CHANGES DURING STORING AND ASTRINGENCY REMOVAL OF PERSIMMON FRUIT (*DIOSPYROS KAKI* L.)

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Physiological changes during ripening, storing and astringency removal of persimmon fruits were traced. The fruits were stored under normal (NA), controlled (CA) and vacuum atmospheres (VA). The fruits were stored for 100 days and analysed for firmness, acetaldehyde and ethanol content and soluble tannins, both before and after storing. The same analyses were done during the deastringency treatment carried out with a high CO_2 concentration (99.99%) for 20 h at 20 °C. The persimmon fruits stored under NA, CA, VA or treated with high CO_2 for 20 h accumulated acetaldehyde and ethanol in the fruit tissue. CA conditions caused the highest acetaldehyde accumulation; vacuum conditions the lowest. Ethanol content increased 20 fold during storage; the highest accumulation was observed in vacuum stored fruit followed by CA (3% CO_2–2% O_2 and 0.5% CO_2–2% O_2) conditions. Astringency removal treatment caused an immediate increase of acetaldehyde and ethanol, nearly to the same extent as in conventionally ripened or stored fruit. The amount of soluble tannins, the main cause of an astringent taste, decreased during storage, and it did much faster during deastringency removal at the same level. The sensory evaluation test revealed that fruit treated with high CO_2 was preferred to conventionally ripened fruit.

Keywords: acetaldehyde, astringency removal, ethanol, persimmon, storing, tannins

Persimmon fruit (*Diospyros kaki* L.) originates from Southeast Asia, but is now cultivated in other regions with suitable climatic conditions. Its popularity is most prominent in Japan, where it ranks fifth in fresh fruit production with non-astringent cultivars. Persimmon has at least 1000 cultivars, which are classified into the following four groups:
– Non-astringent and pollination constant (PCNA) cultivars have dark tannin spots.
– Non-astringent and pollination variant (PVNA) cultivars have dark tannin spots and may become astringent when seedless.
– Astringent and pollination constant (PCA) cultivars have no dark tannin spots.
– Astringent and pollination variant (PVA) cultivars may become non astringent when pollinated (HULME, 1971).

Non-astringent cultivars are consumed as fresh fruits, but astringent cultivars are edible only after the removal of astringency or as a dried fruit (ITOO, 1971). Non-astringent cultivars contain up to 1% soluble tannins, which start to decrease before harvest and ripening. The astringent cultivars contain many more soluble tannins than non-astringent cultivars at their maximum value in July, then they decrease gradually during on-tree maturation and reach 2% at harvest time (ITOO, 1971).

The astringent type persimmon fruit is not edible at harvest due to the presence of soluble tannins that cause an astringent taste in the mouth (KAYS, 1991). Astringent taste is a consequence of the coagulation of tannins with salivary proteins that results in insoluble rough complexes. Soluble tannins are made up from catechin, catechin-3-galate, galocatechin and galocatechin-3-galate (MATSUO & ITOO, 1978). Tannins are also found in other plant parts; ehtyl acetate extract from persimmon leaves contains catechin and galocatechin (SEONG, 1986). The fruit becomes edible during postharvest ripening, when soluble tannins polymerise and become insoluble (MATSUO & ITOO, 1982). Normal postharvest ripening during shelf life includes natural tannin polymerisation, but also fruit softening; edible naturally non-astringent fruits are very soft and thus susceptible to mechanical and microbiological spoilage.

On-tree fruit ripening is associated with the accumulation of acetaldehyde (AA) and ethanol (ET) in postclimacteric 'Bartlett' pears (KE & VAN GORSEL, 1990) and cherries (VIDRIH et al., 1998). A much higher accumulation of acetaldehyde and ethanol occurs during anaerobic metabolism at very low O_2 and/or high CO_2 concentration (>80%) in persimmons (PESIS et al., 1988) or cherries (VIDRIH et al., 1998). Low O_2 stressed 'Bartlett' pears increase the synthesis of acetaldehyde and ethanol (KE & VAN GORSEL, 1990).

