

# Effect of active modified atmospheres on the quality of non-astringent persimmons (Caqui Giombo) *D. kaki* Thunb. when stored under refrigerated conditions

M.R. De Moraes\*, É.R. Daiuto\*, R.L. Vietes\*, N.C. Cardoso\*, R.E. Smith\*\* <sup>(1)</sup>

\* Faculty of Agronomic Sciences, UNESP Botucatu, C.P. 237, Botucatu 18610307, SP, Brazil.

\*\* U.S. FDA, 11510 W 80st, Lenexa, KS 66224, USA.

**Key words:** Caqui Giombo, *Diospyros kaki* L., firmness, persimmon, postharvest.

**Abstract:** The effects of active modified atmospheres were evaluated in ‘Giombo’ persimmons with tannins already removed (non-astringent) and stored at 0°C and 85-90% relative humidity for 35 days. The goal was to maintain quality and delay ripening. The fruits were picked by hand when they were about 50% green, sanitized and subjected to different mixtures of CO<sub>2</sub> and O<sub>2</sub>. Fruits were wrapped in thin film plastic made of nylon + polyethylene and analyzed every seven days for weight loss, respiratory activity, coloration, titratable acidity, soluble solids, ratio, pH, firmness, pectin methyl esterase and polygalacturonase enzyme activities, reducing sugars, ascorbic acid and astringency index. Refrigerated storage and active modified atmospheres were effective in conserving the quality of ‘Giombo’ persimmons. The fruits submitted to the highest CO<sub>2</sub> concentrations (7 and 8%) had the lowest weight loss and respiratory intensity with a delay in the climacteric peak.

## 1. Introduction

Persimmons are the edible fruits of deciduous trees in the genus *Diospyros*, many of which originated in China (Martínez-Calvo *et al.*, 2012), even though some species are native to other parts of the world, including *D. lotus*, or date plum, which is native to southwest Asia and southeast Europe. The fruit was called “fire of the gods”, or *Diospyros* by the ancient Greeks, thus the name of the genus. Persimmons produce popular and nutritious fruits that have become traditional crops in Korea and Japan and are found throughout the world (Veberic *et al.*, 2010; Dembitsky *et al.*, 2011; Giordani *et al.*, 2011). They are sweet and are relatively good sources of carotenoids, vitamin C, polyphenols and proanthocyanidins (Gu *et al.*, 2008; Dembitsky *et al.*, 2011; Giordani *et al.*, 2011). Moreover, persimmons have been reported to have health benefits in both traditional and western medicine (Giordani *et al.*, 2011). For example, persimmons of the Triumph variety improved lipid metabolism and atherosclerosis indices in rats that were fed a diet that was high in cholesterol (Gorinstein *et al.*, 1998; 2011).

The most cultivated species is the *D. kaki* Thunb. (Martínez-Calvo *et al.*, 2012; USDA, 2012), also known as Chinese persimmon, Japanese persimmon, kaki, caqui and

*Diospyros kaki* L. (Martínez-Calvo *et al.*, 2012; USDA, 2012). There is also a species (*D. virginiana*) that is native to eastern North America (Celik and Ercisli, 2008). Persimmons are climacteric fruits (Veberic *et al.*, 2010). That is, the amount of sugars (particularly sucrose) and total carotenoids increase in the final stages of ripeness and firmness, while soluble tannins and titratable acidity levels decrease, resulting in improved flavor (Candir *et al.*, 2009). In the northern hemisphere, they are harvested between September and December (Dembitsky *et al.*, 2011). On the other hand, the Brazilian caqui cultivar called ‘Giombo’ is very productive and matures late, with fruits being picked from March to the end of May (Martins and Pereira, 1989). This relatively short harvest season, coupled with the lack of information about storage, limits its expansion and causes losses in the final processing and marketing of the fruit (Donazzolo and Brackman, 2002).

Persimmon cultivars are classified into four groups: pollination-constant astringent, pollination-variant astringent, pollination-constant non-astringent, and pollination-variant non-astringent (Campo-Dall’Orto *et al.*, 1996; Celik and Ercisli, 2008). Persimmons have also been divided into a volatile-independent group (VIG, corresponding to the pollination-constant non-astringent group) and the volatile dependent group (VDG, consisting of the pollination-constant astringent, pollination-variant astringent and pollination variant non-astringent types) (Giordani *et al.*, 2011). Tannins in the VIG type are usually

<sup>(1)</sup> Corresponding author: robert.smith@fda.hhs.gov

Received for publication 13 March 2013

Accepted for publication 25 May 2013

relatively high in molecular weight and soluble in water. Moreover, their concentrations are maximum at an early stage of development and are <1% of the fresh weight. On the other hand, the VDG types contain tannins that are usually soluble in water, have a lower molecular weight and are not palatable at harvesting time. The seeds of pollination-variant non-astringent cultivars can exude ethanol, which makes the water-soluble tannins insoluble (Giordani *et al.*, 2011). All persimmons are edible when soft, but they can be astringent at harvest time (Giordani *et al.*, 2011). Parthenocarpic fruits of pollination-variant non-astringent cultivars, and both seeded and parthenocarpic fruits of astringent cultivars are edible only after removing the astringency artificially or when soft, overripe or dried. This happens when low molecular weight, soluble tannins are made water-insoluble, probably by binding with pectins (Giordani *et al.*, 2011). Astringency can be removed by storing persimmons in a modified atmosphere containing elevated amounts of CO<sub>2</sub> or ethanol (Del Bubba *et al.*, 2009; Edagi *et al.*, 2009) and refrigeration can delay the ripening of persimmons picked when only half-ripe (Vieites *et al.*, 2012).

