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# Long-term cold storage and vaccumpackage to removal asdringency and maintain firmness of 'Giombo' persimmon

Ferraz Monteiro, Mariana; Tessmer, Magda Andréia; Kluge, Ricardo Alfredo Long-term cold storage and vaccum-package to removal asdringency and maintain firmness of 'Giombo'

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Reportes Frutas

# Long-term cold storage and vaccum-package to removal asdringency and maintain firmness of 'Giombo' persimmon

Almacenamiento en frío a largo plazo y envasado al vacío para eliminarlo en caso de emergencia y mantener la firmeza del caqui 'Giombo'

Mariana Ferraz Monteiro <sup>1</sup> University of São Paulo, Brasil moreau mariana@hotmail.com Redalyc: https://www.redalyc.org/articulo.oa? id=81367929005

Magda Andréia Tessmer <sup>2</sup> University of São Paulo, Brasil magdatessmer@yahoo.com.br

Ricardo Alfredo Kluge <sup>3\*</sup> University of São Paulo, Brasil rakluge@usp.br

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## ABSTRACT:

The 'Giombo' persimmon is a cultivar very cultivated in Brazil and consumed crisp and without astringency. Many methods used for the removal of astringency cause loss of fruit firmness. In this research was studied the application of vaccum-package during cold storage of fruit to removal of astringency and at the same time to maintain the firmness of pulp. The treatments applied were: 1) fruit treated with ethanol (1.75 mL Kg<sup>-1</sup>) during 12 h at 22°C in hermetic chamber; 2) fruit packaged in polyamide polyethylene (PA-PE) (0.30 cm x 0.50 cm in size and 20 mm thick) and vacuum sealed; 3) Control fruit, without treatment with ethanol and no packaged. Fruit were stored at 5±1°C and 90±5% relative humidity (RH) during 90 days. After 30 days of cold stored fruit were placed to room temperature (22°C and 70% RH) and evaluated every 2 days during 8 days. In PA-PE treatment the package was removed immediately after the removal of fruit from cold storage. Fruit from control and those treated with ethanol could only be stored for 30 days. After, these fruit showed symptoms of chilling injuries. In the evaluation of 30 days, fruit treated with ethanol were non astringents from the fourth day at room temperature. Fruit packaged into PA-PE were non astringents from second day at 22°C while the control fruit remained astringents during the eight day. After 60 days of cold storage, vacuum PA-PE promoted deastringency of fruit from fourth day at room temperature and from second days after 90 days of storage. The use of the vacuum packaging associated with the cold storage can be used to reduce the astringency and maintain the firmness of fruit. Also, this association of methods can preserve the shelf life of fruit until 90 days.

KEYWORDS: Diospyros kaki, modified atmosphere, condensed tannins, acetaldehyde.

#### RESUMEN:

El caqui 'Giombo' es una variedad muy cultivada en Brasil y se consume crujiente y sin astringencia. Muchos métodos utilizados para eliminar la astringencia provocan la pérdida de firmeza de la fruta. En esta investigación se estudió la aplicación de envasado al vacío durante el almacenamiento en frío de la fruta para eliminar la astringencia y al mismo tiempo mantener la firmeza de la

### Notas de autor

- 1 University of São Paulo, Luiz de Queiroz College of Agriculture, Brasil. moreau\_mariana@hotmail.com
- 2 University of São Paulo, Luiz de Queiroz College of Agriculture, Brasil. magdatessmer@yahoo.com.br
- $3^* \quad \text{Corresponding author: University of S\~{a}o Paulo, Luiz de Queiroz College of Agriculture, Brasil. rakluge@usp.br}$