Besides natural deastringency that occur during normal ripening of persimmon fruit, the absence of O_2 (balance N_2) or CO_2 content above 80% provokes the astringency removal in persimmon fruit (PESIS & BEN-ARIE, 1984). CO_2 enriched atmospheres provoke the accumulation of acetaldehyde and ethanol in Bartlett pears when CO_2 exceeds 50% (KE & VAN GORSEL, 1990). PESIS and BEN-ARIE (1984), PESIS and co-workers (1988), demonstrated a positive correlation between the atmospheric CO_2 content and the accumulation of acetaldehyde in fruit tissue. The ethanol accumulation did not follow the pattern of acetaldehyde; the final ethanol concentration was nearly the same, independent of CO_2 concentration. PESIS and BEN-ARIE (1984) suggested that CO_2 depresses the conversion of acetaldehyde to ethanol. Higher acetaldehyde content enables the most effective astringency removal. A higher CO_2 concentration always produces more acetaldehyde and makes astringency removal faster. Temperature plays an important role in anaerobic metabolism. Higher temperature makes commodities more susceptible to anaerobic metabolism; more acetaldehyde and ethanol accumulate at higher temperatures.

The storing of fruit in acetaldehyde vapours causes the accumulation of both acetaldehyde and ethanol to the same extent as in fruit stored in N_2 or in CO_2. On the other hand, ethanol vapours provoke the accumulation of both acetaldehyde and ethanol in fruit tissue. Fruits are capable of transforming the exogenous acetaldehyde into ethanol and vice versa, as demonstrated by VIDRIH and co-workers (1999).

Acetaldehyde is much more toxic to the fruit cell, compared to ethanol. To prevent injuries, the cell transforms toxic acetaldehyde into the less toxic ethanol that is the final product of anaerobic respiration. Many injuries are known to be associated with acetaldehyde accumulation in pome fruits (WILKINSON & FIDLER, 1973); CO_2 injuries in the apple (SMAGULA & BRAMLAGE, 1977). When anaerobic conditions last too long, fruit damage may result such as blossom-end browning, skin browning or internal breakdown.

Naturally non-astringent cultivars contain ten times more acetaldehyde than astringent cultivars (NAKAMURA, 1973). Acetaldehyde and ethanol contents were much higher in PCNA or PVNA cultivars than in PVA or PCA cultivars at harvest (CHOI, 1977, CHOI & SOHN, 1977). On the other hand, PVA and PCA cultivars have much higher amount of soluble tannins (SUGIURA et al., 1979) and thus are astringent. Acetaldehyde and ethanol concentration in seeds usually follows that in mesocarp; some authors propose that seeds are the primary source of acetaldehyde and ethanol (SUGIURA et al., 1979). Acetaldehyde is known to play a decisive role in the process of natural or high CO_2 induced de-astringency (MATSUO & ITOO, 1977, SUGIURA et al., 1979, SUGIURA & TOMANA, 1983, MATSUO & ITOO, 1981a, b, MATSUO & ITOO, 1982).

Kaki tannin polymerises by reacting with acetaldehyde in vitro, as demonstrated by MATSUO and ITOO (1982). pH plays an important role; a low pH (below 3.5) causes rapid tannin gelation; the minimum rate of gelation was observed at pH 4–5; gelation was again stimulated in the 6–8 pH range (MATSUO & ITOO, 1982). In vitro tannin polymerisation within persimmon fruit tissue was demonstrated to depend mostly on acetaldehyde concentration.

The aim of this work was to study the post harvest behaviour of persimmon cultivars grown in Slovenia. The emphasis was given to the processes accompanying the removal of astringent taste, either during the storage of persimmon fruit or during its exposure to high CO_2 treatment.

1. Materials and methods

1.1. Raw material

Six cultivars (Fuji, Hachyia, Lycopersicon, Kaki Tipo, Kurokuma, and Thiene) of persimmon fruit (*Diospyros kaki*, L.) were harvested at the end of October 1997 in commercial orchard. According to BELLINI (1982) cultivars Fuji, Hachyia and Lycopersicon belong to astringent pollination constant group (PCA), while Kaki Tipo, Kurokuma and Thiene belong to astringent pollination variant (PVA) group. At harvest all the fruits were still astringent, firm, similar in size and colour and free of diseases.

