

Astringency reduction using ethanol-associated to the storing under refrigeration at 5°C promotes physiological and structural alterations in 'Giombo' persimmons

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Abstract

This study aimed to study the effect of astringency reduction using ethanol and application of 1-MCP in 'Giombo' persimmons, storage in cold chamber at 5°C and to assess its physiological and structural alterations. After the deastringency treatment using ethanol, and the application of 1-methylcyclopropene (1-MCP), the fruits were stored at 5°C and 90% RH during 60 days and shelf life (SL) for 5 days at 22°C. The dosage of 1.70 ml/kg⁻¹ ethanol during 12 hours was efficient for the astringency removal in 'Giombo' persimmons. However, deastringency using ethanol reduces pulp firmness and 1-MCP preserves this characteristic both in detannized fruits and in controls. The increase of soluble pectins and solubilization did not foster the polymerization of the soluble tannins both in control and detannized fruits. 'Giombo' persimmons displayed a severe firmness reduction, increase of soluble pectins, and degradation of the parenchyma cells, gelling of the pulp, increase of the production of ethanol and acetaldehyde after 60 days of storing + 5 days SL, inclusively in control fruits, and those treated with 1-MCP. These results suggest, cultivar 'Giombo' is susceptible to damages at 5°C and that further studies are required using different temperatures to improve the storage.

Keywords: cold-storage, damages, soluble pectins, persimmons, tannins, temperature, 1-MCP

Introduction

The production of persimmons has grown in the last years because of the excellent acceptance of this fruit in the market. Today Brazil ranks as the fourth largest producer of persimmons in the world, with a production of 158,241 tons (Faostat, 2015).

The variety 'Giombo' belongs to the group of pollination variant (PVA), has a clear pulp, and astringency as the fruit develops for parthenocarpy (Campo-Dall'orto et al., 1996). From the commercial point of view, this variety has several advantages, because of its late harvest. Even the same, requires an astringency removal before of the consumption of the fruits

(Neuwald et al., 2009).

The astringency in persimmons is derived from the tannins accumulated in specialized cells of the mesocarp during the development of the fruits (Tessmer et al., 2014). These tannins are called condensed tannins or proanthocyanidins. They are classified as phenolic oligomers derived from the polymerization of flavan-3-ols units (Akagi et al., 2011; Akagi et al., 2009).

'Giombo' persimmons are commercialized and consumed after deastringency. This procedure is performed by the use of ethanol or the exposition to high CO₂ concentrations (Edagi et al., 2009; Salvador et al., 2007). Ethanol promotes the increase of the

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activity of the enzyme alcohol dehydrogenase, and the following production of acetaldehyde, which polymerizes the soluble tannins and minimizes the astringency (Edagi & Kluge, 2009).

The treatments with ethanol and the following production of acetaldehyde may affect the cellular structure, promote the degradation of the cell wall and affect the taste (Chung et al., 2015). The consequence of the is the acceleration of the reduction of pulp firmness, which shortens the shelf life of the fruits (Antoniolli et al., 2000).

In addition to the deastringency, it is necessary to provide the extension of the period of preservation of the fruits and preserve their quality. Refrigeration and 1-MCP are used for this purpose. 1-MCP blocks the site of action of the ethylene, delays the maturation of the fruits and the process of senescence (Pérez-Munuera & Hernando, 2009; Blankenship & Dole, 2003). On the other side, storing at low temperatures may cause damages associated with the cold in susceptible cultivars and promote symptoms as the gelling of the pulp (Besada et al., 2014).

As considering the information provided above, the aimed to verify the effect of astringency reduction using ethanol and application of 1-MCP in 'Giombo' persimmons and storage at 5°C and to assess its physiological and structural alterations.

Material and Methods

'Giombo' persimmons were harvested in a commercial orchard located in Mogi Das Cruzes (SP) (23° 31' S; 46° 11' W) at an altitude of 742m at April 2012. After the harvesting, the fruits were transported to the laboratory of Postharvest Physiology and Biochemistry ESALQ/USP. The fruits were strictly selected as refers to their size, color and mechanical damages.

The following treatments were performed: (T1) Control (without deastringency and without the application of 1-MCP); (T2) 1.000 ml l⁻¹ of 1-MCP during 12 hours; (T3) 1.70 ml kg⁻¹ of ethanol during 12 hours and (T4) 1.70 ml kg⁻¹ of ethanol during 12 hours and following treatment using 1.000 ml l⁻¹ of 1-MCP during 12 hours.

