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**EFFECT OF TEMPERATURE DURING AND IMMEDIATELY AFTER CO₂-
DEASTRINGENCY TREATMENT ON INTERNAL FLESH BROWNING
AFTER COLD STORAGE OF PERSIMMON FRUIT.**

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23 ABSTRACT

24 Currently, a major cause of postharvest loss of ‘Rojo Brillante’ persimmon, the variety
25 mainly cultivated in the Mediterranean Region, is “internal flesh browning”
26 manifestation after storage or shipping at low temperature, whose causes remain
27 unknown. ‘Rojo Brillante’ is an astringent low temperature-sensitive cultivar. Thus fruit
28 is routinely submitted to high CO₂ treatment to remove astringency, and also to 1-MCP
29 treatment before being stored to retard flesh gelling and drastic softening, the main
30 chilling injury symptoms. This study investigates the influence of temperature during
31 CO₂ deastringency treatment and immediately after its application on the incidence of
32 ‘internal flesh browning’ in persimmon fruit. Our results revealed for the first time that
33 the temperature immediately after the CO₂ deastringency treatment was the main factor
34 implied in this alteration. The fruit transferred directly to cold storage after the CO₂
35 treatment showed ‘internal flesh browning’ after 41 storage d at 1 °C, while a 24- hour
36 attemperation period at 20 °C before storage prevented this disorder from appearing.
37 The main effect of the attemperation period was the enhanced release of CO₂ from fruit
38 after the CO₂ treatment, which resulted in less acetaldehyde (AcH) accumulating after
39 24 h. Moreover, the temperature of the CO₂ application was observed to influence
40 ‘internal flesh browning’ severity as AcH accumulated at higher concentrations in the
41 fruit treated at 20 °C than at 12 °C. Our preliminary hypothesis is that AcH can act as a
42 precursor of reactive oxygen species that would be implied in this disorder’s
43 development.

44 **Keywords:** persimmon, attemperation period, flesh browning, gas exchange, respiration

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48 1. INTRODUCTION

49 Currently, one of the most important causes of postharvest losses of ‘Rojo Brillante’
50 fruits is the manifestation of an internal flesh browning disorder, whose causes remain
51 unknown. The onset of this browning is located in the internal fruit area, around the
52 core zone, and it extends to the more external flesh areas. This disorder has been mainly
53 observed after prolonged cold storage and long shipping at low temperature, which can
54 initially lead us to think that it is a chilling injury symptom. However, ‘Rojo Brillante’
55 chilling injury has been widely studied (Salvador et al., 2004; Pérez-Munera et al.,
56 2009) and the internal browning reported herein has never been described as a chilling
57 injury symptom, so other factors than storage temperature must be implied in this
58 disorder manifestation.

59 It is worth to note that despite its chilling injury sensitiveness, ‘Rojo Brillante’
60 persimmons are usually stored and shipped at temperatures between 0-1 °C as fruit are
61 previously subjected to treatment with 1-methylcyclopropene (1-MCP) that
62 considerably delays the main chilling injury symptom, a drastic fruit softening (Besada
63 et al., 2008; Pérez-Munera et al., 2009).

64 On the other hand, ‘Rojo Brillante’ persimmons are routinely submitted to a CO₂-
65 treatment to remove astringency before commercialisation. The recommended CO₂-
66 treatment conditions for ‘Rojo Brillante’ persimmons are 95 % CO₂-20 °C-24 h
67 (Salvador et al., 2007). However, the CO₂-treatment is quite commonly applied at
68 ambient temperature because treatment chambers do not always have temperature
69 control. This implies that the CO₂-treatment temperature can vary between 20 °C to 12
70 °C from the beginning to the end of the season.

71 In the case that fruit must be kept at low temperature for long periods, applying the
72 CO₂-treatment after cold storage rather than before it is recommended to preserve fruit

firmness. Nevertheless, this is not possible when fruit is sent in cold containers overseas as in this case the deastringency treatment needs to be applied before shipment. In these cases, one recommended handling practice is to attemperate fruit for a 12-24-hour period immediately after the deastringency treatment, before being transferred to cold storage. This attemperation period helps the deastringency process to be completed, mainly when applied at lower temperatures or for shorter durations than those recommended (Besada et al., 2010). However due to logistical issues, this practice is not always carried out in packing houses.