The heart-shaped 'Hachiya' cultivar is the most popular pollination-constant astringent persimmon, while the 'Fuyu' is a popular pollination-constant non-astringent cultivar in Japan (Celik and Ercisli, 2008).

The Brazilian 'Giombo' cultivar is in the variable denomination which includes fruits that are yellow and contain tannins. Seedless fruits keep their astringency even when ripe, so the tannins must be removed artificially. The biggest inconvenience in accelerating the ripening process to remove tannins is that it diminishes the shelf life (Edagi *et al.*, 2009). According to Antonioli *et al.* (2000), this can compromise the firmness of the pulp when stored for a long period.

Refrigerated storage is among the practices used to maintain the quality of fruits for a short length of time (Vieites *et al.*, 2012); it can prolong the useful storage time, but the majority of workers show that it should not exceed 35 days to remain safe (Ben-Aire and Zutkhi, 1992; Brackmann and Saquet, 1995; Chitarra and Chitarra, 2005).

Other methods have been tested to extend the shelf life of fruits, with modified atmospheres standing out. Also plastic films can increase CO<sub>2</sub> and decrease O<sub>2</sub> however the concentrations of these gases are not controlled and vary with time, temperature, type of plastic and respiratory rate (Sargent *et al.*, 1993). According to Ferri *et al.* (2004) storing 'Fuyu' persimmons at 0°C maintains the firmness of the pulp for 90 days, but when only using refrigerated storage the shelf life is less than 30 days.

Another approach is to use a modified active atmosphere in which the initial concentration of gases inside the packaging is controlled. 'Giombo' persimmons have not yet been tested under these conditions, therefore the objective of this study was to test the effects of a modified active atmosphere on the cold storage of 'Giombo' persimmons that have had the tannins removed, making them non-astringent.

## 2. Materials and Methods

'Giombo' persimmons were from the Sacramento Agropastoril Ltda, Avaré (SP), located at a latitude of 23°05'56"S, longitude 48°55'33"W and altitude of 780 m, with an annual precipitation of 1500-1700 mm yr<sup>-1</sup>, annual temperature between 20 and 24°C and soil classified as purple oxysoil (structured earth, purple, oxidized). The fruits were collected by hand when they were at stage 3 of maturity, medium-ripe, about 50% green. To remove the tannins, fruits were collected in plastic boxes and exposed to ethanol fumes at a concentration of 6.6 ml kg<sup>-1</sup>, in chambers at 25°C for 48 h. Fruits were then submitted to the following gas mixtures: 0.03% CO<sub>2</sub> and 21% O<sub>2</sub> (T1= control; 5% CO<sub>2</sub> and 4% O<sub>2</sub> (T2); 6% CO<sub>2</sub> and 4% O<sub>2</sub> (T3); 7% CO<sub>2</sub> and 4% O<sub>2</sub> (T4) and 8% CO<sub>2</sub> and 4% O<sub>2</sub> (T5). The fruits were wrapped in plastic wrappers made of nylon and polyethylene and stored refrigerated at 0±0.5°C and 85-90±5% relative humidity for 35 days, and were analyzed every seven days. For the control group (non-destructive), two whole fruits were analyzed five times and for the destructive group, two fruits were cut into pieces and then analyzed in triplicate.

### Loss of mass (%)

The weight of fruits was measured with an analytical balance and the results expressed as a percentage.

### Respiratory activity

The liberation of CO<sub>2</sub> was measured following the method of Bleinroth *et al.* (1976), using a saturated solution of barium hydroxide and 0.1 N potassium hydroxide. The respiratory rate was calculated using the equation:

$$TCO_2 = 2.2 (V_o - V_1) \cdot 10/P \cdot T$$

where T CO<sub>2</sub> = respiratory rate (ml of CO<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup>);

V<sub>o</sub> = volume of HCl needed to titrate the potassium hydroxide solution before and V<sub>1</sub>, after absorbing CO<sub>2</sub> (ml);

P = mass of the fruits; T = time of respiration;

2.2 = equivalent weight of CO<sub>2</sub> (44/2), multiplied by the concentration of HCl;

10 = adjustment for the total amount of KOH used.

Analyses were carried out in triplicate.

### Titrateable acidity

Expressed as gram equivalents of malic acid per 100 g of pulp (g of malic acid 100 g<sup>-1</sup>), obtained by titrating 5 g of homogenized pulp diluted to 100 ml with distilled water with 0.1 N NaOH, using a phenolphthalein indicator, in conformance with Odair *et al.* (2008).

### Soluble solids

Made using a Palette ATAGO PR-32 refractometer and expressed as (°Brix), in conformance with Odair *et al.* (2008).

### Maturity index (ratio)

Determined from the ratio of soluble solids to titrateable acidity (2008).

## pH

Measured using a model 300 pH meter in conformance with Odair *et al.* (2008).

## Sugars

Measured using the methods of Somogy (1945) and Nelson (1944) and a Micronal B 382 spectrophotometer to measure the absorbance at 535 nm.