pulpa. Los tratamientos aplicados fueron: 1) fruto tratado con etanol (1,75 mL Kg<sup>-1</sup>) durante 12 ha 22°C en cámara hermética; 2) fruta envasada en poliamida polietileno (PA-PE) (0,30 cm x 0,50 cm de tamaño y 20 mm de espesor) y sellada al vacío; 3) Fruta de control, sin tratamiento con etanol y sin envasar. La fruta se almacenó a 5.1°C y 90-. 5% de humedad relativa (HR) durante 90 días. Después de 30 días de almacenamiento en frío, la fruta se colocó a temperatura ambiente (22°C y 70% HR) y se evaluó cada 2 días durante 8 días. En el tratamiento con PA-PE, el paquete se retiró inmediatamente después de sacar la fruta del almacenamiento en frío. Las frutas del control y las tratadas con etanol solo pudieron almacenarse durante 30 días. Posteriormente, estas frutas mostraron síntomas de lesiones por frío. En la evaluación de 30 días, los frutos tratados con etanol fueron no astringentes desde el cuarto día a temperatura ambiente. Las frutas envasadas en PA-PE no fueron astringentes desde el segundo día a 22°C mientras que las frutas de control permanecieron astringentes durante el octavo día. Después de 60 días de almacenamiento en frío, el PA-PE al vacío promovió la remoción de la astringencia de la fruta desde el cuarto día a temperatura ambiente y desde el segundo día después de 90 días de almacenamiento. El uso del envasado al vacío asociado con el almacenamiento en frío se puede utilizar para reducir la astringencia y mantener la firmeza de la fruta. Además, esta asociación de métodos puede preservar la vida útil de la fruta hasta 90 días.

PALABRAS CLAVE: Diospyros kaki, atmósfera modificada, taninos condensados, acetaldehído.

# **INTRODUCTION**

The 'Giombo' persimmon is classified as pollination-variant and astringent (PVA) type. It is one of the most cultivated in Brazil, and its preferred form of consumption is crisp-texture and without astringency. The astringency in persimmon is caused by the accumulation of proanthocyanidins (condensed tannins) present in the vacuole of de parenchyma cells of de pulp (Tessmer et al., 2014). These tannins are polyphenolic compounds derived from catechin and important for the plant and human (Santos-Buelga and Scalbert, 2000; Akagi et al., 2011).

Several simple methods have been used to remove the astringency of tannic persimmons, such as the use of ethanol, acetic acid, ethylene and calcium carbide (Edagi and Kluge, 2009). These methods stimulate the production of acetaldehyde by tissue of fruit, via alcohol dehydrogenase activity, and the formed acetaldehyde reacts with the condensed soluble tannins, inducing their polymerization and insolubilization, and consequently reducing the astringency of the flesh fruit (Oshida et al., 1996; Ito, 1971; Sugiura and Tomana, 1983). In Brazil, the most usual method for removal of astringency in 'Giombo" persimmon is the use of ethanol vapours. This method consists in maintaining the fruit confined in chamber until the evaporation of a certain amount of ethanol (Antoniolli et al., 2000, Edagi and Kluge, 2009).. This is a method very fast to removal of astringency, but it becomes a problem because of the rapid loss of fruit firmness when exposed to ethanol. The loss of firmness of this cultivar makes the fruit shriveled and unmarketable.

The use of high levels of carbon dioxide  $(CO_2)$  applied in chamber can be used to remove the astringency of persimmon since it produces effect similar to those of the simplest methods that is the accumulation of acetaldehyde in the tissues of fruit (Vidrih et al., 1994). However, its application demands the use of sealed chambers with rigorous control of temperature and  $CO_2$  levels inside them. This often increases costs to the producers.

The use of modified atmosphere (MAP) using plastic package also can be used to promote removal of astringency of tannic persimmon, depending of alterations obtained itself in relation to levels of  $O_2$  and  $CO_2$ . Low level of  $O_2$  and/or high of  $CO_2$  into package promote anaerobiosis and accumulation of acetaldehyde in fresh pulp. The low oxygen triggers the pyruvate decarboxylation while high carbon dioxide activates the malate via. Both mechanisms cause accumulation of acetaldehyde in fruit pulp making it not astringent (Pesis and Ben-Arie, 1986; Yamada et al., 2002; Edagi & Kluge, 2009). Pesis et al. (1988) showed that 'Triumph' persimmon stored at  $20^{\circ}C$  and packaged in polyethylene films had their astringency reduced faster when  $CO_2$  was present inside the package. However, the quality was lost in the first week of storage, whereas the fruit quality and the firmness were maintained for two weeks in vacuum packaged fruit. Antoniolli et al. (2003) have used low-density polyethylene bags in cold stored 'Giombo' persimmon for 90 days in an attempt



to reach the astringency and keep fruit quality. They found that polyethylene does not completely remove the astringency and the quality was maintained only for 30 days. After this period, they have observed a dramatic reduction of fruit firmness.