In this context, the CO₂ deastringency treatment, and the handling conditions to which the fruit is subjected after it are most probably the factors implied in the internal browning that ‘Rojo Brillante’ persimmons display after cold storage. Hence this study aimed to investigate the effect of CO₂-treatment temperature and the attemperation period between the deastringency treatment and the transfer to cold storage on the incidence of persimmon flesh browning.

2. MATERIAL AND METHODS

2.1. Plant material and treatments

Persimmon (*Diospyros kaki* Lf.) cv. Rojo Brillante fruit were harvested in l’Alcudia (Spain) at midseason. After harvest, fruit were taken to the Instituto Valenciano de Investigaciones Agrarias (IVIA), where they were selected according to homogenous colour and absence of external damage. One lot of 20 fruit was analysed to determine the fruit maturity stage at harvest [colour index of 6.8, firmness of 53 N and soluble tannins content of 0.6 % FW]. The remaining fruit were submitted to the 1-MCP treatment (500 nL L⁻¹ of 1-MCP for 24 h at room T^a). Then fruit were divided into two homogenous lots. One lot was submitted to the CO₂ deastringency treatment under

standard conditions (95 % CO₂-20 °C-24 h), while the other lot was submitted to the CO₂-treatment at 12 °C (95 % CO₂-12 °C-24 h). Then the fruit from each treatment were once again divided into two lots: one was directly transferred to a 1 °C-storage chamber for up to 41 d; the other was kept for 24 h at 20 °C (attemperation period) before being transferred to the storage chamber at 1°C.

In this way, four different treatments were assayed:

➤ *12°C-D-fruit*: The deastringency treatment was applied at 12 °C and then fruit were transferred directly to the storage chamber at 1 °C (HR 85 %).

➤ *12°C-AT-fruit*: After applying the deastringency treatment at 12 °C, fruit were attemperated for 24 h at 20 °C before being transferred to the storage chamber at 1 °C (HR 85 %).

➤ *20°C-D-fruit*: The deastringency treatment was applied at 20 °C and then fruit were directly transferred to the storage chamber at 1 °C (HR 85 %).

➤ *20°C-AT-fruit*: After the deastringency treatment applied at 20 °C, fruit were attemperated for 24 h at 20 °C before being transferred to the storage chamber at 1 °C (HR 85 %).

All the fruit were stored for 41 d at 1 °C, plus a 3-day shelf-life period at 20 °C, and were periodically evaluated throughout the assay period.

According to the diagram shown in Figure 1, 20 fruit of each treatment were removed from the chambers and evaluated at the following time points:

Day 1- fruit were evaluated immediately after the CO₂ treatment (0 h) and after 2, 4, 6 and 24 h. The evaluated parameters were the following: soluble tannins content, carbon

dioxide (CO₂), ethanol (EtOH) and acetaldehyde (AcH) production of the whole fruit, and the EtOH and AcH concentrations in juice.

Day 2- fruit were re-evaluated 48 h after completing the CO₂-treatment. At this time point, the fruit corresponding to the 12 °C-D and 20 °C-D treatments had been kept for 2 d at 1 °C, while the 12 °C-AT and 20 °C-AT-fruit had been kept for 1 d at 20 °C (attemperation period), plus 1 d at 1 °C. The determined parameters were the same as those mentioned for Day 1.

Day 14 and Day 41- after 14 and 41 d of storage at 1 °C, fruit were re-evaluated. Moreover after 41 d, one lot of 20 fruit was transferred to 20 °C to simulate a 3-day shelf-life period at 20 °C. In addition to the parameters mentioned for Day 1, the incidence of the external and internal disorders was determined after 14 and 41 d of storage, and the subsequent shelf-life period.

Figure 1. Diagram of the performed assay. 1-MCP: application of the 1-metilciclopropene treatment; CO₂: application of the deastringency treatment at 12 °C or 20 °C; Attemp: attemperation period (24 h - 20 °C); SL: shelf-life period of 3 days at 20 °C.