## Ascorbic acid

Determined by adding 30 ml of 4% oxalic acid to 30 g of pulp and titrating with 0.5% DPI-2,6-dichlorophenolindophenol with results expressed as ml of ascorbic acid 100 ml<sup>-1</sup> of pulp (MAPA, 2011).

## Astringency index

Determined using the method of Gazit and Levy (1963) and modified by Vitti (2009) in which one of the sides of the cut fruit is placed on a piece of filter paper that has been impregnated with a solution of 5% FeCl<sub>3</sub>. Soluble tannins react and turn the paper dark, which is then analyzed visually on a scale of 1 to 5, with 5 being the darkest and most astringent.

## Coloration

Measured in a Konica Minolta (Chroma meter, CR 400/410) colorimeter over the spectral region of 380 to 780 nm. The reflectance reading was obtained with an angle of observation of 2° and illumination C. The color was expressed by a system of rectangular coordinates: L\* a\* and b\* in conformance with the CIE (*Comission Internatinale de E'clairage*), where L\* is the percent luminosity (0% = black and 100% = white), a\* represents the colors red (+) or green (-) and b\* the colors yellow (+) or blue (-).

## Firmness

Measured using a Texture Analyzer (Stevens – LFRA texture analyzer) with a penetration distance of 10 mm and a velocity of 2.0 mm sec<sup>-1</sup>, and using a TA 9/1000 fixture and a pressure of 15 gram of force per cm<sup>2</sup> (gf cm<sup>-2</sup>).

## Enzyme activity

Activities of polygalacturonase (PG) and pectin methylesterase (PME) were determined by the methods of Albershein *et al.* (1967) and Ahmed and Labavitch (1980).

The Tukey test at a 5% probability level was used to compare results, as recommended by Gomes (2000) and by linear regression analysis for weight loss.

## 3. Results and Discussion

The respiratory activity of 'Giombo' persimmon fruits increased during the storage periods, as shown in Figure 1. Concomitantly, there was a steady weight loss, depending on the treatment (Table 1). The fruits exposed to 7 and 8% CO<sub>2</sub> showed less weight loss than the other treatments. In order to have acceptable surface shrinkage for fresh fruits (Finger and Vieira, 2002) the maximum tolerated weight loss should be between 5 and 10%, thus the weight losses of 'Giombo' persimmons found in this study are acceptable.

It was also observed that the respiratory rate was less at the highest concentrations of CO<sub>2</sub> (6, 7 and 8%) compared

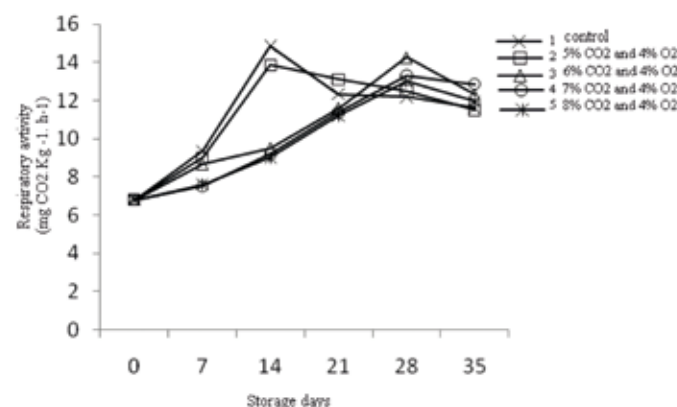


Fig. 1 - Respiratory activity (mg CO<sub>2</sub> Kg<sup>-1</sup> h<sup>-1</sup>) of 'Giombo' persimmons with tannins removed and submitted to modified atmospheres and stored at 0°C, 85-90% relative humidity for 35 days at different concentrations.

Table 1 - Weight loss (%) of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Storage days	control	Treatment				Averages
		5%CO <sub>2</sub> 4%O <sub>2</sub>	6%CO <sub>2</sub> 4%O <sub>2</sub>	7%CO <sub>2</sub> 4%O <sub>2</sub>	8%CO <sub>2</sub> 4%O <sub>2</sub>	
7	0.105±0.024 Aa	0.236±0.189 Ca	0.136±0.184 Aa	0.038±0.011 Aa	0.024±0.093 Aa	0.108±0.028
14	0.144±0.09 Aa	0.323±0.127 a	0.216±0.112 Aa	0.098±0.019 Aa	0.093±0.047 Aa	0.175±0.053
21	0.186±0.104 Aa	0.353±0.226 Ca	0.261±0.108 Aa	0.123±0.026 Aa	0.118±0.059 Aa	0.208±0.155
28	0.190±0.023 Ab	0.695±0.068 Ba	0.270±0.120 Ab	0.143±0.046 Ab	0.130±0.068 Ab	0.286±0.141
35	0.268±0.119 Ab	1.041±0.262 Aa	0.355±0.220 Ab	0.221±0.017 Ab	0.222±0.063 Ab	0.421±0.353
Averages	0.149±0.115	0.441±0.281	0.206±0.098	0.104±0.057	0.098±0.015	

Small letters compare averages of different treatments on each day.

Upper case letters compare averages between different days.

