superficial scald in 'Granny Smith' apples. LWT Food Sci. Technol. 54, 535–541.
Huelin, F.E., Coggiola, I.M., 1968. Superficial scald, a functional disorder of stored apples. IV. Effect of variety, maturity, oiled wraps and diphenylamine on the concentration of α-farnesene in the fruit. J. Sci. Food Agric. 19, 297–301.
Huelin, E.E., Murray, K.E., 1966. Farnesene in the natural coating of apples. Nature 210, 1260–1261.
Isidoro, N., Almeida, D.P., 2006. α-Farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylamine. Postharvest Biol. Technol. 42, 49–56.
Jobling, J., McGlasson, W.B., Dilley, D.R., 1991. Induction of ethylene synthesizing competency in Granny Smith apples by exposure to low temperature in air. Postharvest Biol. Technol. 1, 111–118.
Ju, Z., Curry, E., 2000a. Lovastatin inhibits α-farnesene synthesis without affecting ethylene production during fruit ripening in 'Golden Supreme' apples. J. Am. Soc. Hortic. Sci. 125, 105–110.
Ju, Z., Curry, E., 2000b. Evidence that α-farnesene biosynthesis during fruit ripening is mediated by ethylene regulated gene expression in apples. Postharvest Biol. Technol. 19, 9–16.
Ju, Z.G., Yuan, Y.B., Liu, C.L., Zhan, S.M., Wang, M.X., 1996. Relationships among simple phenol, flavonoid and anthocyanin in apple fruit peel at harvest and scald susceptibility. Postharvest Biol. Technol. 8, 83–93.
Ju, Z.Q., Duan, Y.S., Ju, Z.G., 2000. Mono-, di-, and tri-acylglycerols and phospholipids from plant oils inhibit scald development in 'Delicious' apples. Postharvest Biol. Technol. 19, 1–7.
Knee, M., Looney, N.E., Hatfield, S.G., Smith, S.M., 1983. Initiation of rapid ethylene synthesis by apple and pear fruits in relation to storage temperature. J. Exp. Bot. 34, 1207–1212.
Larrigaudière, C., Vendrell, M., 1993. Short-term activation of the conversión of 1-aminocyclopropane-1-carboxylic acid to ethylene in rewarmed Granny Smith apples. Plant Physiol. Biochem. 31, 585–591.
Larrigaudière, C., Graell, J., Salas, J., Vendrell, M., 1997. Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. Postharvest Biol. Technol. 10, 21–27.
Larrigaudière, C., Pinto, E., Lentheric, I., Vendrell, M., 2001. Involvement of oxidative processes in the development of core browning in controlled atmosphere stored pears. J. Hortic. Sci. Biotechnol. 76, 157–162.
Lurie, S., Watkins, C.B., 2012. Superficial scald, its etiology and control. Postharvest Biol. Technol. 65, 44–60.
Lurie, S., Lers, A., Shacham, Z., Sonego, L., Burd, S., Whitaker, B., 2005. Expression of α-farnesene synthase AFS1 and 3-hydroxy-3-methylglutaryl coenzyme A reductase HMG2 and HMG3 in relation to α-farnesene and conjugated trienols in 'Granny Smith' apples heat or 1-MCP treated to prevent superficial scald. J. Am. Soc. Hortic. Sci. 130, 232–236.
Martinez, M.V., Whitaker, J.R., 1995. The biochemistry and control of enzymaticbrowning. Trends Food Sci. Technol. 6, 195–200.
Misra, K., Seshadri, T.R., 1967. Chemical components of the fruits of Psidium uava. Phytochemistry 7, 641–645.
Pechous, S.W., Whitaker, B.D., 2004. Cloning and functional expression of an (E,E)-farnesene synthase cDNA from peel tissue of apple fruit. Planta 219, 84–94.
Pechous, S.W., Watkins, C., Whitaker, B.D., 2005. Expression of α-farnesene synthase gene AFS1 in relation to levels of α-farnesene and conjugated trienols in peel tissue of scald susceptible 'Law Rome' and scald-resistant 'Idared' apple fruit. Postharvest Biol. Technol. 35, 125–132.
Pesis, E., Ibanez, A.M., Phu, M.L., Mitcham, E.J., Ebeler, S.E., Dandekar, A.M., 2009. Superficial scald and bitter pit development in cold-stored transgenic apples suppressed for ethylene biosynthesis. J. Agric. Food Chem. 57, 2786–2792.
Rao, M.V., Watkins, C.B., Brown, S.K., Weeden, N.F., 1998. Active oxygen species metabolism in 'White Angel' × 'Rome Beauty' apple selections resistant and susceptible to superficial scald. J. Am. Soc. Hortic. Sci. 123, 299–304.
Rowan, D.D., Hunt, M.B., Fielder, S., Norris, J., Sherbun, M.S., 2001. Conjugated trienes oxidation products of α-farnesene induce symptoms of superficial scald on stored apples. J. Agric. Food Chem. 49, 2780–2787.
Rudell, D.R., Mattheis, J.R., 2009. Superficial scald development and related metabolism is modified by postharvest light irradiation. Postharvest Biol. Technol. 51, 174–182.
Rudell, D.R., Mattheis, J.P., Hertog, M., 2009. Metabolomic change precedes apple superficial scald symptoms. J. Agric. Food Chem. 57, 8459–8466.
Shaham, Z., Lers, A., Lurie, S., 2003. Effect of heat or 1-methylcyclopropene on antioxidative enzyme activities and antioxidants in apples in relation to superficial scald development. J. Am. Soc. Hortic. Sci. 128, 761–766.
Silva, F.J.P., Gomes, M.H., Fidalgo, F., Rodrigues, J.A., Almeida, D.P., 2010. Antioxidant properties and fruit quality during long-term storage of 'Rocha' pear: effects of maturity and storage conditions. J. Food Qual. 33, 1–20.
Veltman, R.H., Sanders, M.G., Persijn, S.T., Peppelenbos, H.W., Oosterhaven, J., 1999. Decreased ascorbic acid levels and brown core development in pears (*Pyrus communis* L. cv. Conference). Physiol. Plant. 1, 39–45.
Whitaker, B.D., Solomos, T., Harrison, D.J., 1997. Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C18-HPLC with UV detection. J. Agric. Food. Chem. 45, 760–765.
Whitaker, B.D., Nock, J.F., Watkins, C.B., 2000. Peel tissue α-farnesene and conjugated trienol concentrations during storage of 'White Angel' × 'Rome Beauty' apple selections susceptible and resistant to superficial scald. Postharvest Biol. Technol. 20, 231–241.
Whitaker, B.D., Villalobos-Acuña, M., Mitcham, E.J., Mattheis, J.P., 2009. Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. Postharvest Biol. Technol. 53, 43–50.
Whitaker, B.D., 1998. Phenolic fatty-acid esters from the peel of 'Gala' apples and their possible role in resistance to superficial scald. Postharvest Biol. Technol. 13, 1–10.
Whitaker, B.D., 2007. Oxidation products of alpha-farnesene associated with superficial scald development in d'Anjou pear fruits are conjugated trienols. J. Agric. Food Chem. 55, 3708–3712.
Zubini, P., Baraldi, E., De Santis, A., Bertolini, P., Mari, M., 2007. Expression of anti-oxidant enzyme genes in scald-resistant 'Belfort' and scald susceptible 'Granny Smith' apples during cold-storage. J. Hortic. Sci. Biotechnol. 82, 149–155.