agronomy

MDPI

Communication

Structural Changes Caused by CO_2 or Ethanol Deastringency Treatments in Cold-Stored 'Giombo' Persimmon

Nariane Q. Vilhena [1], Magda Andréia Tessmer [2], Isabel Hernando [3], Ricardo Alfredo Kluge [2], Amparo Quiles [3] and Alejandra Salvador [1,*]

[1] Instituto Valenciano de Investigaciones Agrarias, Postharvest Department, 46113 Valencia, Spain
[2] ESALQ, Department of Biological Sciences, University of São Paulo, Piracicaba 13418-900, SP, Brazil
[3] Food Technology Department, Universitat Politècnica de València, 46022 Valencia, Spain
* Correspondence: salvador_ale@gva.es

Abstract: Persimmon cv. Giombo is astringent at harvest and must be subjected to astringency removal treatment. To date, the most widespread treatment for this variety involves applying ethanol instead of high CO_2 concentrations, which is the usual treatment with other varieties. This study aims to evaluate the effect of high CO_2 or ethanol concentrations as deastringency treatments on the quality and flesh structure of 'Giombo' persimmon during cold storage. The deastringency process was faster in the fruit treated with CO_2 than with ethanol. One day after treatment, the CO_2-treated fruit showed lower soluble tannin levels than those detected sensorially for this variety, while with the ethanol-treated fruit, these values were obtained after 25 storage days plus the shelf-life period. The tannin insolubilisation process was observed by light microscopy. Loss of flesh firmness during storage was more pronounced when fruit were previously treated with ethanol than with CO_2. This is closely related to greater parenchyma degradation during storage caused by ethanol treatment, which was observed by a microstructural study by cryo-scanning electron microscopy. Therefore, as deastringency treatment for 'Giombo', applying CO_2 instead of ethanol treatment is recommended for better fruit quality, especially when fruit are to be cold-stored.

Keywords: fruit anatomy; astringency; *Diospyros kaki* Thunb.; CO_2; deastringency

Citation: Vilhena, N.Q.; Tessmer, M.A.; Hernando, I.; Kluge, R.A.; Quiles, A.; Salvador, A. Structural Changes Caused by CO_2 or Ethanol Deastringency Treatments in Cold-Stored 'Giombo' Persimmon. *Agronomy* **2022**, *12*, 2464. https://doi.org/10.3390/agronomy12102464

Academic Editors: Giorgia Liguori and Noemi Tel-Zur

Received: 15 September 2022
Accepted: 8 October 2022
Published: 11 October 2022

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) cv. Giombo is an astringent cultivar that is produced mostly in Brazil. Nowadays, this country is the fourth persimmon world producer with a planted area of 8148 ha and a production up to 157,000 tons/year [1,2]. This cultivar belongs to the pollination-variant and astringent (PVA) group, which develop by parthenocarpy and is completely seedless [3]. Like all PVA cultivars, 'Giombo' fruit contain high levels of soluble tannins (ST), which are responsible for fruit astringency, and thus, applying deastringency postharvest treatment is necessary for fruit to be marketed with high firmness [4,5].

Astringency in persimmon is promoted by condensed tannins, or proanthocyanidins, which are the phenolic oligomers that result from the polymerisation of flavan-3-ol units [6,7] and accumulate in the vacuole of the tannic cells present in fruit parenchyma. Several methods have been used to remove persimmon astringency [8,9]. These treatments stimulate the accumulation of volatile compounds in fruit flesh, such as ethanol and acetaldehyde, while triggering fruit anaerobic respiration. These substances induce ST to polymerise and become insoluble, which is a process that results in loss of astringency [10,11]. One of the more commercial treatments involves employing 95% CO_2 for 24 h at 20 °C.

However, in some producing countries such as Brazil, deastringency treatment by exposing persimmon to ethanol vapour is the most widespread practice because it is a less costly alternative with good results. This treatment is usually applied in a concentration

around 1.70 mL per kg of fruit for 24 h at room temperature [11–13]; nevertheless, these conditions can vary among producers. It was observed for 'Giombo' that changes in ethanol concentration, duration of the treatment and temperature of the chamber can highly influence the treatment result, affecting fruit final quality [12,14,15].

'Giombo' maturation is later, which is an advantageous aspect to prolong the persimmon season until cold storage as well as for being a potential variety to be commercialised in other countries when no local fruit is available. Nevertheless, as with other cultivars, 'Giombo' is sensitive to cold storage and develops chilling injury when submitted to low temperatures for a long time. The main chilling injury symptom is flesh softening, which occurs when fruit are transferred from cold storage to shelf-life conditions [5].