2.2. Analytical determinations

2.2.1. Firmness and colour

The firmness and colour index of the fruit at harvest were determined on 20 fruit following the methodology described by Novillo et al. (2014). Colour was expressed as $IC = 1000a/Lb$, where L, a and b are Hunter parameters. Firmness was expressed as load in Newton (N) to break flesh at two equidistant locations in the equatorial region of each fruit after epicarp removal.

2.2.2. Soluble tannins, AcH and EtOH in juice

The soluble tannins content (ST), the AcH and EtOH concentrations in juice and sensory astringency, were determined on three individual fruit per treatment at each analysis time. To this end, three fruit were cut into four longitudinal quarters. Two of the opposite quarters were sliced and frozen (-20 °C) to later determine ST by the Folin-Denis method, as described by Taira (1996). Soluble tannin content was expressed as % of fresh weight. One of the remaining quarters was placed in an electric juice extractor (Moulinex model 753, Spain) and was filtered through cheesecloth. The obtained juice was used to determine the AcH and EtOH concentrations (three juices per treatment and analysis time). The AcH and EtOH concentrations were analysed by headspace gas chromatography following the conditions described by Besada et al. (2016). The results were expressed as mg 100 mL⁻¹.

2.2.3. Sensory evaluation of astringency

The remaining fruit quarter underwent a sensory evaluation of astringency, which was performed at the Sensory Laboratory of the Postharvest Department (IVIA) by a panel of experts. Three expert judges with more than 8 years of experience in astringency evaluation rated the astringency of the persimmon samples following a 5-point scale, which went from 0 meaning non-astringent to 4 denoting intensely astringent. The fruit quarter was peeled, and cut in 1.5 cm thick slices that were placed in glass cups identified by randomly assigned 3-digit codes; each judge analyzed individually the

three fruits of each lot. Samples were served at room temperature and panellists were provided with crackers and a glass of water for palate cleansing, which they used between samples.

2.2.4. *CO₂, AcH and EtOH release*

The CO₂, AcH and EtOH productions of whole fruit were determined from three other individual fruit from each lot. To this end, fruit were individually sealed in 1-litre glass jars. On Day 1, fruit were kept enclosed for 30 min, while the measurement time was 2 h on Days 2, 14 and 41. As explained above, in the analyses corresponding to Day 1-0 h, the glass jars were kept at 20 °C or 1 °C, depending on the treatment. In the analysis carried out after 2, 14 and 41 d of cold storage, the glass jars were kept at 1 °C, while fruit were enclosed at 20 °C in the analysis performed after the shelf-life period. The CO₂, AcH and EtOH productions were analysed by injecting 1 mL of headspace in a Perkin Elmer gas chromatograph according to the conditions described by Novillo et al. (2014). CO₂ release was expressed as mL kg⁻¹ h⁻¹ while AcH and EtOH release as µL kg⁻¹ h⁻¹.

2.2.5. *Browning incidence and severity*

External and internal brownings were visually evaluated. Browning severity was determined according to the scale shown in Figure 2.

Figure 2. Browning Scale. Scale used to evaluate internal browning severity, in which 0= absence, 1=slight browning in less than 50% of the pulp, 2= intense browning in less

than 50% of the pulp, 3=intense browning in 50-70% of the pulp, and 4=intense browning in more than 70% of the pulp.

To evaluate the incidence and severity of the disorder, the following browning index (BI) was calculated according to Khademi et al. (2013)

$$BI = \frac{\sum [(browning\ severity) \times (n^{\circ}\ of\ fruit\ at\ each\ browning\ severity)]}{4 \times total\ n^{\circ}\ of\ fruit}$$

2.2.6. Statistical Analysis

Data were subjected to an analysis of variance. At each evaluation period, the mean values of the four assayed treatments were compared by the least significant difference test ($P = 0.05$) using the Statgraphics Plus 5.1 software application (Manugistics Inc., Rockville, MD, USA).