The vacuum-package is another method to modify the atmosphere by using plastic films. The removal of the air from the package induces anaerobic respiration and raises the production of acetaldehyde. Monteiro et al. (2017) have use polyamide polyethylene (PA-PE) plastic to promote vacuum atmosphere in 'Giombo' persimmon. These authors found that PE-PA reduces astringency faster and maintains fruit firmness for a longer time at room temperature (22°C and 70% TH). The use of vacuum atmosphere using plastic films during cold storage of persimmon astringent cultivars has not yet been studied. Thus, considering the importance of achieving complete removal of astringency, keep fruit firmness and therefore its conservation for longer time, this study aimed to associate the use of vaccum-package with cold storage in 'Giombo' persimmon. The combination of these two methods can benefit the productive sector of 'Giombo' persimmon.

#### MATERIAL AND METHODS

Fruit of 'Giombo' persimmon were harvested in Mogi das Cruzes, São Paulo state, Brazil (23.31'16"S; 46.11'19"W; climate subtropical cwa). The selected fruit were on accordance with the Classification Standards, Standardization and Identity of Persimmon (Brazilian Horticulture Modernization Program, 2000).

The treatments applied were: 1) fruit treated with ethanol (1.75 mL Kg<sup>-1</sup>) during 12 h at 22°C in hermetic chamber; 2) fruit packaged in polyamide polyethylene (PA-PE) (0.30 cm x 0.50 cm in size and 20 mm thick) and vacuum sealed; 3) Control fruit, without treatment with ethanol and no packaged. Fruit were stored at 5±1.C and 90±5% relative humidity (RH) during 90 days. After 30 days of cold stored fruit were placed to room temperature (22°C and 70% RH) and evaluated every 2 day during 8 days. In PA-PE treatment the package was removed immediately after the removal of fruit from cold storage.

For determination of astringency index the fruit received a transverse incision on the equatorial region, splitting it in half. For the evaluation, one of the halves was placed in contact with a filter paper previously washed in a solution of ferric chloride (CAMPO-DALL'ORTO et al., 1996). The tannin, in his soluble form, reacts with ferric chloride, becoming dark. The evaluation of the papers was made by visual analysis, where grades from 1 to 5 were given to each stamped fruit, where 1 = not astringent; 2 = slightly astringent; 3 = moderate astringent; 4 = astringent; 5 = very astringent.

The soluble tannin content was determined according with adaptations of methodology of Agostini-Costa et al. (1999), using Folin-Ciocauteau (50%), and the absorbance of 725 nm (Biochrom spectrophotometer, model Libra S22) was used for readings. The results were expressed in grams of gallic acid per 100 grams of pulp (g  $100 \, \text{g}^{-1}$ ).

The determination of acetaldehyde was carried out according Davis & Chace (1969): 1 g of pulp was sealed in 40 mL glass bottles and kept in a freezer at -80°C until analysis. The bottles were incubated in a water bath for 30 minutes at 50°C, and 1 mL of the air in the bottles was collected with a syringe and injected into a Thermo Quest Finnigan Trace GC 2000 gas chromatograph with flame ionization detector (FID) and Porapak-N column. Chromatograph settings were: oven at 140 °C for 8 minutes, then raised to 180°C by 20°C/min, held for 2 min for cleaning up the column; injector at 150°C; detector at 180 °C; pressure of 190 kPa (constant) and N. flow at 70 mL min<sup>-1</sup>. The results were expressed as  $\mu g$  of acetaldehyde per g fresh weight ( $\mu g$  g<sup>-1</sup> fw).

The firmness was measured using a digital penetrometer (Sammar, model TR 85261.0472), with 8 mm tip. Two readings were made on opposite sides of the fruit equatorial region after the removal of a small portion



of the peel. The results were expressed in Newton (N). A commercialization limit acceptance of 15 N was established to 'Giombo' persimmon (Brazilian Horticulture Modernization Program, 2000).

The experimental design was completely randomized, with three treatments and five replicates of eight fruit. The results were submitted to the minimum significant difference analysis in a multiple comparison test, in which differences between the two treatments higher than the sum of two standard deviations were considered significant at 5% probability.