The softening that takes place during storage can be accelerated by the previous deastringency treatment. It is known that high CO_2 concentrations lead to cell wall degradation, which may be associated with firmness loss after treatment application [16]. Likewise, deastringency with ethanol can be accompanied by reduced firmness during the postharvest life [13].

To date, no studies have compared the effect of CO_2 and ethanol deastringency treatment on the flesh structural degradation that occurs during the cold storage of 'Giombo' persimmon. Hence, in the present paper, a microstructure study was carried out to evaluate the effect of high CO_2 and ethanol concentrations as deastringency treatments on 'Giombo' persimmon quality during cold storage.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Persimmon cv. Giombo were harvested in the commercial stage from the experimental germplasm bank of ANECOOP Cooperative in Valencia (Spain) and transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA), where they were carefully selected for uniformity of size, colour and lack of defects.

Fruit were divided into 15 lots of 15 fruit and placed in commercial plastic crates. One lot was characterised at harvest. Half the lots were submitted to CO_2 treatment (CO_2 95%, 20 °C, 90% RH, 24 h) and the other half were submitted to ethanol treatment (ethanol 1.70 mL kg^{-1}, 20 °C, 24 h). One lot per treatment was analysed 1 day after the deastringency process. After deastringency treatments, fruit were stored at 1 °C up to 40 days. One lot of each treatment was evaluated after 15, 25 or 40 cold storage days. At each time point of cold storage, one lot per treatment was transferred to 20 °C and evaluated after 5 days to simulate a shelf-life period.

The following measurements were taken: external colour, firmness, total soluble solids (TSS), sensory evaluation. Fruit samples were frozen to determine soluble tannins (ST) content. Microstructural analyses of flesh were performed by light microscopy (LM) and cryo-scanning electron microscopy (Cryo-SEM) techniques.

2.2. Skin Colour and Flesh Firmness

Fruit skin colour was evaluated by a Minolta Colorimeter (Model CR-300, Ramsey, NY, USA). The L, a, b Hunter parameters were measured on the skin of each fruit on two opposite zones, and the results were expressed as external colour index (CI = 1000 a/Lb) [16]. Flesh firmness was determined by a Texturometer Instron Universal Machine, model 4301 (Instron Corp., Canton, MA, USA) using an 8 mm flat plunger. Fruit firmness values were taken from 15 fruit per lot on opposite sides. The results were expressed as the force in Newtons (N) required to break flesh after removing peel.

2.3. Total Soluble Solids and Soluble Tannins

Fruit were longitudinally cut into four. Two opposite quarters were used to determine total soluble solids and the other two were used to collect samples, which were frozen at −20 °C to later analyse the ST content. To determine total soluble solids, fruit juice was

extracted with an electric juice extractor and filtered. Measurements were recorded by a refractometer (Atagomod. PR1), and the results were expressed as °Brix.

The ST concentrations were determined using the Folin–Ciocalteu reagent according to the method of Taira and Ono [17]. For quantification purposes, the following were used: 7.5 mL of distilled water, 1 mL aliquot of extract, 0.5 mL of Folin–Ciocalteu reagent (50%) and 1 mL of supersaturated sodium carbonate solution. The absorbance of the resulting solution was measured at 725 nm by a spectrophotometer (Thermo Scientific Multiskan® Spectrum, Thermo Fisher Scientific Oy, Vantaa, Finland). The results were expressed as a percentage of dry weight (% DW).

2.4. Astringency Sensory Analysis

Fruit's astringency level was sensory evaluated by a semitrained panel made up of 6–8 people who were familiar with persimmon astringency. A 4-point scale was used, where 1 = no astringency and 4 = intense astringency. Samples were presented to panel members on trays labelled with random 3-digit codes and served at room temperature (25 °C ± 1 °C). Milk was provided for palate-rinsing between samples.

2.5. Microstructural Study

The microstructural study was performed with two fruit per replicate. For the LM analysis, tissue sections were taken from the equatorial portion of fresh fruit using a stainless blade. Sections were placed on histological slides and stained with vanillin-HCl (1:1, v/v) to identify tannins [18]. Tannins react with hydrochloric vanillin to give a red colour. Cutting promotes the extravasate of tannins from tannin cells when they come in a soluble form. Images were taken by LM (Nikon Eclipse E800 V-PS100E, Tokyo, Japan).