3. RESULTS

3.1. External and internal disorders and fruit firmness

The visual evaluation of fruit revealed the absence of external disorders throughout the study period. After 41 d at 1 °C plus the shelf-life period, the fruit from the four assayed treatments showed a similar external quality to that noted at harvest.

The fruit internal evaluation showed that any flesh disorder was manifested in the fruit that was attempered after the CO₂ treatment for 24 h prior to cold storage (12 °C-A and 20 °C-A) (Fig 3). Nevertheless, the fruit transferred directly to cold chambers (12 °C-D and 20 °C-D-fruit) showed flesh browning symptoms after 41 d at 1°C (12 °C-D and 20 °C-D-fruit). The browning index revealed that the incidence and severity of internal

flesh browning were more marked in those fruit submitted to the CO₂ treatment at 20°C (BI=0.24) than in those treated at 12°C (BI=0.18) (data not shown). The browning indices increased in both cases after the 3-day shelf-life period at 20 °C when, once again, the disorder manifestation was more marked in the 20 °C-D-fruit (BI=0.46) than in 12 °C-D-fruit (BI=0.32).

Figure 3. Internal Browning. The insides of the fruits submitted to the CO₂-treatment at 12 °C or 20 °C, and then transferred directly to cold storage or after an attemperation period lasting 24 h at 20 °C. The pictures were taken after 41 d at 1 °C, plus 3 d at 20 °C. Internal browning was evaluated according to the scale displayed in figure 2.

Regarding fruit firmness, mean value at harvest time was 53 N. Not relevant changes were detected during cold storage, but fruit from all treatments suffered softening to values close to 30 N during the shelf-life period. Statistical analysis of firmness values revealed not significant differences among treatments after this period. This pattern of firmness loss, in which the main changes are observed after transferring fruit treated

with 1-MCP from low to moderate temperature, has been previously described for ‘Rojo Brillante’ (Pérez-Munera et al., 2009; Novillo et al., 2015)

3.2. Soluble tannins and sensory astringency

As shown in Figure 4, the deastringency treatment lowered soluble tannins (ST) from 0.6 % at harvest to 0.2-0.25 % once treatment ended (D1-0h). At this time point, the ST concentration was slightly lower in the fruit treated at 20 °C than in those treated at 12 °C. In all cases, the panellists detected “medium astringency” in the fruit (data not shown). After 2, 4 and 6 h following treatment, a gradual decrease in soluble tannins took place in all the treatments, and the decline in ST was faster in the fruit treated at 20 °C than at 12 °C. Moreover in both cases, the tannins insolubilisation process was accelerated with the attemperation period. Thus 6 h after the deastringency treatment ended, the 20 °C-A-fruit had an ST content of 0.02 %, evaluated by the panellists as ‘non-astringent’, while the 20° C-D-fruit obtained values of 0.06 % and the panellists detected “residual astringency”. At this time point, all the fruit treated at 12 °C had an ST content of 0.1 % and were evaluated as “medium astringency”. After 24 h, all the treatments achieved ST values close to 0.02 % and were evaluated as ‘non-astringent’. These values did not further change during the remaining storage time.

Figure 4. Soluble Tannins. Evolution of the concentration of soluble tannins (% FW) in the persimmon fruit submitted to the CO₂-deastringency treatment (95% CO₂) at 12 °C and 20 °C, and then transferred directly to cold storage at 1 °C (12 °C-D and 20 °C-D) or attemperated for 24 h at 20 °C (12 °C-A and 20 °C-A) before being cold stored for 41 d at 1 °C, plus a 3-day shelf-life period at 20 °C. Vertical bars represent the LSD intervals ($p = 0.05$).

3.3. Acetaldehyde (AcH) and ethanol (EtOH) concentrations in fruit juice

The CO₂ treatments applied at both 12 °C and 20 °C resulted in marked AcH accumulation in fruit, with values coming close to zero at harvest and at around 1-1.5 mg 100mL⁻¹ immediately after treatment (D1-0h), with no statistical differences among treatments (Fig. 5A). For the next 6 h, gradual AcH accumulation was observed in all the fruit.

