Application of a new wax containing ethanol as a method to remove persimmon astringency during cold storage

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Research highlights

- Wax containing ethanol is effective to remove packaged persimmon astringency during cold storage
- CO_2 deastringency treatment may cause quality loss and flesh browning after prolonged cold storage
- New patented method can replace CO_2 deastringecy treatment when prolonged cold storage is needed

Abstract

Nowadays the treatment based on applying high CO_2 concentrations to fruit is the main method used in astringent persimmon prior to being commercialized, but it can cause quality problems for fruit during cold storage. The aim of this study was to evaluate the effectiveness of a recently patented astringency removal method based on applying a new wax whose formulation includes ethanol before commercial packaging. During two seasons, three treatments were evaluated in cv. Rojo Brillante and Triumph: 1) CO_2- standard treatment; 2) waxed and packed in plastic film according to the patented method; 3) packed in plastic film without any treatment. During a third season, the new method's effectiveness in removing astringency was evaluated under industrial conditions. After treatments fruit was stored at 0°C for 15, 21 and 30 days before being transferred at 20°C to simulate a 5-days shelf-life. All the fruit treated with the new wax completely lost astringency after 30 days at 0°C, and commercial firmness was maintained. At the end of the storage, fruit quality was substantially higher in fruit submitted to the new treatment. CO_2-treated fruit, manifested internal browning after 30 storage days and shelf-life, while this disorder was not detected in waxed fruit.

Keywords: deastringency, cold storage, wax, ethanol, 'Rojo Brillante', 'Triumph'

Introduction

Persimmon cultivars are divided into two categories, astringent and non-astringent, depending on their astringency level at harvest. Astringent persimmons are not edible when harvested due to the presence of high soluble tannin concentrations, which produce a dry puckering sensation in the mouth (Besada and Salvador 2018). 'Triumph' and 'Rojo Brillante' are the main cultivars from the Mediterranean region, both of which are astringent at harvest.

The most habitual treatment used to remove astringency in persimmon is based on exposing fruit to 95-98% CO_2 at 20 °C and 90% RH for 24 h (Besada and Salvador 2018). The effectiveness of this method lies in the fact that it triggers anaerobic respiration in fruit, which gives rise to the accumulation of acetaldehyde. The reaction between this acetaldehyde and the soluble tannins responsible for astringency leads tannins to become insoluble, hence they lose their astringent character (Arnal and Del Río 2003; Salvador et al. 2007). Although the effectiveness of this deastringency method has been widely studied, a high CO_2 concentration affects the parenchyma structure, causing cell membrane degradation, which can lead to loss of flesh firmness after treatment. Thus, in the case the fruit has to be cold stored, it is recommended to remove the astringency after conservation to preserve the fruit firmness. Nevertheless, it is noteworthy that in some marketing scenarios, such as shipments to overseas countries, CO_2 treatment is necessarily applied before using refrigerated transport. Under these conditions, the risk of fruit quality loss during cold storage is high. Indeed, the fruit softening and internal browning that appears during prolonged refrigerated transport is one of the most important problems that is limiting the market to overseas countries.

In this context, interest has been shown in finding alternative deastringency methods that can be applied in cold storage management scenarios to avoid quality losses associated with the currently employed CO_2 treatment. To respond to this demand, the present work takes as a

starting point two postharvest technologies used for different purposes that have never been combined. On the one hand, it is known that exposing fruit to ethanol has an effect of removing astringency. Ethanol vapor was one of the first treatments to be assayed to remove astringency in the persimmon fruit industry (Taira et al. 1989). Although this treatment still continues to be used in countries like Brazil (Tessmer et al. 2018), it is being replaced with CO_2 treatment as the latter requires less time to remove astringency and fruit firmness is better preserved.

On the other hand, edible waxes are used to carry some ingredients like antioxidants, antifungal and antimicrobial agents for different purposes in different fruits (Nair et al. 2018; Rojas-Graü et al. 2007). In persimmon no information on this regard exits, only some studies have reported the use of waxes to reduce softening and weight loss (Blume et al. 2008; Carvalho da Silva et al. 2011).

In this context, the hypothesis based on producing an edible wax as a carrier for ethanol was the starting point to reach research collaboration between the Instituto Valenciano de Investigaciones Agrarias (IVIA) and the Fomesa Fruitech S.L.U. Company. The final objective was to find a new deastringency method based on ethanol that can be easily applied and that does not have negative effects on the fruit quality during low-temperature storage and subsequent shelf-life. The studies carried out allowed to develop an ethanolic wax that could remove fruit astringency during cold storage when combined with fruit packaging, and this process has been recently patented (P-101459).

The objective of this work was to evaluate the effectiveness of this new method to remove persimmon astringency during cold storage, and to determine its effect on other important physico-chemical quality parameters as color, firmness, total soluble solids, chilling injury and browning incidence. This study was part of the studies submitted for approval of the patent.

Materials and Methods

Fruit source and treatments

This study was conducted in three consecutive seasons from 2017 to 2019. In the first and second season the treatments were tested under lab conditions while in the third season the assays were carried out under industrial conditions.