For the Cryo-SEM analyses, cubes (3 mm^3) were cut from the equatorial area perpendicularly to the main axis of the persimmon flesh with a stainless-steel cutter. Cubes were then immersed in slush nitrogen (−210 °C) and transferred to a cryo-trans (CT 15,000 C from Oxford Instruments, Oxford, UK), linked with a JEOLJSM 5410 scanning electron microscope (JEOL, Tokyo, Japan), which operated at a temperature below −130 °C. Samples were cryofractured at −180 °C and etched at −90 °C. Observations under the microscope were made at 15 kV and at a working distance of 15 mm.

2.6. Statistical Analysis

Data were subjected to analyses of variance (ANOVA) after testing normality with a Komolgorov–Smirnov test and homoscedasticity with Levene's test. The multiple comparisons between means were determined using the least significant difference ($p \leq 0.05$) with the Statgraphics Centurion XVII.I software application (Manugistics Inc., Rockville, MD, USA).

3. Results and Discussion

'Giombo' is an astringent variety with a high ST content, which make applying astringency removal treatment before marketing necessary. In the present study, ST came close to 1.07% at harvest. One day after deastringency treatment with high CO_2 concentrations, the tannin content drastically dropped to values of 0.08% (Figure 1). However, this decrease in the ethanol-treated fruit was less marked with values of 0.8%. The sensorial evaluation revealed that the astringency of the CO_2 fruit was undetectable (sensorial value = 1), while the ethanol-treated fruit were qualified by the sensory panel as astringent (sensorial value = 3.2) (data not shown).

During cold storage and the following shelf-life periods, the CO_2-treated fruit had ST values between 0.07% and 0.05%, while the ST content of the ethanol-treated fruit gradually lowered throughout storage. During this treatment, ST dropped to values of 0.4% and 0.2% after 15 cold storage days and the posterior shelf life, respectively. At these times, the sensorial panel detected slight-medium astringency (sensorial values between 1.8 and 2.4). After 25 days at 1 °C, the fruit's ST content was 0.13% and "residual astringency" was detected. Only after the subsequent shelf life were fruit evaluated by panellists as "absence

of astringency" with ST values between 0.07% and 0.06%. No changes were observed after 40 days.

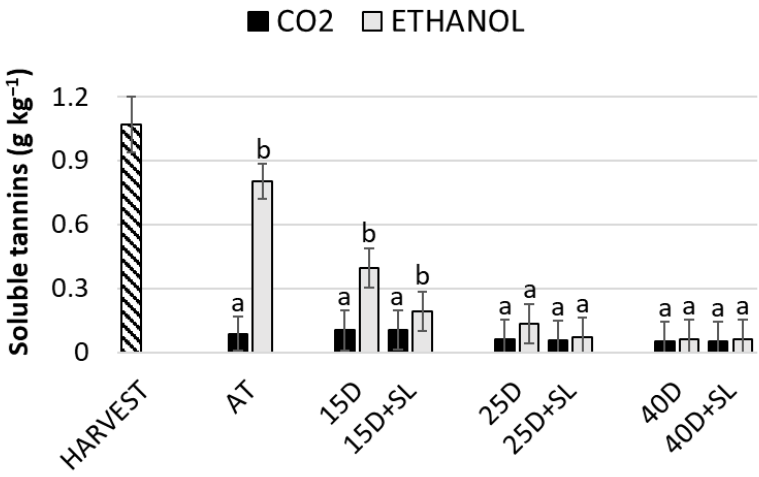

Figure 1. Changes in the soluble tannins of the 'Giombo' persimmon treated with CO_2 or ethanol during cold storage (1 °C) and after shelf-life simulation (5 more days at 20 °C). AT: One day after deastringency treatment, D: Days of cold storage; SL: Shelf-life simulation. The vertical bar represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction treatment–storage days). Different letters above bars indicate significant differences among treatments at each moment of evaluation ($p \leq 0.05$).

According to Antoniolli et al. [12] and Tessmer et al. [13], the ST content in 'Giombo' should be below 0.1% so as not to detect astringency. Likewise, in non-astringent cultivars, tannin concentrations have been reported to be below 0.1% at harvest [19]. Nevertheless, it should be noted that the level of tannins whose astringency is not sensorially detectable depends on cultivars. For example, for the astringent cultivar 'Rojo Brillante', the tannin content must be below 0.04% to guarantee non-astringent fruit [16,20,21].

By means of LM and vanillin hydrochloric staining, tannic cells were observed on persimmon flesh. At harvest, tannins had extravasated from the cell vacuole, and they were dispersed throughout flesh and deposited on the whole section with an intense red colour (Figure 2A). One day after CO_2 treatment, most tannins remained inside tannic cells, which indicates the polymerisation of ST (Figure 2B) to non-astringency levels (see Figure 1).