It is noteworthy that after 24 h, AcH concentration sharply dropped in the AT-fruit to values below 1 mg 100mL⁻¹, while AcH increased in the fruit transferred directly to cold

265 storage (D-fruit). This increase was more marked in the fruits treated with CO₂ at 20°C
266 (20 °C-D) (5 mg 100mL⁻¹) than in those treated at 12 °C (12 °C-D) (3 mg 100mL⁻¹).

267 On Day 2, a drop in the AcH concentration took place to values that came close to 1 mg
268 100mL⁻¹ for the CO₂-treated fruit at 12°C, with no differences between the AT-fruit and
269 the D-fruit. Nevertheless, the CO₂-treated fruit at 20°C had increased AcH content, and
270 the D-fruit obtained higher values than the AT-fruit.

271 As storage advanced, all the fruit obtained values between 2.5 and 5 mg 100mL⁻¹, with
272 no differences between the AT-fruit and D-fruit. Only when fruit were transferred and
273 stored during the shelf-life period did the AcH values become significantly higher in the
274 fruit transferred directly to cold storage.

275 Regarding EtOH concentration (Fig. 5B), the CO₂-treatment temperature had a clear
276 effect as the CO₂-treated fruit at 20 °C had higher values than those CO₂-treated at 12
277 °C, as shown by all the measurements taken until D2. During the first 6 h, the effect of
278 the attemperation period was not evident, and it was only after 24 h when this practice
279 was seen to have a clear effect on EtOH accumulation. At this point, and similarly to
280 that observed in AcH, EtOH content was higher in the fruit directly transferred to cold
281 storage. These differences between D-fruit and A-fruit disappeared at D-2, but the CO₂-
282 treated fruit at 20 °C still had a higher EtOH concentration than that CO₂-treated at 12
283 °C. No relevant differences were detected among treatments for the next 14 storage
284 days. However at 41 storage days, EtOH content did not change in D-fruit, but dropped
285 in A-fruit. After the shelf life, the EtOH level of the D-fruit CO₂-treated at 20°C did not
286 change, but the other treatments brought about a lower EtOH content, but no differences
287 among them were observed.

Figure 5. Acetaldehyde and Ethanol Concentration in Juice. Evolution of the AcH (A) and EtOH (B) concentration in fruit juice (mg 100mL⁻¹) in the persimmon fruit submitted to the CO₂-destringency treatment (95 % CO₂) at 12 °C and 20 °C, and then transferred directly to cold storage at 1 °C (12 °C-D and 20 °C-D) or attemperated for 24 hours at 20 °C (12 °C-A and 20 °C-A) before being cold stored for 41 d at 1 °C, plus a 3-day shelf-life period at 20 °C. Vertical bars represent the LSD intervals ($p = 0.05$).

297

298 3.4. CO₂, AcH and EtOH release

299 The release of CO₂ from the fruit treated at 12 °C and 20 °C is shown in Figure 6. At
300 harvest, fruit had values of 7.2 mL kg⁻¹ h⁻¹. The CO₂ deastringency treatment applied at
301 both temperatures led to a drastic increase in CO₂ with values above 120 mL kg⁻¹ h⁻¹.
302 These high CO₂ values have been associated with the diffusion of the CO₂ that had
303 accumulated inside fruit during treatment. That is, once fruit were moved from the CO₂
304 application chamber to an air atmosphere environment, the CO₂ detained by fruit was
305 released. This is why the term ‘CO₂ release’ is used in this study rather than ‘CO₂
306 production’.

307 In the fruit treated at 12 °C, slight differences between AT-fruit and D-fruit were
308 detected immediately after treatment (0 h), with CO₂ values of 190 and 150 mL kg⁻¹ h⁻¹,
309 respectively. However in the fruit treated at 20 °C, CO₂ release was clearly more
310 marked in those submitted to an attemperparation period than in those transferred directly
311 to cold storage (230 and 140 mL kg⁻¹ h⁻¹, respectively). During the subsequent periods,
312 CO₂ release gradually decreased, and the measurements taken until 6 h showed a greater
313 CO₂ release in A-fruit than in those transferred directly to 1 °C.