During the application of the treatments, fruits were packaged in leaked plastic boxes

and placed inside a hermetic box measuring 0.50 X 0.80 X 0.47m. Ethanol was placed in the inferior part of the box, hermetically sealed to provide the gaseous mixture and kept at 22°C. 1-MCP (SmartFresh®, Rohm and Hass Inc.) was used in the form of hidratable powder (0.14% of active principle). 1-MCP was added in a 10 mL sealed glass flask adding 5 ml of distilled water with a syringe and opened in the interior of the chamber to permit its volatilization.

After the treatments, the fruits were stored at 5°C + 90% RH. The fruits were taken out from this condition at 30 and 60 days and kept at 22°C for 5 days to simulate the shelf life (SL) at 22°C. The analyses were performed after the harvesting, and before the treatments (day zero), before the entrance into the chamber (soluble tannins) and after the removal of storage + SL.

Astringency index and soluble tannins content

Astringency index was determined by the method of Campo-Dall'orto et al. (1996). This method assesses the impression of one of the two parts of the fruits that have been cut in the equatorial region in a filter paper previously soaked with 5% Ferric Chloride (FeCl₃). The tannin, in its soluble form, reacts with the Ferric Chloride, becoming darker. The impressions were evaluated using the rating scale: 1= not tannic; 2= slightly tannic; 3= moderately tannic; 4= tannic; 5 very tannic.

Soluble tannins content were assessed by spectrophotometry, using the Follin-Ciocalteu (50%) reagent, according to the Taira (1995) technique. The extract for soluble tannins was prepared with 1 g of minced pulp and centrifuged adding 80% methanol, filtered, and adjusted with distilled water until 100 ml. From this solution, a 1 ml aliquot was withdrawn. To this aliquot was added the Follin-Ciocalteu (50%), reagent and 1 ml of a supersaturated solution of sodium carbonate, and 7.5ml of distilled water. Reading was performed in a Biochrom Libra S22 Spectrophotometer, with absorbance at 725 nm. The results were expressed as g 100 g⁻¹.

Histochemical test with hydrochloric vanillin

Fresh tissue sections from the equatorial region of the fruits were performed using a

stainless blade. Fruit sections were placed on histological slides, tainted with vanillin in 10% chloride acid to localize the tannins (Vázquez-Gutiérrez et al., 2011), and covered with the coverslip. Pictures were taken using a Leica DM LB microscope coupled with the Leica DC 300 F digital camera and computer processed for the image editing.

Ethanol and acetaldehyde production

Ethanol and acetaldehyde concentration was assessed according to Davis & Chace (1969). 1 g of pulp was sealed in a 40 ml glass vial and stored at -26°C until the analysis. The vials were placed in bain-Marie during 30 minutes at 50°C. After this, 1 ml of air from any vial was sampled and injected in the gas chromatograph ThermoFinnigan Thermoquest GC Trace 2000 with flame ionization detector (FID) with Porapak N column. The results were expressed as $\mu\text{g g}^{-1}$.

Pulp firmness

Pulp firmness was assessed using a digital penetrometer Sammar 85261.0472 TR, equipped with an 8 mm diameter tip. The readings of the firmness were performed twice in each fruit, on the opposite side of the equatorial region of the fruit, after the removal of the peel. The results were expressed as Newtons (N).

Pectins

The extract for total and soluble pectins was prepared using 2 g of frozen fruit pulp, and its determination was performed according to Bitter & Muir (1962). According to this technique is used 1 ml of the extract, 3.6 ml of H_2SO_4 solution, containing 0.0125M of sodium borate and 60 μl of 3-phenylphenol (90%). The readings were performed in a spectrophotometer at 520 nm wavelength and compared to a standard curve of the galacturonic acid. The results were expressed as $\text{mg } 100 \text{ g}^{-1}$.

Statistical analysis

The experimental design was completely randomized with five repetitions of five fruits each treatment. Data were submitted to the analysis of variance (ANOVA). Means were compared by the Tukey test ($P < 0.05$) using the program SASM-Agri.