Nevertheless, 1 day after ethanol treatment, a large region with content extravasation was observed (Figure 2C), which is in accordance with the still high ST concentration observed at that time, as shown in Figure 1. After 15 storage days plus shelf life, the ethanol-treated fruit presented lower dispersed content with material accumulation closer to tannic cells (Figure 2D). This finding corresponds to the slight-medium astringency noted with this fruit. After 25 days plus shelf life, tissue presented greater delimitation of the tannic cells dispersed in the degraded parenchyma. Tannic cells were elongated, and content was completely insolubilised with intact walls, which indicate a complete loss of astringency at 40 days plus shelf life (Figure 2E,F).

In both 'Giombo' and 'Rojo Brillante', the tannin insolubilisation process inside tannin cells during fruit maturation has been previously observed by LM [19].

Flesh firmness is the most important quality attribute to be taken into account after submitting persimmon to deastringency treatment [22]. It is also an essential parameter to be maintained during fruit storage and commercialisation.

At harvest, fruit flesh firmness was 35.6 N (Figure 3A). After 15 storage days, fruit firmness obtained an average of 28 N with non-significant differences between the fruit submitted to both deastringency treatments. After the posterior shelf life, this value lowered to 17 N and 13.7 N in the fruit treated with CO_2 and ethanol, respectively. After 25 cold storage days, the CO_2-treated fruit had a firmness of 28 N, but that of the ethanol-treated

fruit was 21.4 N. After shelf life, the CO_2 fruit had lower values of 13.4 N, while the ethanol-treated fruit values dropped to 8.7 N, which is a value that is below the commercial limit for persimmon [23]. After 40 cold storage days, the CO_2-treated fruit firmness was still higher than 15 N, but values came close to 7 N for the ethanol-treated fruit, and the posterior shelf-life period gave a firmness of 5.6 N with no differences between treatments.

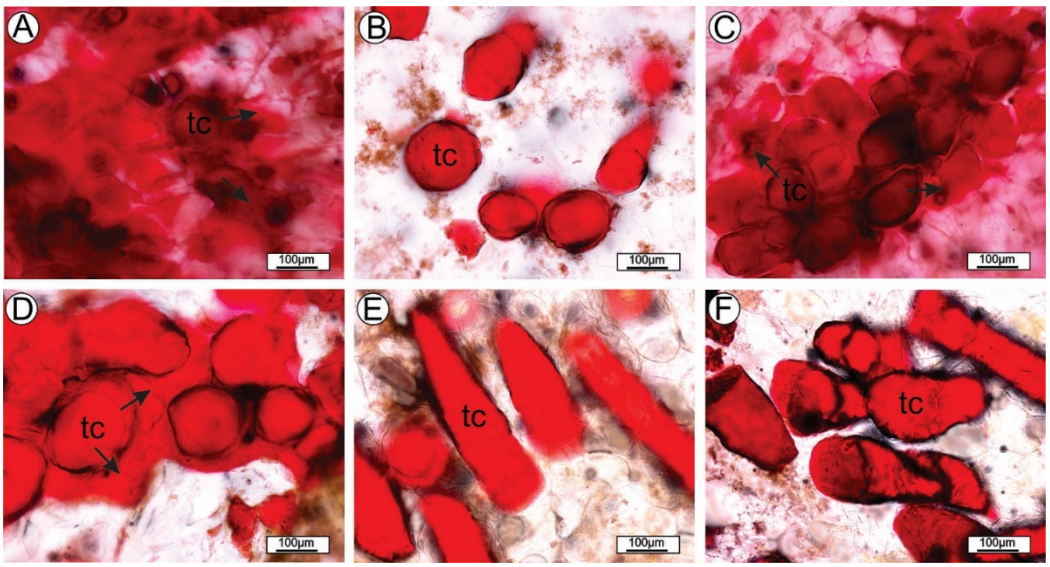

Figure 2. Tissue sections of 'Giombo' persimmon flesh. (**A**) Harvest, (**B**) 1 day after CO_2 treatment, (**C**) 1 day after ethanol treatment, (**D**) Ethanol-treated fruit after 15 d at 1 °C + 5 d at 20 °C, (**E**) Ethanol-treated fruit after 25 d at 1 °C + 5 d at 20 °C; (**F**) Ethanol-treated fruit after 40 d at 1 °C + 5 d at 20 °C. Cells with extravasated tannin content (**A**,**C**,**D**), tannins accumulate near tannic cells (**B**) and tannic cells intact dispersedly in the degraded parenchyma (**E**,**F**). tc: tannic cell.