314 After 24 h, the CO₂ level significantly lowered in all the fruit with values of about 25
315 mL kg⁻¹ h⁻¹. On Day 2, CO₂ release lowered to similar values to those recorded at
316 harvest in all the treatments. During the subsequent storage period, the values remained
317 quite constant, and it was only when fruit were transferred to 20 °C after 41 d, in order
318 to simulate commercialisation that a slight increase in CO₂ was observed, with no noted
319 differences among treatments.

320

321

Figure 6. CO₂ Release. Evolution of the CO₂ release (mL kg⁻¹ h⁻¹) in the persimmon fruit submitted to the CO₂-deastringency treatment (95 % CO₂) at 12 °C and 20 °C, and then transferred directly to cold storage at 1 °C (12 °C-D and 20 °C-D) or attemperated for 24 h at 20 °C (12 °C-A and 20 °C-A) before being cold stored for 41 d at 1 °C, plus a 3-day shelf-life period at 20 °C. Vertical bars represent the LSD intervals ($p = 0.05$).

As explained for CO₂, the AcH and EtOH values presented in Fig. 7A and 7B correspond to the release of the gases that accumulated inside fruit during the CO₂ treatment. During the first 4 h in the CO₂-treated fruit at 20 °C, the release of AcH and EtOH was more marked in AT-fruit than in D-fruit. However, in the CO₂-treated fruit at 12 °C, no differences between treatments were observed until 6 h. All the fruit showed a marked decrease after 24 h, with no differences among treatments. From this day onwards, the level of these two volatiles dropped in all the fruit.

Figure 7. Acetaldehyde and Ethanol Release. Evolution of the AcH (A) and EtOH (B) release ($\mu\text{L kg}^{-1} \text{h}^{-1}$) in the persimmon fruit submitted to the CO_2 -deastringency treatment (95% CO_2) at 12 °C and 20 °C, and then transferred directly to cold storage at 1 °C (12 °C-D and 20 °C-D) or attemperated for 24 h at 20 °C (12 °C-A and 20 °C-A) before being cold stored for 41 d at 1 °C, plus a 3-day shelf-life period at 20 °C. Vertical bars represent the LSD intervals ($p = 0.05$).

4. DISCUSSION

In the present study, the browning index results revealed that only the fruit transferred directly to coldstorage displayed flesh browning after 41 days, indicating that, the attemperation period is a crucial factor to avoid the development of this flesh disorder. According to our results Min et al. (2018) reported browning incidence in 'Niuxin' persimmons transferred directly from CO₂-treatment to cold storage chamber while this disorder was not observed in fruit that have been not submitted to the deastringency treatment before storage. More recently, Win et al. (2019) reported browning manifestation after cold storage of 'Sangjudungsi' persimmons previously treated with 1-MCP and CO₂. The authors did not describe the conditions between CO₂-treatment and fruit transfer to cold storage, but it is quite possible that fruit were transferred directly to cold chambers.

It is well-known that the astringency removal process using CO₂ consists in two phases: 1) an induction phase in which fruit must be maintained for a minimum period at high CO₂ concentrations. During this phase, AcH and EtOH are accumulated as result of the anaerobic respiration and tannins-AcH reaction leads to a tannins insolubilization process; 2) a second phase in which astringency keeps gradually disappearing and presence of CO₂ is not essential (Gazit and Adato, 1972). These two phases are temperature-dependent (Matsuo and Ito, 1977).

Accordingly, our results showed that the tannins insolubilisation reaction that took place immediately after CO₂-treatment was faster when the process was performed at 20 °C than at 12 °C. Moreover, during the first 24 horas after CO₂-treatment a clear effect of the temperature exposure on tannins insolubilisation was also observed as the decline in soluble tannins was faster in the fruit kept at 20 °C (20 °C-A and 12 °C-A) than in those directly moved to cold storage (20 °C-D and 12 °C-D).