Results and Discussion

Astringency and structure of the tannins during refrigerated storage

The dosage of 1.70 ml kg^{-1} of ethanol during 12 hours was sufficient for the astringency removal in 'Giombo' persimmons, in agreement with Edagi et al. (2009) and Monteiro et al. (2012). We observed a significant reduction of the soluble tannins of $0.085 \text{ g } 100\text{g}^{-1}$ in the fruits treated with ethanol and $0.185 \text{ g } 100\text{g}^{-1}$ in the fruits treated with ethanol and 1-MCP after the treatments + 1 day at 22°C and before the entrance in the AR. During the period of AR at 5°C, the fruits with the astringency reduced, both those treated with ethanol and ethanol + 1-MCP had their soluble tannins content cut to about $0.050 \text{ g } 100\text{g}^{-1}$ and astringency index note 1.0 at 30 and 60 days of storage + 5 days of SL (Figures 1A and B).

In the control fruits, even the significant reduction of the soluble tannins content and astringency index at 30, 60 days in storage + 5 days SL, the results were not yet acceptable for human consumption (Figures 1A and B). The persistence of the astringency in these fruits may be associated with the low storage temperature and, in the case of 1-MCP, to the delay in the ripening. Terra et al. (2014) also described that, as 1-MCP is applied alone, it delays the astringency reduction of 'Giombo' persimmons during the storage.

Structural analyses confirmed the qualitative results of the tannins. Before applying the treatments, mesocarp sections submitted to the hydrochloric vanillin test showed intense red color, which indicates the extruded of soluble tannins in the tissue (Figure 2A). Even after 30 days at 5°C + 5 days of SL, in not-deastringency fruit and those treated with 1-MCP, soluble tannins extruded from the vacuole, suggesting the persistence of non-polymerized tannins (Figures 2B and C). The extruded of the tannins was similar to what we observed after 60 days at 5°C + 5 days of SL. On the other side, deastringency fruit, the content of tannin was limited to the vacuole of the cells, indicating its complete polymerization (Figure 2D and I).

As considering the loss of parenchyma structure, even if the sections were transversal to the pericarp, most of the tannin cells were

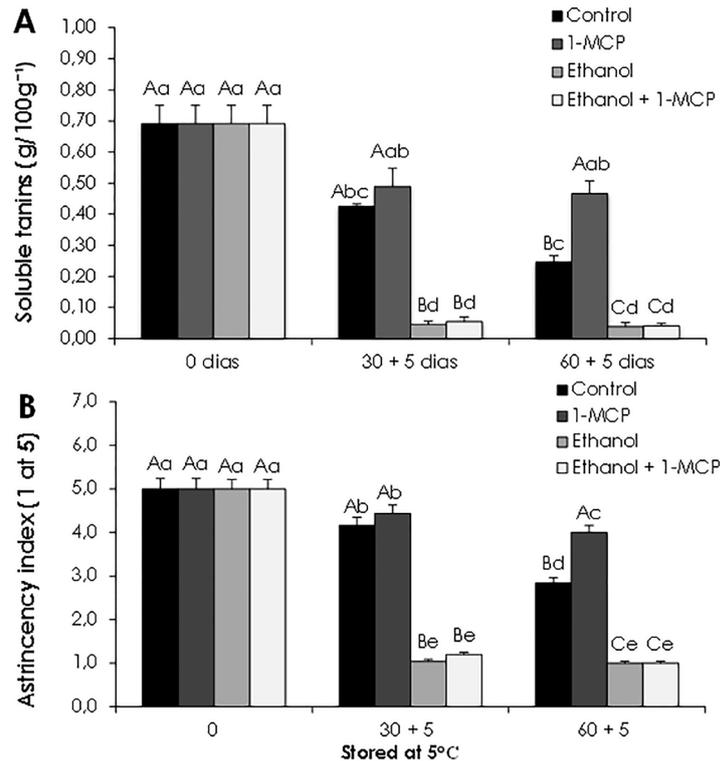


Figure 1. Soluble tannins (A) and astringency index (B) in 'Giombo' persimmons stored at 5°C and 90% RH of stored during 60 days + 5 days of SL at 22°C. Day 0: Characterization of the fruit after the harvest. Capital letters compare the treatment in each assessed period. Lower-case letters compare the treatments during storage. The same letter indicates no significant difference by the Tukey Test ($P < 0.05$). Vertical bars represent the standard error of the average ($n=4$).