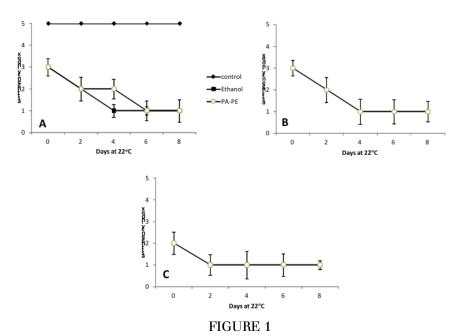
#### RESULTS AND DISCUSSION

Fruit from control and those treated with ethanol could only be stored for 30 days. After, these fruit showed symptoms of chilling injuries (CI), featured by translucent of pulp, external browning and intense softening (Grant et al., 1992). In fact, CI is one of the main limiting factors to long-term storage of persimmon. In previous studied it was found that 'Giombo' persimmon can be cold storage up to 30 days. After this, the development of CI reduces dramatically the fruit quality (Antoniolli et al., 2003).

In the evaluation of 30 days, fruit treated with ethanol were non astringents from the fourth day at room temperature. Fruit packaged into PA-PE were non astringents from second day at 22°C while the control fruit remained astringents during the eight day (Fig. 1A).

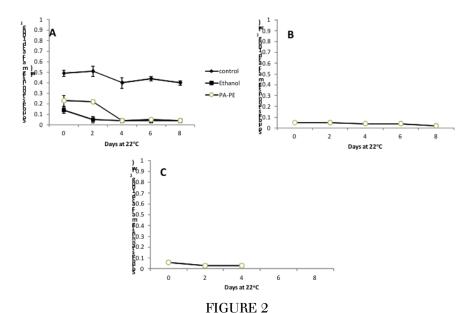
After 60 days of cold storage, vacuum PA-PE promoted deastringency of fruit from fourth day at room temperature (Fig. 1B) and from second days after 90 days of storage (Fig 1C).

The soluble tannins content in control fruit show slight decrease during exposure at room temperature after 30 days of cold storage, while fruit treated with ethanol show decrease faster than fruit packaged in PA-PE (Fig 2A). According to citation by Vidrih et al. (1994), the content of soluble tannins below 0.1 g 100 g<sup>-1</sup> becomes fruit non-astringent. In our study, values below this were observed after two and four day at room temperature for fruit treated with ethanol and those packed in PA-PE, respectively. Fruit kept in PA-PE maintained low values of soluble tannins after 60 and 90 day of cold storage (Fig 2B and 2C).



Adstringency index in 'Giombo' persimmon treated with ethanol or mantained in polyethylene-polyamide (PA-PE) package during 30(A); 60(B) and 90(C) days at  $5^{\circ}C$  and 90-95% RH. Day 0 = value immediately after removal from the cold storage. Value at harvest = 5. Vertical bars indicate the standard error (n = 5).

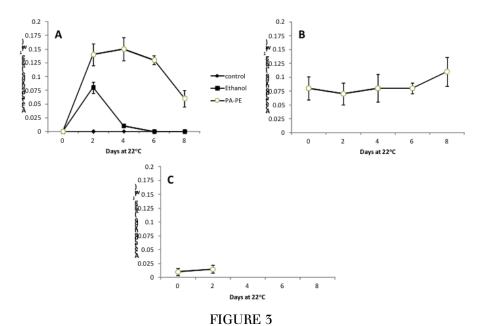




Soluble tannins in 'Giombo' persimmon treated with ethanol or mantained in polyethylene-polyamide (PA-PE) package during 30(A); 60(B) and 90(C) days at 5°C and 90-95% RH. Day 0 = value immediately after removal from the cold storage. Value at harvest = 0.49 g 100 g<sup>-1</sup>. Vertical bars indicate the standard error (n = 5).

The results found to acetaldehyde content were very significant (Fig. 3). After 30 day, fruit kept in PA-PE during cold storage showed a drastic increase when exposed at 22°C, reaching near to 0.15  $\mu g \, g^{-1}$  on fourth day, and decreased by near to 0.060  $\mu g \, g^{-1}$  on eighth day (Fig 3A). Fruit treated with ethanol also showed increase of acetaldehyde content; however the maximum observed was on second day (near to 0.075  $\mu g \, g^{-1}$ ). The acetaldehyde content in fruit packaged in PA-PE show value of 0.075 to 0.1  $\mu g \, g^{-1}$  after 60 days (Fig 3B) and very low values (less of 0.025  $\mu g \, g^{-1}$ ) after 90 days of cold storage, no being more detected from the fourth day.