371 It is noteworthy that the CO₂-treatment to which fruit were subjected led to not only
372 AcH and EtOH accumulating in fruit flesh, but also CO₂. The ability of the fruit to
373 continue accumulating or releasing these gases during the hours that followed the CO₂-
374 treatment was also highly depended on the temperature after treatment. So, maintaining
375 fruit for 24 h at 20 °C after being submitted to the deastringency treatment resulted in a
376 faster release of these three gases out of the fruit compared to fruit transferred directly
377 to coldstorage. This fact, first described in this study, is explained by the effect of
378 temperature and pressure in the diffusion coefficient of a gas (Welty et al., 1984).
379 Diffusion increases with increasing temperature as molecules move more rapidly, and
380 decreases with increasing pressure, which packs more molecules in a given volume and,
381 thus, makes it harder for them to move.

382 The lower ability to release CO₂ at low temperature led to the anaerobic respiration
383 lasted longer in the fruit transferred directly to low temperature (D-fruit), with the
384 consequent accumulation of AcH, compared with the fruit maintained at 20°C (A-fruit).
385 So, this AcH accumulation in fruit flesh during the first 24 h after CO₂-treatment seems
386 to be the key to browning development in the fruit. Moreover, the browning severity
387 was higher in those fruits CO₂-treated at 20°C (D-20°C), in which the highest AcH
388 concentrations were detected after 24 h.

389 The link connecting the accumulation of higher AcH levels triggered by anoxia
390 situation and flesh browning manifestation, that herein was observed in the D-fruit has
391 been previously suggested for other fruit by different authors. So, a situation of anoxia
392 in the central fruit region has been associated with the development of browning
393 disorders in pear (Ho et al., 2010). Moreover, the greater sensitivity of pear to develop
394 internal browning, has been associated with the lesser ability tissues to facilitate gas
395 exchange (Verboten et al., 2008) and as observed herein, CO₂ diffusivity in pear has
396 also been reported to be strongly influenced by temperature (Ho et al., 2006).

Moreover, the effect of anoxia to cause unrecoverable cell damage and flesh disorders (Ho et al., 2009) has been related to a smaller amount of detoxification enzymes against harmful reactive oxygen species (ROS) (Drew 1997; Purvis, 2001; Apel and Hirt, 2004). In such a process, AcH accumulation when fruit are exposed to high CO₂ concentrations seems to play an important role in tissue browning and cell death (Fan et al., 2005; Zhan et al., 2015) as it can act a promoter of ROS generation.

In persimmon, a tannins oxidation process by ROS mediated by AcH has been previously linked to other kind of browning disorders associated with mechanical damage (Novillo et al., 2014). Therefore, it is possible that the high AcH concentration herein linked to the ‘internal flesh browning’ manifestation acts as a source of ROS generation. However, cell membranes should prevent the reaction of ROS and the tannins allocated in the vacuole of the so-called ‘tannins cells’ (Gottreich and Blumenfeld, 1991; Yonemori et al., 1997). In this sense, the main structural changes that ‘Rojo Brillante’ undergo during cold storage have been described as loss of cell wall integrity and low intercellular adhesion (Pérez-Munera et al., 2009). This would explain why a relatively long storage period, lasting some 40 d, is necessary for internal flesh browning to be manifested. Only after cell membranes lose their integrity due to low temperature conditions can the ROS generated by AcH mediation oxidise tannins with the subsequent browning manifestation. While high AcH concentration accumulated in flesh at the beginning of storage seems to be the primary cause of browning disorder, an EtOH accumulation was detected during browning manifestation. Similarly, the EtOH concentration has been suggested as a potential predictor of the flesh browning manifestation risk during prolonged apple fruit storage as it has been associated with flesh browning induced by heat stress (Fan et al., 2005).

In summary the results obtained in this study revealed for the first time that temperature at which the fruit is exposed after CO₂-treatment is the main factor implied in ‘internal

flesh browning' manifested in 'Rojo Brillante' persimmon after cold storage. An
attemperation period at 20 °C during 24 h after deastringency treatment facilitates the
diffusion of CO₂ out of fruit, which results in lower AcH accumulation, which seems to
play a key role in preventing browning disorder manifestation. Therefore, in the case
that persimmon have to be cold stored, it is necessary to implement an attemperation
period after the deastringency treatment to avoid internal browning manifestation.

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