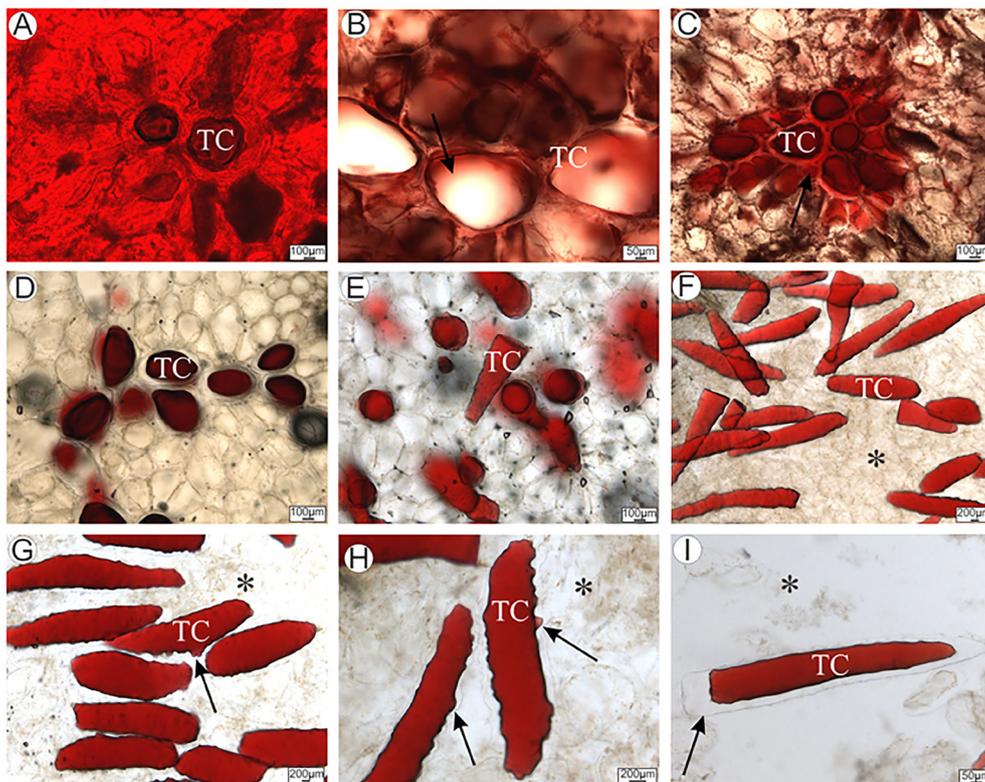


Figure 2. Hydrochloric vanillin test in transversal sections of 'Giombo' persimmons treated and stored at 5°C and 90% RH during 60 days + 5 days of SL at 22°C. Treatments T1, T2, T3, T4. **A:** Before the astringency reduction treatment **B:** CT with vacuole without tannin content, because of the extruded (arrow) in T1 at 30 days. **C:** CT and tannin content (arrow) in T2 at 30 days. **D:** Insoluble tannin and limited to the TC in T3 at 30 days. **E:** Intact tannin cells dispersed in the parenchyma in T3 at 60 days. **F:** Insoluble tannin and limited to the TC in T4 at 60 days. **G-H:** CT with projections of the cell wall (arrows) in T3 at 60 days. **I:** CT with intact cell wall and vacuole filled with tannin (a) in T4 at 60 days. TC = tannin cell, (*) degraded parenchyma.

intact and dispersed in the parenchyma (Figures 2F and G). In some tannin cells, we observed projections of the cell wall accompanied by the vacuole (Figures 2G and H) and with the intact cell wall (Figure 2I). These projections in the tannin cells were visible in de-astringent fruits and are associated to the mechanic effect in the tonoplast created by the polymerization of the tannins during the process of astringency reduction (Salvador et al., 2007).

Association between firmness and pectin degradation

'Giombo' persimmons displayed average firmness of 44.6 N after the harvest (Figure 3A). At 30 days at 5°C + 5 days SL, deastringent fruit displayed a fairly reduced firmness (10.9 N), in comparison with deastringent fruit and 1-MCP (25.7 N). The structural analysis showed that even the firmness reduction in the deastringent fruit, parenchyma cells were still intact at 30 days (Figure 2D), probably because of the low solubility of the pectins (Figure 3B). After 60 days at 5°C + 5 days SL, the firmness reduction in these treatments was drastic, reaching values as low as 10 N. Figure 2E shows the disruption of the parenchyma cells which coincides with firmness reduction as a consequence of the astringency reduction. Decreasing levels of firmness were also described by Antonioli et al. (2000) in 'Giombo' persimmons treated with ethanol at 3.85 ml kg⁻¹ during 24, 36 or 48 hours and stored during ten days at 25°C.

Firmness was higher in the control fruits and those fruits treated with 1-MCP for 30 days at 5°C + 5 days SL. In these samples firmness was respectively 20.7 and 29.7 N. At 60 days + 5 days SL, also happened the firmness reduction below 10 N, with the exception of those fruits treated with 1-MCP (Figure 3A), with the disruption of the parenchyma in a similar form as we observed in the astringency reduced fruits (data not shown).