First season (S1). 'Rojo Brillante' persimmons were harvested in November in a commercial maturity stage from an orchard in Valencia (Spain) before being immediately transported to the IVIA's Postharvest Laboratory Technology Center. Fruit were carefully selected for their uniform size and external color. Then they were separated into homogenous lots of 12 fruits, and were placed on plastic alveoli trays in a cardboard fruit box. Three of these lots were used to evaluate the physico-chemical quality of fruit at harvest.

The remaining lots of fruit were subjected to the following three treatments (12 lots per treatment):

1) fruit submitted to the traditional CO_2 treatment with 95% CO_2 at 20ºC for 24h (CO_2);

2) fruit waxed and packed in plastic film according to the patented method (Wax+Film);

3) fruit packed in plastic film with no wax application, used as the control (Film).

After applying treatments, all the fruit were stored at 0ºC with 85%-90% RH for up to 30 days. Periodically after 15, 21 and 30 days, three lots of each treatment were removed from cold storage for analysis. In addition, after 30 days three other lots of fruit were transferred to 20 ºC for 5 days to simulate the shelf-life period, after which they were evaluated.

Second season (S2). During this season, experiments were carried out in the same way as during the first season, but in this case they were performed with persimmons 'Rojo Brillante' and 'Triumph'. The fruit of both cultivars were harvested in the commercial maturity stage in December from two orchards in Valencia. During this season, a 5-day shelf-life at 20°C was simulated after 15, 21 and 30 days of cold storage periods.

Third season (S3). During this season, the effectiveness of the new method to remove astringency was evaluated under industrial conditions with 'Rojo Brillante'. To this end, after

harvest fruit were transported to a commercial packing house (Natural Hand S.L.) where they were carefully selected for their uniform size and external color. Then fruit were separated into homogenous lots of 16 fruits and were placed on plastic alveoli trays in a commercial cardboard fruit box. Four treatments were assayed: 1) fruit submitted to the traditional CO_2 treatment with 95% CO_2 at 20 ºC for 24 h (CO_2) in industrial chambers; 2) fruit waxed and packed in plastic bags according to the patented method (Wax+Film); 3) fruit packed in plastic films with no wax application, used as the control (Film); 4) fruit waxed and boxes stacked on a wooden pallet, strapped with macroperforated stretch film by a commercial automatic strapping machine (Wax+Pallet). Here the objective was to generate a kind of film package by strapping pallets, whereby a cardboard sheet was placed on top of the pallet (Wax+ Pallet) and it was film-strapped until almost the whole pallet surface was covered. After applying treatments, boxes were placed in the cold chambers at the Natural Hand Company at 0-1 °C with 85%-90% RH for up to 30 days. After 30 days, three lots of each treatment were evaluated. Three other lots of fruit were transferred to 20 ºC for 5 days to simulate the shelf-life period, after which they were evaluated.

In all cases after harvest the fruit were treated with 1-MCP under commercial conditions (500 nL/L of 1-MCP for 24 h at room T). This treatment is commercially applied to persimmons before they are submitted to low temperature in order to delay chilling injury symptoms (Salvador et al. 2004).

CO_2 treatment was carried out in closed containers (467 L), which contained 95% CO_2 for 24 h at 20 ºC and 90% RH. These conditions were established by passing a stream of air containing 95% CO_2 through containers.

In the first and second season, in the lab, wax was applied uniformly to the fruit surface with a sprayer at a rate of 12.5 mL wax per kg of fruit. Wax and plastic film (Xtend®, StePac L.A. Ltd) were provided by the Fomesa Fruitech S.L.U. The application system for the wax in the industry (third season) was composed of two main parts, the pressurizer pump and the

spraying gun. The main objective of this application system was to create a fine nebulization of the wax, creating a thin coating of the product on the surface of the fruit. The pressurizer pump is in charge of using pressured air from a compressor to pressurize the wax through an 8 mm pipe to the spraying guns. Once the wax is pressured, the spraying guns are able to make a fine nebulization of the wax on the surface of the fruit. Regarding the spraying gun, we used an 80º R nozzle, which allows us to make a diffusion of the wax at an 80º opening angle and a flow rate of 2.5 gallons per hour.

At harvest, and after different cold storage periods as well as the shelf-life, the determined physico-chemical and sensory parameters were as follows: external color, firmness, soluble tannins content (ST), sensory astringency and off-flavors, acetaldehyde (AcH) and ethanol (EtOH) in juice. After cold storage, the CO_2 and EtOH concentrations inside plastic films were measured.

For a better understanding, the treatments and conditions tested in each season are shown in the table 1.

Fruit quality assessments

Determinations were made with 12 fruits, three replicates per treatment. After the low-temperature storage periods and the subsequent shelf-life, the following were evaluated: external color, flesh firmness, soluble tannin contents (ST), sensory astringency and off-flavors, internal and external disorders, AcH and EtOH in juice, EtOH and CO_2 in plastic bags.

Skin color was determined by a Minolta colorimeter (Model CR-300; Minolta, Ramsey, NY). Hunter parameters “L,” “a,” and “b,” were measured and the results were expressed as Color Index = (1000a)/(Lb) (Salvador et al. 2007). Two measurements were taken on the opposite equatorial area of each fruit.