Averages followed by at least one letter in common do not differ statistically.

to fruits exposed to 5% CO<sub>2</sub> and 4% O<sub>2</sub> which reached a peak on the 14th day of storage. The fruits exposed to 6, 7 and 8% CO<sub>2</sub> had maximum respiratory activity after 28 days. The elevated concentrations of CO<sub>2</sub> inhibited the respiratory activity of the fruits.

The color of the fruits was not statistically different in fruits treated differently, as shown in Tables 2-4. No darkening was seen on the surface of the fruits. The values of a\* in the apical region of the fruits was less than that of the median or basal regions. Negative a\* values were found only on the first day, indicating the presence of a green color and verifying that the fruits did ripen during storage. This is in agreement with data reported by Chitarra and Chitarra (2005) who reported that the change in color is associated with ripening, which is a standard attribute for determining fruit quality. The increase in a\* and b\* color indices each reflected changes from yellowish-green to orangish-red.

Brackmann *et al.* (1997) reported that persimmons stored refrigerated at 5°C did not show an appreciable change in color since low temperatures inhibit the biosynthesis of carotenoids. The differences in color seen in the present study were due to differences at the time of collection. Danieli *et al.* (2002) reported that 'Fuyu' persimmons that were collected when still yellowish-green eventually changed to red. Still, there were no such changes in the treatments in the current study. This is commercially important since coloration is a primary quality standard.

The amounts of soluble solids, titratable acidity and the ratio between the two are reported in Table 5, which shows that there is no significant change, regardless of dose of CO<sub>2</sub> and storage time.

Working with the same cultivar, Antoniolli *et al.* (2000) found that there was little change in the amount of soluble solids, as confirmed also by the current study. Murray and Valentini (1998) reported that limits in the precision of the method and the many factors that affect soluble solids make it difficult at times to establish interactions between the process of maturation and the content of soluble solids. Thus, the amount of soluble solids serves best as a standard of quality rather than an index that measures the effects of storage.

The present study also verified that there is a slow increase in the titratable acidity of the fruits during storage, with values ranging from 0.07 to 0.10 grams equivalents of malic acid per 100 g of pulp. According to Costa and Balbino (2002), the increase in titratable acidity is due to the formation of galacturonic acid during the process of breaking down cell walls, which occurs during fruit storage. The ratios of soluble solids to titratable acidity varied little during storage.

The pH of the samples oscillated between 5.49 and 5.87, as shown in Table 6. This is similar to the results reported by Blum *et al.* (2008) who found no change in pH or acidity in 'Giombo' persimmons that were covered with carnuba wax during cold storage. Therefore, the modified atmospheres used in the current study did not affect the pH very much.

One of the main concerns about storage is the rapid loss of firmness of the pulp, which makes fruits commercially unacceptable. Thus, the fact that the present study shows only small variations in firmness, as shown in Table 7, is important, and can be explained as a combination of cold storage in a modified atmosphere, each of which decrease metabolism and prolong shelf life.

Table 2 - Luminosity (%) of caquis 'Giombo' persimmons with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Days of storage	Luminosity		
	Reg. apical	Reg. mediana	Reg. basal
0	45.3 ab	48.9 ab	46.6 a
7	44.3 b	48.4 b	45.6 ab
14	44.8 b	48.2 b	45.2 ab
21	45.3 ab	48.8 ab	44.7 b
28	46.2 a	49.3 a	45.2 ab
35	45.5 ab	49.3 a	46.0 ab

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 3 - Color a\* in 'Giombo' persimmons with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Storage days	Color a*		
	Reg. apical	Reg. media	Reg. basal
0	-0.1 d	0.5 d	1.4 d
7	2.5 cd	3.0 c	7.5 c
14	2.8 cd	3.1 c	7.3 c
21	6.7 b	9.8 b	11.8 b
28	12.5 a	15.8 ab	15.8 ab
35	15.6 a	18.9 a	18.3 a

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 4 - Color b\* in 'Giombo' persimmons with tannins removed, submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Storage days	Color b*		
	Apical	Medium	Basal
0	34.5 a	39.0 a	32.9 a
7	31.2 b	37.7 abc	32.1 a
14	32.6 b	36.7 bc	31.9 a
21	29.0 c	35.0 c	26.7 b
28	32.6 b	37.4 bc	28.1 b
35	32.1 b	38.9 abc	32.3 a