Even the firmness reduction in deastringent fruit, and in the controls, we observed the effect of 1-MCP on the preservation of the firmness of both groups. Terra et al. (2014) confirm, 'Giombo' fruit with reduced astringency, and treated with 1-MCP displayed higher firmness at the end of the analysis period in comparison with those fruit treated only with the use of the

ethanol.

At the end of the 60 days storage period we observed a significant increase of soluble pectins (Figure 3B) in deastringent fruit, either treated or not with 1-MCP, associated with a reduction of the firmness (Figure 3A).

According to Taira et al. (1997), soluble pectins can form covalent and non-covalent bonds with the soluble tannin molecules, forming insoluble tannin polymers and removing the astringency of the fruit. In the control fruits and in those fruits treated with 1-MCP, even the increase of the solubilization of the pectins, soluble tannins content and astringency index kept high, suggesting the incapacity of the pectins to polymerize the soluble tannins.

Even more, associated to the drastic reduction of the pulp firmness, increase, and solubilization of the pectins, as highlighted by the cellular disruption of the parenchyma, the 'Giombo' persimmons displayed gelatinous and translucent pulp, and an increase in the concentration of ethanol and acetaldehyde after the withdrawal from the storage, in both fruit with astringency reduction, or not. These results highlight that the cultivar 'Giombo' is susceptible to cold storage damages during storage at 5°C.

According to Besada et al. (2014), Pérez-Munuera & Hernando (2009), persimmons cultivars such as 'Rojo Brillante' are susceptible to the cold and display a drastic reduction of the firmness and translucent pulp during storage at temperatures close to 5°C.

It is important to point out; persimmons are commercialized in two forms: either crispy or soft, and the preferences of the consumers determine their consumptions. According to Kato (1990), the firmness of the persimmons should be between 14 and 25N to be suitable for the consumption. Above this interval, fruits shall be considered too crisp. Below this interval, are too soft. Even the same, this study aimed to obtain fruit with elevated firmness after the astringency reduction and storage. In this form, the shelf life can be extended and the manipulation of the fruit can be eased.

Ethanol and acetaldehyde concentration

Clear pulp persimmons, such as the

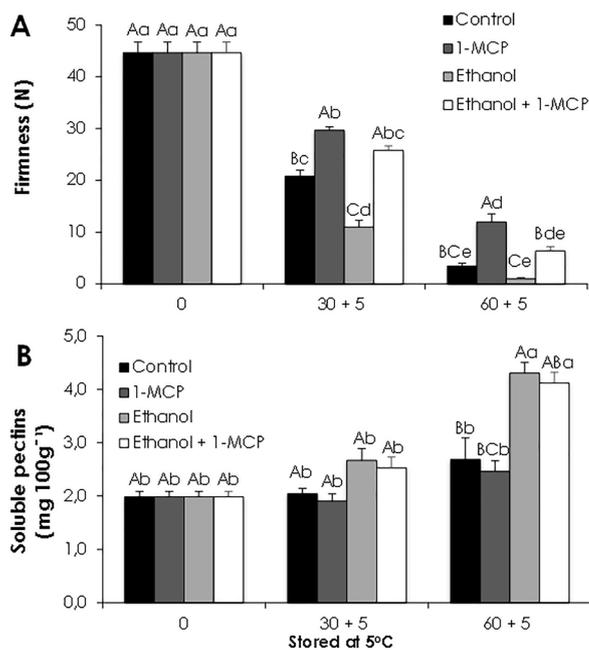


Figure 3. Firmness (A), and Soluble pectins (B) in 'Giombo' persimmons treated and stored at 5°C, and 90% RH during 60 days + 5 days of SL at 22°C. Day 0: Characterization of the fruit after the harvest. Capital letters compare the treatment in each assessed period. Lower-case letter compares the treatments during storage. The same letter indicates no significant difference by the Tukey Test ($P < 0.05$) Vertical bars represent the standard error of the average ($n=4$).

'Giombo' variety (PVA) have no seeds. Their ethanol and acetaldehyde production is low at ripening, as displayed in the figures 4A and B. For this reason, the treatment using ethanol or CO₂ is required for the production of acetaldehyde and to promote the process of astringency reduction in these fruit (Besada et al., 2012; Pesis & Ben-Arie, 1984).