Flesh firmness was evaluated by a texturometer (model 4301, Instron Corp., Canton, Mass., USA) using an 8-mm plunger. The crosshead speed during firmness testing was set at 10 mm/min. The results were expressed as load in Newton (N) to break flesh at two equidistant locations in the equatorial region of each fruit after epicarp removal.

Immediately after taking the firmness measurements, fruits were cut into half and any internal disorder was evaluated. The incidence of external and internal disorders was evaluated visually on all the fruit in each lot according to Khademi et al. (2013). Skin browning severity was evaluated on four scales as: 0, no browning; 1, slight browning; 2, moderate browning; 3, severe browning. Similarly, flesh browning was rated on a 4-point scale according to the browning intensity detected in the central fruit part as: 0, no browning; 1, slight browning (less than 30% of the fruit flesh surface was brown); 2, moderate browning (more than 30% and less than 60% of the fruit flesh surface was brown); 3, severe browning (more than 60% of the fruit flesh surface was brown). The browning index (BI) was calculated as BI= ∑[(browning severity) X (no. of fruit at each browning severity)] / 3 X total no. of fruit.

After disorders evaluation, six fruits per lot were cut to obtain four quarters. Two opposite quarters were frozen at -21°C until the ST analyses. ST content was evaluated by the Folin-Denis method described by Arnal and Del Río (2004). The results were expressed as a percentage or g/100g FW. One of the remaining quarters was used in the sensory evaluation and the other was employed to obtain juice for the AcH and EtOH determinations.

The sensory evaluation was performed by 8 trained panelists who were asked to evaluate astringency levels and the presence of off-flavors. Sensory sessions were carried out in a specifically adapted room, where panelists were seated in individual evaluation booths. Fruits were peeled and sliced (longitudinal slices, 1.5 cm wide), and three slices from three different fruits were presented per sample to compensate for variability. Water was provided to cleanse palates between samples. Samples were served in random order to the panelists in 50-mL stainless steel soufflé cups, identified by a unique three-digit number. Each fruit was tasted

by at least three panelists. A 5-point scale was used for the sensory astringency evaluation: 1 = no astringency; 2 = residual astringency; 3 = slight astringency; 4= moderate astringency; 5 = astringency. A value below 1.5 guarantees the non-astringency of the fruit, and therefore is considered commercially acceptable (Besada et al., 2016; Munera et al., 2017). Off-flavors were evaluated as Presence/Absence.

In order to determine the AcH and EtOH concentrations, one quarter of the six fruits per lot was placed in an electric juice extractor (model 753, Moulinex, Spain) and the obtained juice was filtered through cheesecloth. AcH and EtOH production was measured with three samples per replicate of juice samples, obtained as previously mentioned and analyzed by headspace gas chromatography. Five milliliters of the filtered juice were transferred to 10-mL vials with crimp-top caps, sealed with TFE/silicone septa, and frozen (-21) until analyzed. For the analysis, samples were put in a water bath at 20 ºC for 1 h, followed by heating at 60 °C for 10 min. A 1-mL headspace sample was withdrawn from the vials and injected into the gas chromatograph (model 2000, Perkin-Elmer, Norwalk, Conn., USA), equipped with a flame ionization detector (FID) and a 0.32 cm × 1.2 m Poropak QS 80/100 column. The injector was set at 175 °C. EtOH and AcH were identified by comparing the retention times with those of a standard solution. The results were expressed as mg/100 mL.

Prior to opening the plastic bags, the EtOH and CO_2 concentrations were determined. For this purpose, a BD Plasticpak syringe was placed inside the plastic bag and 1 mL of the generated atmosphere was extracted carefully so as not to damage fruit. This volume was injected into the gas chromatograph (model 2000, Perkin-Elmer, Norwalk, Conn., USA) and the same previously explained pattern was followed. For EtOH, the results were shown as µL/L and as % of gas in the atmosphere for CO_2.

Statistical Analysis

Data were subjected to an analysis of variance, based on two factors (treatment × storage duration). Multiple comparisons between means were determined by the least significant

difference test ($P \leq 0.05$) using the Statgraphics Plus 5.1 software application (Manugistics Inc., Rockville, MD, USA).

Results and Discussion

Soluble tannins (ST) and sensorial astringency evaluation

At harvest, the ST content in 'Rojo Brillante' was close to 0.65-0.70% during all three studied seasons (Figure 1a, 1b, 1d). The ST values for 'Triumph' were slightly higher at harvest time, 0.9% (second season) (Figure 1c). These values were in the same range found by most previous studies conducted on both cultivars, which have been related to high astringency levels in fruit (Besada et al. 2014; Novillo et al. 2015; Salvador et al. 2007).

As expected, the ST content dropped significantly after being treated with a high CO_2 concentration (CO_2 treatment). In all cases after 1 day of the treatment (DA), the ST concentration lowered to values between 0.01% and 0.03%. The panelists evaluated fruit astringency with scores of 1 (non-astringent fruit) (Figure 1e, 1f, 1g, 1h).

In the fruit packed in plastic film with no added wax (Film), ST slightly dropped, over all after 30 cold storage days and the shelf-life that followed the different cold storage periods. Despite of this decrease, in all cases, the ST values remained very high, which resulted in fruit having high levels of sensory astringency (astringency scores ranged between 3.9 and 4.8).