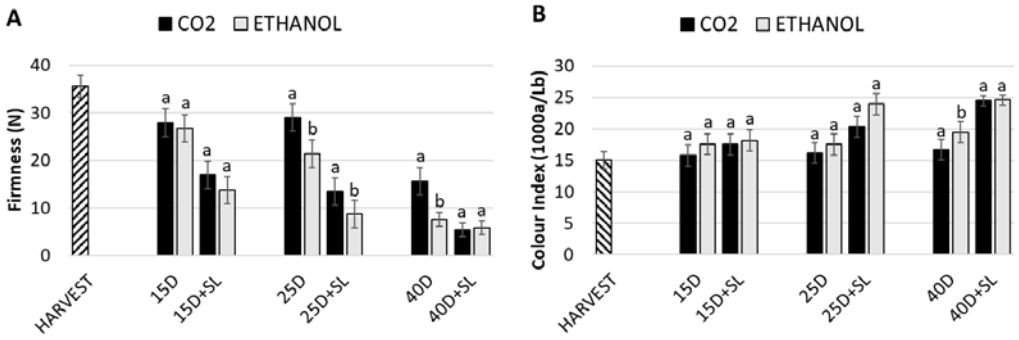

Figure 3. Changes in the (**A**) firmness and (**B**) external colour of the 'Giombo' persimmon treated with CO_2 or ethanol after cold storage (1 °C) and after shelf-life simulation (5 more days at 20 °C). D: Days of cold storage; SL: Shelf-life simulation. The vertical bar represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction treatment–storage days). Different letters above bars indicate significant differences between treatments at each moment of evaluation ($p \leq 0.05$).

A previous study conducted with Japanese and Chinese varieties noted that the flesh softening caused by deastringency treatment was also more pronounced in the fruit treated with ethanol than in those treated with CO_2 [24]. A sharp decrease in flesh firmness was observed when fruit were transferred from 1 °C to shelf-life conditions regardless of the previous deastringency treatment, which is associated with a chilling injury (CI) symptom reported for persimmon fruit [22].

A strong negative correlation between external colour and flesh firmness during fruit maturation has been reported for some persimmon varieties [16,19]. At harvest, fruit had a colour index of 15, with an increase noted during storage (Figure 3B). After 15 storage

days, the values slightly increased with values close to 17 but with no large differences between treatments. Only after 25 days at 1 °C plus 5 days at 20 °C did the CO_2-treated fruit obtain lower CI values than those treated with ethanol, with a colour index of 20.3 and 24 for the treatments with CO_2 and ethanol, respectively. After 40 storage days, there was no difference between the CO_2- and ethanol-treated fruit whose average colour was 24.5 after the last shelf-life period.

The flesh firmness loss during storage and after deastringency treatments was closely associated with microstructural parenchyma degradation. After 15 days at 1 °C, the fruit from both treatments displayed quite a structured parenchyma, with rounded cells and small air-filled intercellular spaces (Figure 4A,B,E,F). Cells had a compact mass, which indicates the presence of insoluble tannins inside their vacuoles as a consequence of the deastringency process. Nevertheless, in the fruit from the CO_2 treatment (Figure 4A,B), more cells with insolubilised material were observed compared to the ethanol-treated fruit (Figure 4E,F). After the shelf-life period, slight cell compaction loss was observed in the CO_2-treated fruit, and more changes were detected in the flesh structure of the ethanol-treated fruit (Figure 4C,D,G,H). The parenchyma appeared more deteriorated with intercellular spaces invaded by soluble material. The cell membrane was degraded in some cells.

After 25 cold storage days, no major changes were observed in the structure of the CO_2-treated fruit (Figure 4I,J) compared to that observed after 15 days. However, the flesh of the ethanol-treated fruit was more degraded with deformed cells, and there was also clear cell–cell separation in some areas (Figure 4M,N). Flesh degradation was more evident when fruit flesh firmness diminished after the subsequent shelf-life period. Notwithstanding, differences between the CO_2- and the ethanol-treated fruit were found. The flesh integrity of the CO_2-treated fruit was preserved, although some cells were deformed and the spaces between them became bigger (Figure 4K,L). The parenchyma of the ethanol-treated fruit was more deteriorated, and the membranes in some areas were hard to distinguish in the collapsed tissue (Figure 4O,P).