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 5 - Amounts of soluble solids (°Brix), titratable acidity (g malic acid per 100 g of pulp) and ratio in ‘Giombo’ persimmons with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatments	Soluble Solids (SS)						Averages
	Storage days						
	0	7	14	21	28	35	
Control	17±0.3	15.7±0.2	16.9±1.8	17.4±1.5	14.4±1.9	17.4±0.5	16.5±1.6
5%CO <sub>2</sub> 4%O <sub>2</sub>	17±0.3	16.5±0.9	15.8±0.9	16.6±2.0	15.7±1.0	17.0±0.8	16.5±1.1
6%CO <sub>2</sub> 4%O <sub>2</sub>	17±0.3	16.8±0.2	15.9±0.9	16.4±0.7	15.3±0.5	15.6±0.2	16.2±0.8
7%CO <sub>2</sub> 4%O <sub>2</sub>	17±0.3	18.1±1.0	16.8±0.7	16.8±0.3	15.2±0.3	17.0±0.8	16.8±1.0
8%CO <sub>2</sub> 4%O <sub>2</sub>	17±0.3	15.8±0.6	16.9±0.4	16.9±1.6	15.4±1.0	16.1±0.6	16.4±1.0
Average	17A±0.3	16.6A±1.1	16.5A±1.03	16.8A±1.2	15.2B±1.0	16.6A±0.9	
Titratable Acidity (TA)							
Control	0.07±0.01	0.09±0.01	0.07±0.01	0.08±0.05	0.08±0.03	0.08±0.01	0.08±0.02
5%CO <sub>2</sub> 4%O <sub>2</sub>	0.07±0.01	0.06±0.01	0.08±0.01	0.08±0.05	0.10±0.04	0.09±0.01	0.08±0.03
6%CO <sub>2</sub> 4%O <sub>2</sub>	0.07±0.01	0.08±0.01	0.07±0.02	0.07±0.05	0.06±0.02	0.09±0.01	0.07±0.02
7%CO <sub>2</sub> 4%O <sub>2</sub>	0.07±0.01	0.07±0.01	0.06±0.01	0.08±0.03	0.10±0.02	0.14±0.04	0.09±0.03
8%CO <sub>2</sub> 4%O <sub>2</sub>	0.07±0.01	0.07±0.01	0.06±0.01	0.10±0.04	0.08±0.03	0.09±0.01	0.08±0.02
Average	0.07B±0.01	0.07B±0.01	0.07B±0.01	0.08AB±0.04	0.08AB±0.03	0.10A±0.03	
Ratio							
Testemunha	234±27	183±17	250±34	260±114	195±77	230.5±45	225.3±60
5%CO <sub>2</sub> 4%O <sub>2</sub>	234±27	267±48	199±10	279±142	183±75	188.2±27	225.0±71
6%CO <sub>2</sub> 4%O <sub>2</sub>	234±27	221±26	224±32	278±125	265±72	176.4±14	233.2±63
7%CO <sub>2</sub> 4%O <sub>2</sub>	234±27	283±53	300±63	239±73	155±22	131.8±40	223.9±76
8%CO <sub>2</sub> 4%O <sub>2</sub>	234±27	222±24	269±7	220±152	215±77	179.6±29	223.2±66
Averages	234AB±23	235AB±48	248.7AB±47.1	255.1A±107.6	202.4AB±68.8	181.3B±42.8	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 6 - pH of ‘Giombo’ persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatments	Days of storage						Averages
	0	7	14	21	28	35	
Control	5.87±0.28	5.50±0.03	5.52±0.16	5.63±0.13	5.83±0.12	5.91±0.24	5.71a±0.23
5%CO <sub>2</sub> 4%O <sub>2</sub>	5.87±0.28	5.49±0.16	5.44±0.04	5.49±0.08	5.48±0.11	5.60±0.08	5.56b±0.20
6%CO <sub>2</sub> 4%O <sub>2</sub>	5.87±0.28	5.50±0.07	5.48±0.08	5.60±0.12	5.52±0.05	5.68±0.14	5.61ab±0.19
7%CO <sub>2</sub> 4%O <sub>2</sub>	5.87±0.28	5.52±0.11	5.46±0.13	5.44±0.09	5.44±0.02	5.54±0.06	5.55b±0.19
8%CO <sub>2</sub> 4%O <sub>2</sub>	5.87±0.28	5.48±0.07	5.57±0.02	5.45±0.06	5.54±0.10	5.53±0.06	5.58ab±0.18
Averages	5.87A±0.24	5.50B±0.08	5.49B±0.01	5.52B±0.12	5.56B±0.16	5.65B±0.18	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 7 - Firmness (gf.cm<sup>-2</sup>) of ‘Giombo’ persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatment	Storage days						Averages
	0	7	14	21	28	35	
Control	671±48	497±26	515±295	568±207	378±204	378±320	501 b±209
5%CO <sub>2</sub> 4%O <sub>2</sub>	671±48	579±86	651±75	786±43	627±76	732±253	674 ab±123
6%CO <sub>2</sub> 4%O <sub>2</sub>	671±47	652±49	780±65	766±38	627±89	691±199	698 a±100
7%CO <sub>2</sub> 4%O <sub>2</sub>	671±47	607±84	634±47	673±169	551±108	647±179	630 ab±1084
8%CO <sub>2</sub> 4%O <sub>2</sub>	671±47	555±13	661±142	684±170	503±91	694±126	628 ab±1203
Averages	671AB±404	578AB±735	648AB±157	695A±145	537B±141	628AB±232	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

The activities of the enzymes PME and PG exhibited different tendencies during the 35 days of storage, as shown in Tables 8 and 9. In a previous study, enzyme activities decreased, remained constant or increased during maturation, depending on the fruit and method of analysis (Lima *et al.*, 2006). For the fruits tested in this study, there was a nearly linear increase in PME activity with storage time. However, there was little difference in the PME activities of fruits that were exposed to 6, 7 and 8% CO<sub>2</sub>. A reduction in PG activity was also seen. It has been suggested that this can be explained by the lack of substrate for the enzyme or the existence of other multi-enzyme complexes (Abeles and Takeda, 1989). Enzymes such as  $\beta$ -galactosidase and other cellular proteins could be acting in the destruction of the cell walls of the fruits, causing the extravasation of cellular fluids. It is also possible that proteases could have catalyzed the hydrolysis of PG.