However, the ST in the waxed fruit (Wax+Film) gradually lowered during the studied storage period. In the first and second seasons, after 15 days at low temperature, ST drastically decreased, with values close to 0.15%-0.18% in both cultivars. The sensory evaluation revealed that this fruit still exhibited a high astringency level (scores of 4.2-4.5). In all cases from 15 days onward, the ST content continued to lower and reached values below 0.03% after 30 days, when the fruit's astringency scores were lower than 1.5, considered to be

commercial. Similar results were obtained during the third season, when assays were carried out with ‘Rojo Brillante’ under industrial conditions. After 30 cold storage days, the wax+film fruit and wax+pallet fruit obtained ST values close to 0.03%. In both cases, fruit was evaluated as non-astringent.

One point worth mentioning is that in the second season, in which the shelf-life conditions were simulated after all the cold storage periods, just after 15 and 21 days at low temperature the wax+Film fruit values were 0.01% and 0.02% in ‘Rojo Brillante’ and ‘Triumph’, respectively, and the panelists did not detect astringency.

In persimmon fruit the threshold of ST leading to astringency perception depends on the cultivar. ST above 0.1% has been found to confer an astringent taste, and these fruit are not edible in persimmons ‘Kaki Tipo’, ‘Lycopersicom’ and ‘Thiene’ (Vidrih et al. 1994). In the present study, and irrespectively of the treatment to which fruit was submitted, the panelists’ evaluation of fruit not being astringent corresponded to the fruit that had an ST content below 0.03%. This value agrees with those previously reported for both ‘Rojo Brillante’ and ‘Triumph’ (Besada et al. 2010; 2014; Salvador et al. 2007).

Acetaldehyde (AcH) and ethanol (EtOH)

Most methods performed to remove persimmon astringency are based on maintaining fruit under anaerobic conditions or exposing them to products that induce anaerobic respiration. Under these conditions, soluble tannins, responsible for astringency, are polymerized by AcH that accumulates in flesh, hence the deastringency process rate has been positively related to the level of AcH that accumulates in fruit flesh (Pesis et al. 1988; Taira et al. 1989). In ‘Rojo Brillante’, the accumulated AcH concentration after different deastringency treatments has also been closely associated with a drop into the decrease of ST (Besada et al. 2010). Nevertheless, the AcH level required to insolubilize tannins in fruit flesh to reach undetectable astringency values has not yet been established.

296In the present study, both cultivars exhibited very low AcH concentrations at harvest, 297between 0.1 and 0.3 mg/100 mL (Figure. 2a, 2b, 2c, 2d). These values are in the range 298reported in previous studies (Arnal and Del Río 2003; 2004; Besada et al. 2014). As expected 299in the CO_2-treated fruit, AcH significantly increased 1 day after treatment (DA), with values 300of around 4.5 mg/100 mL in both cultivars in the first and second seasons. For the third 301season, the values reached after CO_2 treatment were slightly lower, 3.5 mg AcH/100 mL. For 302all three seasons, AcH levels linked to CO_2-treated fruit showed no relevant changes 303throughout the study period.

304The fruit packed in plastic film with no added wax (Film fruit) had similar values to those at 305harvest throughout cold storage. Although the AcH concentration slightly increased after the 306shelf-life periods, the maximum values did not exceed 0.7 mg/100 mL.

307In the Wax+Film fruit, AcH gradually increased throughout cold storage. So, in the first and 308second season the AcH content obtained values close to 0.8 mg/100 mL after 15 days at low 309temperature, which coincided with a significant drop in ST. From this time point, the AcH 310concentration continued to rise concomitantly with a drop in ST. After 30 cold storage days, 311when fruit was evaluated as non-astringent, the AcH content came close to 2.5 mg/100 mL 312and 3.5 mg/100 mL for ‘Rojo Brillante ‘and ‘Triumph’, respectively. It should be noted that 313similar AcH values were found during the second season (Figure 2b, 2c) in the fruit evaluated 314as being non-astringent after the shelf-life that followed the 15 and 21 cold storage days. 315Under industrial conditions (third season), loss of astringency after 30 cold storage days in 316the Wax+Film fruit was associated with an AcH concentration of 3.5 mg/100 mL. 317Nevertheless, in the palletized fruit (Wax+Pallet), this value was lower, 2 mg/100 mL (Figure 3182d).

319According to these results, an AcH concentration in fruit flesh within the 2 and 3.5 mg/100 320mL range was necessary to achieve a low enough ST content (≤ 0.03%) to not induce 321perceived astringency. This AcH range is consistent with previous studies, in which similar 322AcH values have been measured in non-astringent fruit after CO_2 treatment (Besada et al.

3232010; Novillo et al. 2013; 2015; Salvador et al. 2007). In the present study, this AcH content 324was achieved only 1 day after CO_2 treatment, whereas 30 cold storage days or 15 days plus 325the shelf-life were required to reach the same AcH concentration in the waxed fruit.