At 30 days at 5°C + 5 days of SL at 22 °C, deastringent fruit and deastringent fruit + 1-MCP displayed ethanol (0.60 and 0.67 µg g⁻¹) and acetaldehyde (0.020 and 0.025 µg g⁻¹) (Figures 4A and B) content which could be sufficient for the complete astringency reduction (Figures 1A and B). In the control fruit, and in those treated only with 1-MCP, ethanol and acetaldehyde content kept low during this period (Figures 4A and B).

At 60 days at 5°C + 5 days SL at 22 °C we observed an increase in the ethanol and acetaldehyde production in all the treatments (Figures 4A and B). The increase in the production of these volatile compounds shall be associated to the exposition to high temperatures, and the susceptibility of this cultivar to cold storage damages (Besada et al., 2014; Salvador et al., 2008).

The astringency reduced fruits and

the astringency reduced + 1-MCP, our results suggest that the increase in the ethanol and acetaldehyde production occurred after the withdrawal of the fruits from storage and at 22°C + 5 days of SL at 22 °C.

In the control fruits and those only treated with 1-MCP we also observed a significant increase of ethanol and acetaldehyde, probably after the withdrawal from AR at 60 days of storage, and the transfer to the storage at 22°C. These high volatiles levels were not adequate to reduce the soluble tannins (0.246 g 100g⁻¹ and 0.466 g 100g⁻¹) and the astringency (notes 3 and 4), respectively. Besada et al. (2014) also described a significant increase of the ethanol and acetaldehyde in 'Triumph' persimmons, both in deastringent fruit control, and treated with 1-MCP, stored at 1°C and after 5 days of SL at 22°C in controlled atmosphere (1°C, 4 a 5% O₂+ N₂). These same authors defined the relationship between the accumulation of these volatiles and the higher incidence of cold storage damages. Arnal & Del Río (2004), also described the association between the increase of the levels of volatile compounds and cold storage damages in 'Rojo Brillante' persimmons.

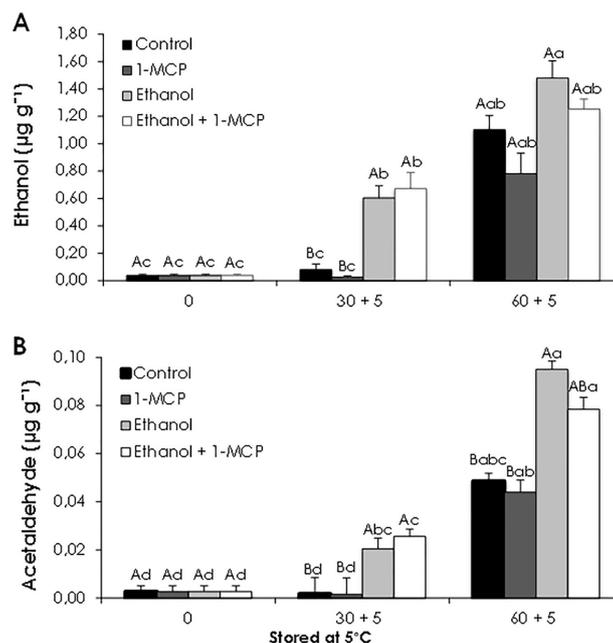


Figure 4. Ethanol (A), and acetaldehyde (B) production in 'Giombo' persimmons treated and stored at 5°C and 90% RH during 60 days + 5 days of SL at 22°C. Day 0: Characterization of the fruit after the harvest. Capital letters compare the treatment in each assessed period. Lower-case letter compares the treatments during storage. The same letter indicates no significant difference by the Tukey Test ($P < 0.05$). Vertical bars represent the standard error of the average ($n=4$).

Conclusions

A 1.7 ml kg^{-1} dose of ethanol during 12 hours was efficient for the astringency removal in 'Giombo' persimmons. Astringency reduction with the use of the ethanol reduced the firmness of the fruits. A complementary application of 1-MCP delays the loss of firmness in deastringent fruit, and in the control. The increase of soluble pectins did not foster the polymerization of the soluble tannins both with and without the application of the ethanol.

'Giombo' persimmons displayed a severe reduction of the pulp firmness, increase of soluble pectins, and degradation of the parenchyma cells, gelling of the pulp, increase of the production of ethanol and acetaldehyde after 60 days of storing + 5 days SL, inclusively in control fruit. These results suggest, cultivar Giombo is susceptible to cold storage damages at 5°C, and that further studies are required using different temperatures to improve the storage.

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