Acetaldehyde content in 'Giombo' persimmon treated with ethanol or mantained in polyethylene-polyamide (PA-PE) package during 30(A), 60(B) and 90(C) days at 5°C and 90-95% RH.

Day  $0 = \text{value immediately after removal from the cold storage. Value at harvest} = 0,0028 \, \pi g$ .

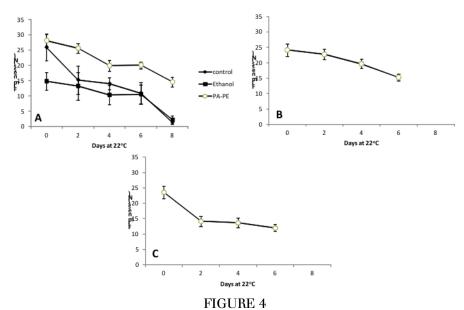
It has been postulated that deastringency is caused by the reaction of acetaldehyde with the condensed soluble tannins and here it probably occurred because the decrease of soluble tannins was coincident with increase of acetaldehyde, mainly in fruit kept in PA-PE during cold storage. Thus, we can state that that the greatest stimulus of acetaldehyde production leads to more rapid loss of fruit astringency. The absence of oxygen inside de vacuum PA-PE package promotes the decarboxylation of pyruvate and consequently the generation of acetaldehyde and ethanol. For the majority of fruit the anaerobiosis leads to loss of quality due the development of off-flavor. In case of PVA type persimmon the formed acetaldehyde probably react rapidly with soluble tannins forming a polymerized molecule least damaging and preventing its conversion to alcohol. Thus, neither the acetaldehyde nor alcohol impairs the quality of fruit. In addition, the conversion of soluble tannins into insoluble tannins is beneficial to fruit quality because removes astringency.

The effect of alcohol on removal of astringency is different from vacuum conditions. The application of alcohol vapors probably stimulates the alcohol dehydrogenase enzyme that converts alcohol into acetaldehyde. The formed acetaldehyde reacts with soluble tannins and fruit lost the astringency.

The fruits firmness was affected by the treatments (Fig 4). Fruit treated with ethanol and the control fruit showed higher decrease of firmness than fruit packaged in vacuum PA-PE during exposure to room temperature after 30 days of cold storage (Fig 4A). At the end of 8 days of room temperature control fruit and fruit treated with ethanol shown very low firmness (< 5 N), while fruit kept into vacuum-package during cold storage have firmness above 15 N. After 60 and 90 days, fruit kept in vacuum-package still had adequate firmness for consumption, around 15 N up to 6 days at room temperature (Fig. 4B and 4C). The softening is a normal process in climacteric fruit after harvest (such as persimmon). This process is regulated primarily by ethylene after harvest and caused by activity of cell wall degrading enzymes such as polygalacturonase (PG), pectin methylesterase (PME),  $\beta$ -D-galactosidase ( $\beta$ -D-Gal),  $\alpha$ -L-arabinofuranosidase ( $\alpha$ -L-Arf) and others (Giovannoni, 2004). The biosynthetic pathway of ethylene includes in the last step the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene by the ACC oxidase (ACO) (Van de Poel and Van Der Straeten, 2014). Also, oxygen appears to be essential for the action of ethylene at the cellular level. Therefore, the higher firmness observed in fruit packaged in PA-PE film is related probably to lower



production and action of ethylene. The absence of oxygen in vacuum package (PA-PE) probably reduced the conversion of ACC in ethylene.



Firmness in 'Giombo' persimmon treated with ethanol or mantained in polyethylene-polyamide (PA-PE) package during 30(A); 60(B) and 90(C) days at 5°C and 90-95% RH. Day 0 = value immediately after removal from the cold storage. Value at harvest = 39,24 N. Vertical bars indicate the standard error (n = 5).

#### FINAL CONSIDERATIONS

We can observe that the use of the vacuum packaging associated with the cold storage can bring two benefits to the quality of persimmon 'Giombo', i.e., to reduce the astringency and maintain the firmness of pulp. Another great benefit of the association between vacuum packaging and cold storage is the increase of shelf life, which benefits the commercialization of the fruit. The cold storage of 'Giombo' persimmon for 90 days, with the preservation of quality, mainly pulp firmness and absence of astringency, had not yet been demonstrated. The combination of the two methods can be promising for growers of 'Giombo' persimmon, which can offer non-astringent fruits with adequate pulp firmness for longer.

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