326Regarding the EtOH content values went below 6 mg/100 mL at harvest in both cultivars 327(Figure 2e, 2f, 2g, 2h). In the CO_2-treated fruit, the EtOH content significantly increased 1 328day after treatment (DA). Values of 35 mg/100 mL and 65 mg/100 mL were detected in 'Rojo 329Brillante' in the first and second season respectively. The EtOH content in ‘Triumph’ (Figure 3302g) was higher and came close to 78 mg/100 mL. These EtOH values remained constant 331throughout cold storage and the subsequent shelf-life periods. The film fruit accumulated 332very little EtOH during storage. Nevertheless, in the Wax+Film fruit, EtOH accumulation 333was in flesh after 15 cold storage days, and values did not relevantly change until the end of 334the study. In ‘Rojo Brillante’, the EtOH values were 80 mg/100 mL during the first season 335and came close to 180 mg/100 mL during the second season. In ‘Triumph’, the EtOH values 336came close to 200 mg/100 mL. In the third season, differences in EtOH were found between 337the Wax+Film and palletized fruit (Wax+Pallet). The Wax+Film fruit showed 150 mg EtOH/ 338100 ml after 30 storage days while the EtOH values of the palletized-fruit were significantly 339lower (close to 50 mg/100 mL).

340In this study, the applied wax formulation contained ethanol. Therefore, the AcH required to 341insolubilize tannins would be produced mainly by alcohol dehydrogenase directly from the 342EtOH that accumulated in flesh, as reported by other authors after studying the effectiveness 343of deastringency treatments by EtOH vapor (Taira et al. 1989; Yamada et al. 2002).

344According to the EtOH measures taken inside plastic bags, an increment 1 day after cold 345storage to values of 0.5 µL/L and 0.7 µL/L was observed in the Wax+film ‘Triumph’ fruit 346and the ‘Rojo Brillante’ fruit, respectively. After 15 days however, this concentration 347dropped to those values found in plastic films with the unwaxed fruit (values close to 0.04) 348(Table 2). The low EtOH concentration that accumulated in plastic films allowed us to 349assume that the gradual increase in AcH in flesh during cold storage would be promoted by

EtOH diffused through skin from wax into the parenchymal tissue, instead of from ethanol vapors. Yamasaki et al. (2017) also reported astringency removal ‘on tree’ using ethanol stickers to release EtOH to the fruit surface.

It is also noteworthy that like other fruits, EtOH accumulation may play an important role in off-flavors developing in persimmon (Pesis 2005). In line with this, Ben-Arie et al. (1991) studied the effect of modified atmospheres during persimmon storage and reported how EtOH that accumulated to levels exceeding 75 mg/100 mL was related to deteriorated taste. Nevertheless, in other studies, values of 100 mg EtOH/100 mL in ‘Rojo Brillante’ or 200 mg/100 mL in ‘Triumph’ had no negative effect on fruit taste (Besada et al. 2014). The maximum EtOH values herein detected came close to 200 mg/100 mL and were, in any case, associated by the panelists with a negative change in fruit flavor (data not shown).

External color and flesh firmness

The two studied cultivars were harvested in all three seasons with homogeneous coloration and color index values between +8 and +11, but no relevant changes in external coloration were detected during the cold storage period (data not shown). Nevertheless, when fruit were transferred to shelf-life conditions, a significant increase took place. Thus the ‘Rojo Brillante’ values came close to +20, and to +15 for ‘Triumph’, after the shelf-life following 30 days storage at 0 °C. In any case, differences among treatments were found.

One of the most important parameters for preservation during the cold storage of persimmon is flesh firmness. It is stressed that most persimmon cultivars have been widely reported to exhibit chilling injury symptoms when stored at low temperatures, and firmness loss is the main chilling injury symptom. Such flesh softening can be exhibited during cold storage at 4–11 °C (Arnal and Río 2004). However, during storage at 0–1 °C, drastic firmness loss occurs mainly when fruit are transferred to shelf-life temperatures (Salvador et al. 2004; 2006). Thus the application of 1-MCP prior to storage has become a common treatment before submitting fruit to low temperatures as it reduces chilling injury symptoms in many persimmon cultivars

(Kim and Lee 2005; Salvador et al. 2004; Tessmer et al. 2019). In the present study, all the fruit were submitted to commercial 1-MCP treatment after harvest to preserve flesh firmness. In the first and second seasons, fruit were harvested with high flesh firmness values of around 40-45 N. In the third season, the initial firmness was slightly lower (close to 35 N). During cold storage, firmness slightly decreased in all cases. After transferring fruit from cold storage to shelf-life conditions, firmness loss became more evident, but flesh firmness remained crispy with values above 25 N during the first and second seasons. It was only during the third season when fruit underwent more marked firmness loss, with values close to 15 N. In any case, differences among the assayed treatments were found.

Internal and external disorders

Throughout the study period, no external disorders were observed. Nevertheless, flesh browning was exhibited only in 'Rojo Brillante' subjected to CO_2 treatment after the shelf-life that following the 30 cold storage days (Figure 3). The Browning Index gave values of 0.2 and 0.3 in the first and the second season, respectively (data not shown). The wax-treated fruit (Wax+Film) did not show any browning (Figure 3).

Different postharvest factors have been associated with internal persimmon browning, such as storage in controlled atmospheres and mechanical damage (Besada et al. 2014; Novillo et al. 2014), and browning symptoms differ depending on the causal factor.