After 40 cold storage days, the flesh structure degraded in the fruit from both treatments, but this effect was stronger in the ethanol-treated fruit (Figure 4Q,R,U,V). More drastic changes occurred after the shelf-life period. The typical cell structure was lost, and the parenchyma was very deteriorated (Figure 4S,T,X,Z). Most cells had lost their integrity, and the cell membrane was indistinguishable in compacted tissue. Although these changes were observed in the fruit from both treatments, they were more evident in the fruit treated with ethanol.

In relation to soluble solids, fruit showed 21.4 °Brix at harvest, which slightly lowered after the astringency removal and subsequent storage periods to values between 18 and 19.2 °Brix. No major differences between treatments were found (data not shown). This reduction may be influenced by the insolubilisation of soluble tannins, which can be quantified together with soluble solids as reported in previous studies [16].

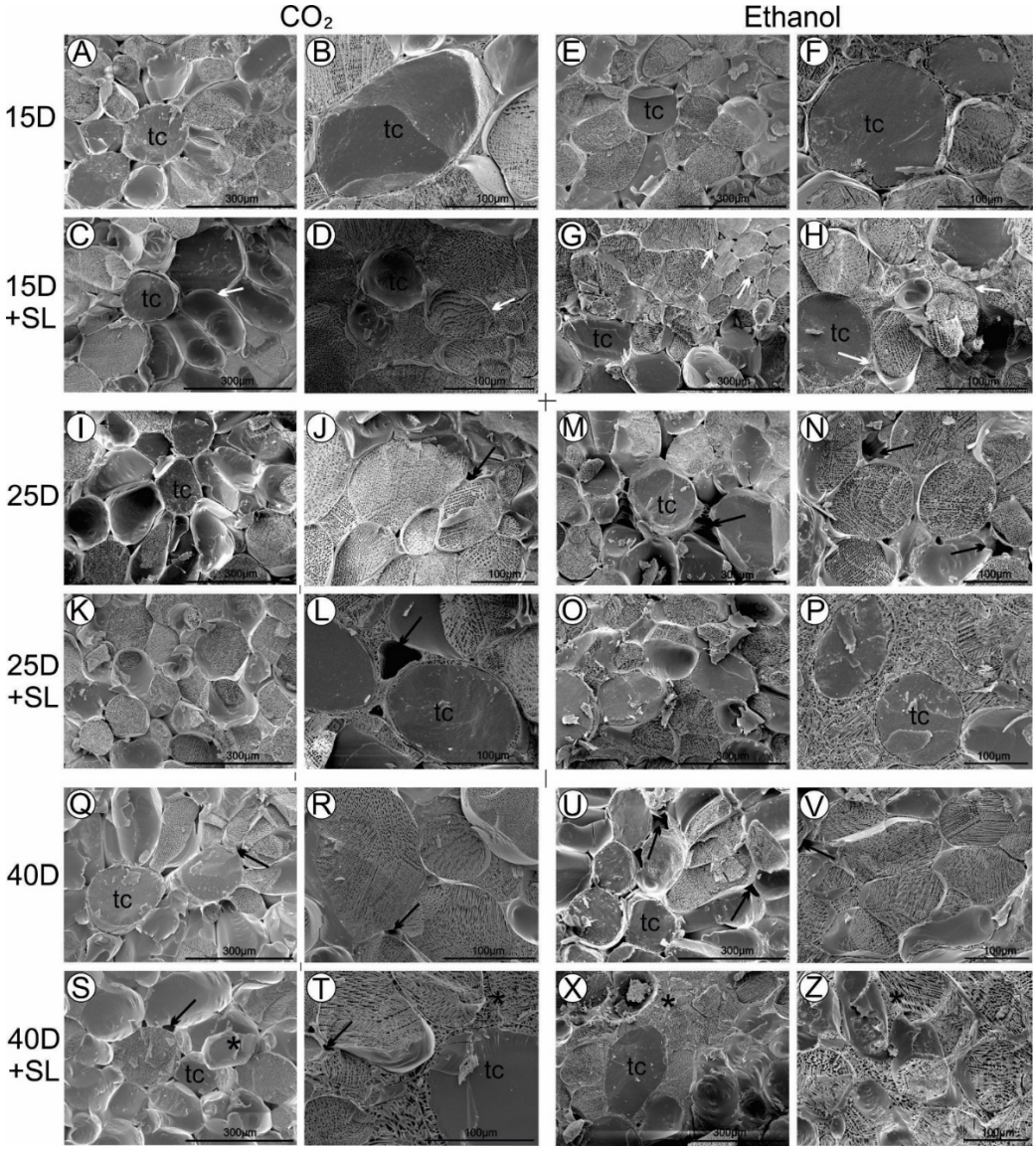

Figure 4. The 'Giombo' persimmon images obtained by cryo-scanning electron microscopy (Cryo-SEM) throughout storage after treatment with CO_2 (**A**–**D**,**I**–**L**,**Q**–**T**) or ethanol (**E**–**H**,**M**–**P**,**U**,**V**,**X**,**Z**). Black arrow: cell separation and spaces formation. White arrow: soluble material in intercellular spaces. TC: tannin cell. *: cell wall degradation. Vertical columns: Deastringency treatments. Horizontal columns: Days (D) of storage at 0 °C or Days of storage at 0 °C plus shelf life (SL) (5 days at 20 °C).