Antunes *et al.* (Antunes *et al.*, 2006) measured the activities of PG and PME in blackberries (*Rubus spp.*) that were stored in different environments for different times. They concluded that the activity of PME increased during storage for all cultivars and storage conditions, while the activity of PG decreased. This was confirmed in the present study on persimmons.

There was a slow decrease in the amount of reducing sugars beginning on the 28th day of storage, as shown in Table 10.

The fruits had ascorbic acid levels ranging from 15.6 to 40.4 mg per 100 ml, as shown in Table 11. The amount of ascorbic acid decreased with time. Silva *et al.*, (2011) evaluated the quality of 'Fuyu' persimmons and verified that covering them with wax did not affect the levels of ascorbic acid. According to Chitarra and Chitarra (2005) vitamin C tends to decrease during the ripening and stor-

Table 8 - Pectin methylesterase (UE.min<sup>-1</sup>.g<sup>-1</sup> of fresh fruit) of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatment	Days of Storage					
	0	7	14	21	28	35
Control	1343aF±85	3782aC±145	3320cE±140	3490aD±155	10898aA±385	10675aB±375
5%CO <sub>2</sub> 4%O <sub>2</sub>	1343aF±85	2766bD±130	6081aA±415	2814cC±133	2057dE±125	4830bB±225
6%CO <sub>2</sub> 4%O <sub>2</sub>	1343aF±85	2446dB±115	2520dA±130	1881eD±114	2074cC±125	1811eE±100
7%CO <sub>2</sub> 4%O <sub>2</sub>	1343aF±85	2607cA±125	22389eB±128	1913dE±123	2056eC±125	1945dD±105
8%CO <sub>2</sub> 4%O <sub>2</sub>	1343aF±85	1751eE±102	3879bA±149	3150bB±150	2174bD±130	2189cC±185

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 9 - Polygalacturonase (min<sup>-1</sup>.g<sup>-1</sup> of fresh fruit) of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatment	Storage days					
	0	7	14	21	28	35
Control	890 aA±143	548±95 aA	591±55 aB	341±55 bC	213±40 aC	206±86 aB
5%CO <sub>2</sub> 4%O <sub>2</sub>	890 aA±143	453±60 aB	239±37 bC	473±71 bB	243±72 aB	143±84 bC
6%CO <sub>2</sub> 4%O <sub>2</sub>	890 aA±143	89±4 bC	200±39 bC	648±14 aA	257±88 aA	394±15 aA
7%CO <sub>2</sub> 4%O <sub>2</sub>	890 aA±143	432±268 aB	257±40 bC	374±30 bB	80±33 dD	175±43 cC
8%CO <sub>2</sub> 4%O <sub>2</sub>	890 aA±143	333±25 aB	673±24 aA	241±83 cC	166±132 bC	102±47 bC

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 10 - Reducing sugars (%) in 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatment	Storage days						Average
	0	7	14	21	28	35	
Control	13.3±1.8	12.1±2.1	13.9±1.6	13.5±1.1	10.8±0.5	11.1±0.4	12.4±1.7
5%CO <sub>2</sub> 4%O <sub>2</sub>	13.3±1.8	13.5±1.3	13.7±0.9	13.9±2.0	12.4±0.6	10.9±0.6	13.0±1.5
6%CO <sub>2</sub> 4%O <sub>2</sub>	13.3±1.8	15.2±1.2	13.8±0.9	12.7±0.5	11.6±0.4	10.0±1.4	12.8±2.0
7%CO <sub>2</sub> 4%O <sub>2</sub>	13.3±1.8	16.0±1.5	13.6±0.7	13.1±0.8	12.0±0.5	11.1±0.5	13.2±1.8
8%CO <sub>2</sub> 4%O <sub>2</sub>	13.3±1.8	14.1±0.6	14.1±1.1	13.0±1.6	12.2±0.9	10.6±0.4	12.9±1.6
Average	13.3±1.8	14.2A±1.9	13.8A±0.93	13.2A±1.2	11.8B±0.8	10.7B±0.8	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

age of many fruits, due to the action of ascorbic acid oxidase, other oxidative enzymes and/or peroxidases.

The astringency index of 'Giombo' persimmons that had their tannins removed and were exposed to modified atmospheres and were stored at 0°C had an index of 1 (no tannins) until the end of storage, which is in agreement with results reported by Núñez-Delicado *et al.* (2003) who reported that ethanol dissolves the tannins.

Refrigerated storage in active modified atmospheres is effective in preserving 'Giombo' persimmons. Fruits exposed to 7 or 8% CO<sub>2</sub> gave the best results. This work should not be taken as reflecting FDA policy or regulations.

## References

ABELES F.B., TAKEDA F., 1989 - *Increased cellulase activity during blackberry fruit ripening*. - HortSci., 24(5): 851.

AHMED E.A., LABAVITCH J.M., 1980 - *Cell wall metabolism in ripening. I cell wall changes in ripening "Bartlett" pears*. - Plant Physiol., 65: 1009-1013.

ALBERSHEIM P., NEVINS D.J., ENGLISH P.D., KARR A., 1967 - *A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography*. - Carbohydr. Res.; 5(3): 340-345.