The herein manifested flesh browning was visualized in the central fruit area, similarly to that reported to be induced by storage in controlled atmospheres (Besada and Salvador 2018). It is noteworthy that this alteration is not new to us because it has often been visualized in those persimmons submitted to CO_2 treatment before being cold-stored for lengthy periods. Indeed, the causes of this disorder are currently being studied by our group.

In other fruits, tissue browning and cell death have been linked to the AcH that accumulates under anaerobic conditions (Fan et al. 2005; Zhang et al. 2015). Accordingly, the browning manifestation herein found in the fruit subjected to CO_2 treatment could be related to AcH

accumulating in flesh. So the CO_2 fruit achieved the maximum AcH level only 1 day after treatment and this high AcH concentration in flesh remained constant throughout the study period. Nevertheless, in the wax-treated fruit, AcH accumulation was gradual and it was only after 30 days when it reached similar levels to those of the CO_2-treated fruit. Thus the period that flesh is exposed to high AcH levels seems crucial for browning development.

Conclusion

The patented process based on new wax is revealed as an effective deastringency treatment during the cold storage of persimmons 'Rojo Brillante' and 'Triumph'. Complete astringency removal is achieved in both varieties with no fruit quality loss after 30 storage days at 0 °C and after the shelf-life period that followed 15 and 21 storage days. Therefore, this method is an alternative to CO_2 treatment when prolonged cold storage is required, as on shipments to far overseas markets.

This study corroborates that applying CO_2 deastringency treatment to persimmon implies an important quality loss risk associated with flesh browning manifestation after prolonged cold storage.