4. Conclusions

This work shows in 'Giombo' persimmon that deastringency with high CO_2 concentrations results in faster tannins insolubilisation than with ethanol. In addition, the parenchyma degradation during cold storage and the subsequent shelf life is more severe in ethanol-treated fruit, resulting in a faster firmness loss than in CO_2-treated fruit. Therefore, fruit treated with CO_2 maintained high firmness after 25 storage days plus shelf life while ethanol-treated fruit, after 15 days, did not show marketable firmness. These results suggest that in the case of Giombo, although ethanol is the commonplace deastringency treatment, it is recommended to introduce a treatment with high CO_2 concentrations to obtain better fruit quality, especially when fruit are to be cold stored.

Author Contributions: Conceptualization, M.A.T. and A.S.; methodology, I.H., A.Q. and A.S.; formal analysis, M.A.T.; investigation, M.A.T. and A.S.; resources, R.A.K. and A.S.; data curation, N.Q.V. and M.A.T.; writing—original draft preparation, N.Q.V. and M.A.T.; writing—review and editing, N.Q.V., M.A.T. and A.S.; supervision, I.H., R.A.K., A.Q. and A.S.; project administration, A.S.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish Ministerio de Economía y Competitividad (Project RTA2017-00045-C02) and the Fondo Europeo de Desarrollo Regional (FEDER).