ANTONIOLLI L.R., CASTRO P.R.C., KLUGE R.A., SCARPARE F.J.A., 2000 - *Remoção da adstringência de frutos de caqui 'Giombo' sob diferentes períodos de exposição ao vapor de álcool etílico*. - Pesq. Agropec. Bras., 35(10): 2083-2091.

ANTUNES L.E.C., GONÇALVES E.D., TREVISAN R., 2006 - *Alterações da atividade da poligalacturonase e pectinametilesterase em amora-preta (Rubus spp.) durante o armazenamento*. - Rev. Bras. Agroci., 12(1): 63-66.

BEN-AIRE R., ZUTKHI Y., 1992 - *Extending the storage life of 'Fuyu' persimmon by modified-atmosphere packaging*. - Hort Sci., 27: 811-813.

BLEINROTH E.W., ZUCHINI A.G., POMPEO R.M., 1976 - *Determinação das características físicas e mecânicas de variedade de abacate e sua conservação pelo frio*. - Coletânea ITAL, Campinas, 7(1): 29-81.

BLUM J., HOFFMANN F.B., AYUB R.A., JUNG D.L., MALGARIM M.B., 2008 - *Uso de cera na conservação pós-colheita do caqui cv. Giombo*. - Rev. Bras. Fruticult. Jaboticabal, SP, 30(3): 830-833.

BRACKMANN A., MAZARO S.M., SAQUET A.A., 1997 - *Frigoconservação de caquis (Diospyrus kaki, L.) das cultivares Fuyu e Rama Forte*. - Ciência Rural, Santa Maria, 27(4): 561- 565.

BRACKMANN A., SAQUET A.A., 1995 - *Efeito da temperatura e condições de atmosfera controlada sobre a conservação de caqui (Diospyrus kaki L.)*. - Rev. Ciência Rural, Santa Maria, 25: 375-378.

CAMPO-DALL'ORTO F.A., OJIMA M., BARBOSA W., ZULLO M.A.T., 1996 - *Novo processo de avaliação da adstringência dos frutos no melhoramento do caquizeiro*. - Bragantia Campinas, 55: 237-243.

CANDIR E.E., OZDEMIR A.E., KAPLANKIRAN M., TOPLU C., 2009 - *Physico-chemical changes during growth of persimmon fruits in the east Mediterranean region*. - Scientia Horticulturae, 121: 42-48.

CELIK A., ERCISLI S., 2008 - *Persimmon cv. Hachiya (Diospyros kaki Thunb.) fruit: some physical, chemical and nutritional properties*. - Intl. J. Food Sci. Nutr., 59: 599-606.

CHITARRA M.I.F., CHITARRA A.B., 2005 - *Pós-colheita de frutos e hortaliças: fisiologia e manuseio*. - Ed. UFLA, Lavras, Minas Gerais, Brasil, pp. 785.

COSTA A.F.S., BALBINO J.M.S., 2002 - *Características da fruta para exportação e normas de qualidade*, pp. 12-18. - In: FOLEGATTI M.I.S., and F.C.A.U. MATSUURA (eds.) *Mamão: pós-colheita. Papaya: Post-harvest Embraapa Informação Tecnológica*. Brasília, DF, Série Frutas do Brasil, 21.

DANIELI R., GIRARDI C.L., PARUSSOLO A., FERRI V., ROMBALDI C., 2002 - *Efeito da aplicação de ácido giberélico e cloreto de cálcio no retardamento da colheita e na conservabilidade de caqui*. - Fuyu. Rev. Bras. Fruticult. Jaboticabal, SP, 24(1): 44-48.

DEL BUBBA M., GIORDANI E., PIPPUCCI L., CINCINELLI A., CHECCHINI L., GALVAN P., 2009 - *Changes in tannins, ascorbic acid and sugar content in astringent persimmons during on-tree growth and ripening and in response to different postharvest treatments*. - J. Food Comp. Anal., 22: 668-677.

DEMBITSKY V.M. POOV., DEMBITSKYA V.M., POOVARODOM S., LEONTOWICZ H., LEONTOWICZ M., VEARASILP SU., TRAKHTENBERG S., GORINSTEIN S., 2011 - *The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites*. - Food Res. Intl., 44(7): 1671-1701.

Table 11 - Ascorbic Acid (mg 100 ml<sup>-1</sup>) in 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatment	Storage days						Averages
	0	7	14	21	28	35	
Control	38±11	44±4	33±7	34±15	20±9	18±7	31±13
5%CO <sub>2</sub> 4%O <sub>2</sub>	38±11	38±2	37±9	37±19	24±11	13±3	31±13
6%CO <sub>2</sub> 4%O <sub>2</sub>	38±11	36±3	34±7	33±18	21±2	18±2	30±11
7%CO <sub>2</sub> 4%O <sub>2</sub>	38±11	46±13	30±6	43±22	36±20	13±0	34±16
8%CO <sub>2</sub> 4%O <sub>2</sub>	38±11	39±2	41±2	19±2	41±11	16±2	32±12
Averages	38AB±9	40A±7	35AB±67	33AB±16	28BC±13	16C±4	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