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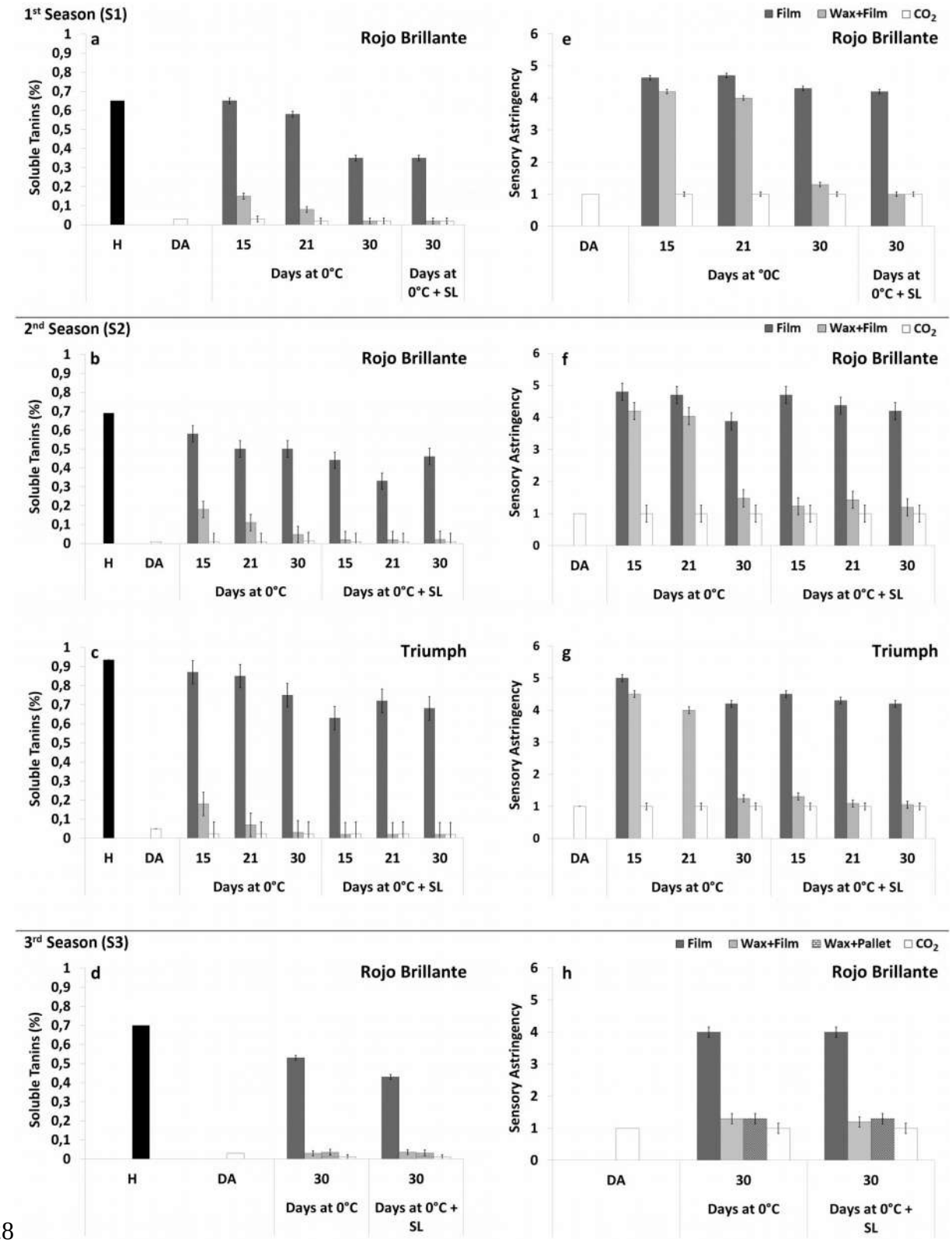
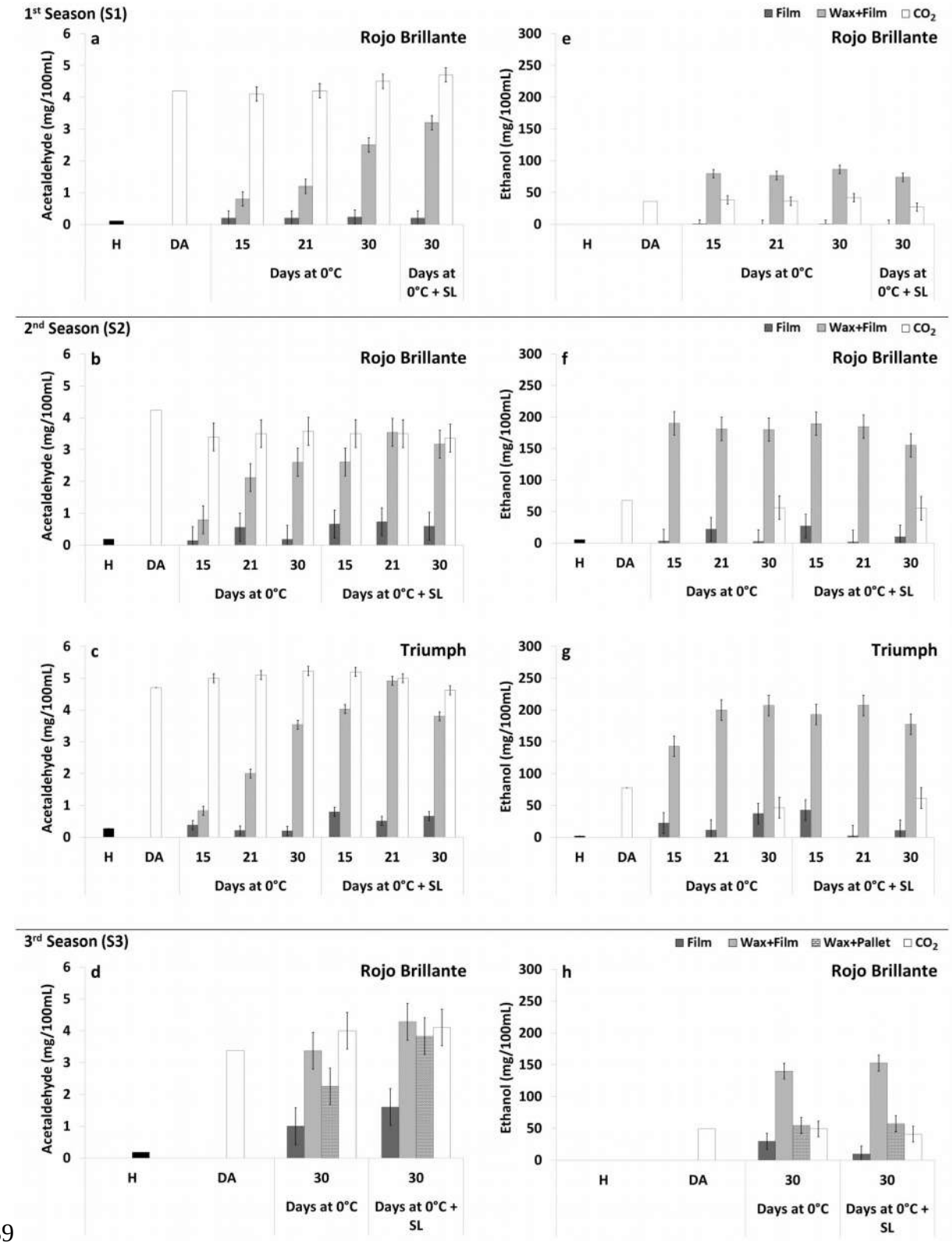


528

529**Fig. 1** Soluble tannins content (a, b, c, d) and sensory astringency evaluation (e, f, g, h) of 530persimmons ‘Rojo Brillante’ and ‘Triumph’ at harvest (H) during storage at 0°C and after the 531subsequent 5-day shelf-life period at 20°C in season 1 (S1), season 2 (S2) and season 3 (S3). 532Vertical bars represent LSD intervals ($p \leq 0.05$). Sensory astringency evaluation was from 1

533= no astringency to 5 = astringency; a value below 1.5 is considered commercially
534acceptable.

535Film: fruit packed in plastic film without wax. Wax+Film: fruit waxed and packed in plastic
536film. CO_2: fruit submitted to CO_2 treatment. Wax+Pallet: fruit waxed and boxes stacked on a
537wooden pallet which was strapped with macroperforated stretch film. DA: 1 day after CO_2
538treatment.



539

540**Fig. 2** Acetaldehyde (a, b, c, d) and ethanol (e, f, g, h) (mg/100 mL) of persimmons 'Rojo
541Brillante' and 'Triumph' at harvest (H), during storage at 0°C and after the subsequent 5-day
542shelf-life period at 20°C in season 1 (S1), season 2 (S2) and season 3 (S3). Vertical bars
543represent LSD intervals ($p \leq 0.05$).