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the Agrupación Nacional de Exportación de Cooperativas Citrícolas (ANECOOP) for supplying the persimmon cultivar fruit herein used. N.Q.V. thanks the Spanish Ministry of Science and Innovation for Grant FPI-INIA (PRE2018-085833).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Andrioli, J.; Scariot, F.J.; Delamare, A.P.L.; Echeverrigaray, S. Morphological characterization and molecular identification of colletotrichum species associated to sweet persimmon anthracnose in Southern Brazil. *Cienc. Rural* **2021**, *51*, 1–10. [CrossRef]
2. FAOSTAT. Available online: http://www.fao.org/faostat/en/#data (accessed on 13 September 2022).
3. Kluge, R.A.; Tessmer, M.A. Caqui—Diospyros kaki. In *Exotic Fruits*; Rodrigues, S., de Oliveira Silva, E., de Brito, E.S., Eds.; Academic Press: London, UK, 2018; pp. 113–119.
4. Tessmer, M.A.; Kluge, R.A.; Appezzato-Da-Glória, B. The accumulation of tannins during the development of "Giombo" and "Fuyu" persimmon fruits. *Sci. Hortic.* **2014**, *172*, 292–299. [CrossRef]
5. Tessmer, M.A.; Appezzato-da-Glória, B.; Kluge, R.A. Evaluation of storage temperatures to astringency 'Giombo' persimmon: Storage at 1 °C combined with 1-MCP is recommended to alleviate chilling injury. *Sci. Hortic.* **2019**, *257*, 108675. [CrossRef]
6. Akagi, T.; Ikegami, A.; Yonemori, K. DkMyb2 wound-induced transcription factor of persimmon (*Diospyros kaki* Thunb.), contributes to proanthocyanidin regulation. *Planta* **2010**, *232*, 1045–1059. [CrossRef] [PubMed]
7. Dixon, R.A.; Xie, D.Y.; Sharma, S.B. Proanthocyanidins—A final frontier in flavonoid research? *New Phytol.* **2005**, *165*, 9–28. [CrossRef] [PubMed]
8. Edagi, F.K.; Kluge, R.A. Remoção de adstringência de caqui: Um enfoque bioquímico, fisiológico e tecnológico. *Ciência Rural* **2009**, *39*, 585–594. [CrossRef]
9. Monteiro, M.F.; Aguila, J.S.D.; Pessoa, C.D.O.; Kluge, R.A. Vacuum Packaging is Efficient to Remove Astringency and to Maintain the Firmness of 'Giombo' Persimmon. *Rev. Bras. Frutic.* **2017**, *39*, 1–6. [CrossRef]
10. Arnal, L.; del Río, M.A. Effect of cold storage and removal astringency on quality of persimmon fruit (*Diospyros kaki*, L.) cv. Rojo brillante. *Food Sci. Technol. Int.* **2004**, *10*, 179–185. [CrossRef]
11. Vitti, D.C.C. Destanização e Armazenamento Refrigerado de Caqui 'Rama Forte' em Função da Época de Colheita. Ph.D. Thesis, Universidade de São Paulo, Piracicaba, Brazil, 2009.
12. Antoniolli, L.R.; Castro, P.R.D.C.E.; Kluge, R.A.; Scarpare Filho, J.A. Remoção da adstringência de frutos de caquizeiro "Giombo" sob diferentes períodos de exposição ao vapor de álcool etílico. *Pesqui Agropecuária Bras.* **2000**, *35*, 2083–2091. [CrossRef]
13. Tessmer, M.A.; Da Glória, B.A.; Kluge, R.A. Astringency reduction using ethanol-associated to the storing under refrigeration at 5 °C promotes physiological and structural alterations in "Giombo" persimmons. *Comun. Sci.* **2018**, *9*, 449–456. [CrossRef]
14. Antoniolli, L.R.; Castro, P.R.C.; Kluge, R.A.; Scarpare Filho, J.A. Astringency removal of 'Giombo' persimmon fruits submitted to different temperatures. *Pesquisa Agropecuária Brasileira* **2002**, *37*, 687–691. [CrossRef]
15. Chiou, D.G.; Kluge, R.A. Destanização de caquis cv. Giombo em diferentes concentrações de etanol e tempos de exposição. In *Simpósio Internacional de Iniciação Científica da Universidade de São Paulo*; USP: São Paulo, Brazil, 2006.
16. Salvador, A.; Arnal, L.; Besada, C.; Larrea, V.; Quiles, A.; Pérez-Munuera, I. Physiological and structural changes during ripening and deastringency treatment of persimmon fruit cv. *"Rojo Brillante"*. *Postharvest Biol. Technol.* **2007**, *46*, 181–188. [CrossRef]
17. Taira, S.; Ono, M. Reduction of astringency in persimmon caused by adhesion of tannins to cell wall fragments. In *I International Persimmon Symposium 436*; ISHS: Chang Mai City, Thailand, 1996; pp. 235–242.
18. Vázquez-Gutiérrez, J.L.; Quiles, A.; Hernando, I.; Pérez-Munuera, I. Changes in the microstructure and location of some bioactive compounds in persimmons treated by high hydrostatic pressure. *Postharvest Biol. Technol.* **2011**, *61*, 137–144. [CrossRef]
19. Tessmer, M.A.; Besada, C.; Hernando, I.; Appezzato-da-Glória, B.; Quiles, A.; Salvador, A. Microstructural changes while persimmon fruits mature and ripen. Comparison between astringent and non-astringent cultivars. *Postharvest Biol. Technol.* **2016**, *120*, 52–60. [CrossRef]
20. Besada, C.; Salvador, A.; Arnal, L.; Martínez-Jávega, J.M. Optimization of the duration of deastringency treatment depending on persimmon maturity. *Acta Hortic.* **2010**, *858*, 69–74. [CrossRef]
21. Munera, S.; Besada, C.; Blasco, J.; Cubero, S.; Salvador, A.; Talens, P.; Aleixos, N. Astringency assessment of persimmon by hyperspectral imaging. *Postharvest Biol. Technol.* **2017**, *125*, 35–41. [CrossRef]

22. Salvador, A.; Arnal, L.; Monterde, A.; Martínez-Jávega, J.M. Influence of ripening stage at harvest on chilling injury symptoms of persimmon cv. Rojo Brillante stored at different temperatures. *Food Sci. Technol. Int.* **2005**, *11*, 359–365. [CrossRef]
23. Besada, C.; Novillo, P.; Navarro, P.; Salvador, A. Effect of a low oxygen atmosphere combined with 1-MCP pretreatment on preserving the quality of "Rojo Brillante" and "Triumph" persimmon during cold storage. *Sci. Hortic.* **2014**, *179*, 51–58. [CrossRef]
24. Yamada, M.; Taira, S.; Ohtsuki, M.; Sato, A.; Iwanami, H.; Yakushiji, H.; Wang, R.; Yang, Y.; Li, G. Varietal differences in the ease of astringency removal by carbon dioxide gas and ethanol vapor treatments among oriental astringent persimmons of Japanese and Chinese origin. *Sci. Hortic.* **2002**, *94*, 63–72. [CrossRef]