- DONAZZOLO J., BRACKMANN A., 2002 - *Efeito do CO<sub>2</sub> em atmosfera controlada na qualidade de caqui (Diospyros kaki, L.) Cv. Fuyu*. - Rev Bras. Agroci. Pelotas, 8: 241-245.
- EDAGI F.K., CHIOU D.G., TERRA F.A.T., SESTARI I., KLUGE R.A., 2009 - *Remoção da adstringência de caquis 'Giombo' com subdosagens de etanol*. - Ciênc. Rural, Santa Maria, 39: 2022-2028.
- FERRI V.C., RINALDI M.M., DANIELLI R., LUCCHETTA L., ROMBALDI C.V., 2004 - *Atmosfera modificada na conservação de caquis (Diospyros kaki, L.) cultivar Fuyu*. - Rev. Bras. Agroci., 10: 111-115.
- FINGER F.L., VIEIRA G., 2002 - *Controle da perda pós colheita de água em produtos hortícolas*. - UFV, Viçosa, pp. 29.
- GAZIT S., LEVY Y., 1963 - *Astringency and its removal in persimmon*. - Israel J. Agr. Res., 13(3): 125-132.
- GIORDANI E., DOUMETT S., NIN S., DEL BUBBA M., 2011 - *Selected primary and secondary metabolites in fresh persimmon (Diospyros kaki Thunb.): A review of analytical methods and current knowledge of fruit composition and health benefits*. - Food Res. Intl., 44: 1752-1767.
- GOMES F.P., 2000 - *Curso de estatística experimental. 14. ed.* - Fundação de Estudos Agrários Luiz de Queiroz FEALQ, Piracicaba, Brazil.
- GORINSTEIN S., BARTNIKOWSKA E., KULASEK G., ZEMSER M., TRAKHTENBERG S., 1998 - *Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol*. - J. Nutr., 128: 2023-2027.
- GORINSTEIN S., LEONTOWICZ H., LEONTOWICZ M., JESION I., NAMIESNIK J., DRZEWIECKI J., PARK Y.S., HAM K.S., GIORDANI E., TRAKHTENBERG S., 2011 - *Influence of two cultivars of persimmon on atherosclerosis indices in rats fed cholesterol-containing diets: Investigation in vitro and in vivo*. - Nutr., 27(7-8): 838-846.
- GU H.-F., LI C.-M., XU Y.-J., HU W.-F., CHEN M.-H., WAN Q.-H., 2008 - *Structural features and antioxidant activity of tannin from persimmon pulp*. - Food Res. Intl., 41: 208-217.
- LIMA M.A.C., ALVES R.E., FILGUEIRAS, H.A.C. 2006 - *Mudanças relacionadas ao amaciamento da graviola durante a maturação pós-colheita*. - Pesq. Agropec. Bras., 41(12): 1707-1713.
- MAPA, 2011 - *Modified Tillman's Method*. - Ministério da Agricultura, Pecuária e Abastecimento <http://www.agricultura.gov.br>.
- MARTÍNEZ-CALVO J., NAVAL M., ZURIAGA E., LLÁCER G., BADENES M.L., 2012. Genet. Resour. Crop. Evol. Published on-line.
- MARTINS F.P., PEREIRA F.M., 1989 - *Cultura do caquizeiro. Jaboticabal*. - FUNEP, pp. 71.
- MURRAY R., VALENTINI G., 1998 - *Storage and quality of peach fruit harvest at different stages of maturity*. - Acta Horticulturae, 465: 455-463.
- NELSON N.A., 1944 - *A photometric adaptation of Somogy method for the determination of Glucose*. - J. Biol. Chem., 153: 375-380.
- NÚÑEZ-DELICADO E., SOJO M.M., GARCÍA-CARMONA F., SÁNCHEZ-FERRER A., 2003 - *Partial Purification of latent persimmon fruit polyphenol oxidase*. - J. Agric. Food Chem., 51: 2058-2063.
- ODAIR Z., NEUS S.P., TIGLEA P., 2008 - *Métodos físico-químicos para análise de alimentos. Physical-chemical methods for analyzing foods*. - Instituto Adolfo Lutz, São Paulo, Brasil.
- SARGENT S.A., CROCKER T.E., ZOELLNER J.J., 1993 - *Storage characteristics of 'Fuyu' persimmons*. - Proc. Florida State Hort. Soc., 106: 131-134.
- SILVA M.C., ATARASSI M.E., FERREIRA M.D., MOSCA M.A., 2011 - *Qualidade pós-colheita de caqui 'Fuyu' com utilização de diferentes concentrações de cobertura comestível. Postharvest quality of 'Fuyu'*. - Ciênc. Agropec. Lavras, 35(1): 144-151.
- SOMOGY M., 1945 - *Determination of blood sugar*. - J. Biol. Chem., 160: 69-73.
- USDA, 2012 - *Germplasm Resources Information Network (GRIN)*. - Website: <http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?14293>
- VEBERIC R., JURHAR J., MIKULIC-PETKOVSEK M., STAMPAR F., SCHMITZER V., 2010 - *Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (Diospyros kaki L.)*. - Food Chem., 119: 477-483.
- VIEITES R.L., PICANÇO N.F.M., DAIUTO É.R., MORAES M.R., 2012 - *Optimum temperature and state of maturity for storing persimmons, Diospyros kaki L., caqui 'Giombo'*. - Nat. Prod. J., 2: 180-187.
- VITTI D.C.C., 2009 - *Destanização e armazenamento refrigerado de caqui «Rama Forte» em função da época de colheita*. - PhD, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brasil.