Film: fruit packed in plastic film without wax. Wax+Film: fruit waxed and packed in plastic film. CO_2: fruit submitted to CO_2 treatment. Wax+Pallet: fruit waxed and boxes stacked on a wooden pallet, which was strapped with macroperforated stretch film. DA: 1 day after CO_2 treatment.

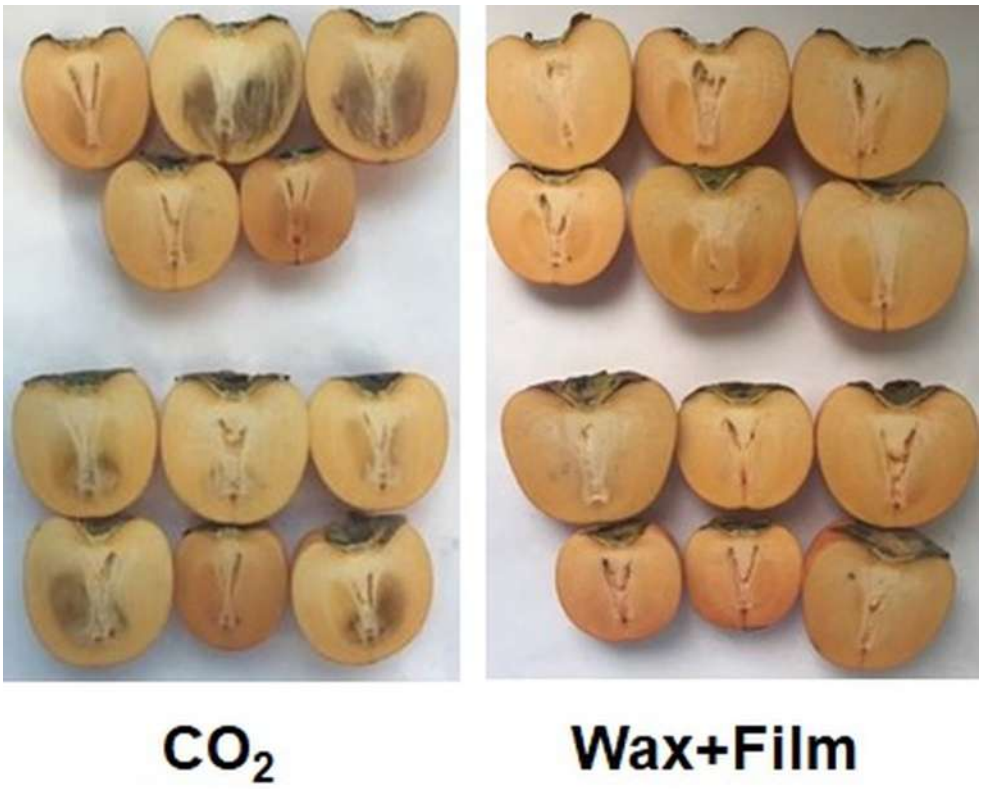


Fig. 3 Internal quality of the 'Rojo Brillante' fruit treated with either CO_2 or the new wax after 30 days of cold storage, plus the 5-day shelf-life at 20 ºC

Table 1

Treatments and conditions tested for ‘Rojo Brillante’ and Triumph’ in the three season studied

Season	Treatments	Cultivar	Cold storage (CS) (0 ºC)			Shelf-life (5d at 20 ºC after CS)		
			15d	21d	30d	15d	21d	30d
S1	CO_2 Wax+Film Film	Rojo Brillante	X	X	X	-	-	X
S2	CO_2 Wax+Film Film	Rojo Brillante Triumph	X	X	X	X	X	X
S3	CO_2 Wax+Film Film Wax+Pallet	Rojo Brillante	-	-	X	-	-	X

Table 2

EtOH (μL/L) and CO_2 (%) of persimmons 'Rojo Brillante' and 'Triumph' during storage at 0 °C in season 1 (S1), season 2 (S2) and season 3 (S3).

*Different letters indicate statistical differences ($p \leq 0.05$).

Film: fruit packed in plastic film without wax. Wax+Film: fruit waxed and packed in plastic film

		Days at 0 °C							
Season (Cultivar)	Treatment	1d		15d		21d		30d	
		EtOH	CO_2	EtOH	CO_2	EtOH	CO_2	EtOH	CO_2
S1	Film	-	-	0a*	0.2b	0a	0.6b	0	0a
(Rojo Brillante)	Wax+Film	-	-	0.02b	0.4b	0.02b	0.7b	0.02b	0.01a
S2	Film	0a	0.63b	0.01a	0.32a	0.01a	0.69b	0.01a	0.36a
(Rojo Brillante)	Wax+Film	0.67d	0.6b	0.08c	0.66b	0.04b	0.57b	0.02a	0.53b
S2	Film	0a	0.36a	0.01a	0.72b	0.01a	0.72b	0.01a	0.42a
(Triumph)	Wax+Film	0.47c	0.49a	0.04b	0.84b	0.04b	0.84b	0.02a	0.51a
S3	Film	-	-	-	-	-	-	0a	-
(Rojo Brillante)	Wax+Film	-	-	-	-	-	-	0.02a	-