1.2. Storage conditions

Each cultivar (40 kg) was stored in a normal atmosphere (NA) at 0 °C in air at 90–95% relative humidity, vacuum atmosphere (70 mm Hg) (VA) or under two different controlled atmosphere (CA) conditions. Controlled atmosphere storage was conducted in a plastic container (1 m^3); fruits were sealed in a plastic container (1 m^3) and a desirable atmosphere was established by streaming the O_2 out by means of N_2. The desired CO_2 level was achieved by means of fruit respiration. Two different CA conditions (3% CO_2–2% O_2) and (0.5% CO_2–2% O_2) were performed; CA conditions were monitored and controlled by a computer (Fruit Controll, Italy).

The vacuum atmosphere (VA) was established in aluminum jars (20 l) by the constant working of a vacuum pump. The fruit was put into the jars, and the desired pressure was maintained at 70 mm Hg by means of controlling the air flow into each jar. The air was continually passed through the water in order to maintain the highest possible relative humidity content in the jars.

The fruits were stored for 100 days.

1.3. Method for astringency removal

Concentration of soluble tannins decrease slowly during normal ripening of persimmon fruit in air at ambient temperature, but is known to decrease more rapidly in a so called deastringency treatment. In the deastringency treatment, the fruits (30 kg of each cultivar) were transferred to 20 °C on the day of harvest. They were sealed in a plastic container (1m^3); a desirable atmosphere (99.99% CO_2) was established by streaming the O_2 out with CO_2. The fruits were treated for 20 h at 20 °C, then transferred to air and maintained at 20 °C for an additional 72 h.

1.4. Determination of firmness

Fruit firmness was measured with a penetrometer (Chatillon, USA) equipped with an 11 mm plunger on the peeled fruit; for each treatment 10 fruits were assayed, and four measurements were made on each fruit.

1.5. Determination of acetaldehyde and ethanol

Acetaldehyde and ethanol contents were measured according to the method described by BONGHI and co-workers (1999). Fruit tissue was frozen under liquid N_2 and kept at –20 °C until further use. Ten g of tissue was homogenised under liquid N_2 and mixed with 10 g of water. The homogenate was then centrifuged; 5 ml of the supernatant was pipetted into a vial, tightly closed with a septum and incubated at 55 °C. One ml of head space gas was taken after half an hour and injected into GC. Five different concentrations of acetaldehyde and ethanol were analysed in the same way in order to establish a calibration curve. The acetaldehyde and ethanol were analysed on a GC (Hewlett Packard 5890 II) equipped with an HP-FFAP (25 m×0.2 mm×0.3 µm) column and FID detector. The temperature programming was isothermal at 40 °C.

1.6. Determination of soluble tannin

Folin-Ciocalteau method was used with gallic acid standards at 6 different concentrations ranging from 20 to 500 mg l^{-1}. Ten g of fruit was homogenized with 10 g of water by means of Ultraturax; suspension was then filtered through four layers of cheese cloth and centrifuged ($2000 \times g$). Supernatant (0.1 ml) was mixed with 1.9 ml of water and 10 ml of Folin-Ciocelteau (1:3) and allowed to stand for 8 min; finally 8 ml of Na_2CO_3 (20%) was added. Absorbance at 765 nm was recorded after 2 h.

1.7. Sensory evaluation

A comparative sensory evaluation by 15 trained panellists was performed. The panellists compared the fruits of the cultivar Fuji exposed to the astringency removal treatment (high CO_2, see 1.3) to naturally ripened fruits of the same cultivar. Naturally ripened fruits were stored in air at 20 °C until an edible taste (not astringent) was achieved.

1.8. Determination of the activity of alcohol dehydrogenase

Alcohol dehydrogenase activity was measured according to the method described by BONGHI and co-workers (1999).

Five g of the mesocarp was homogenized in 10 ml of extraction buffer consisting of 100 mM KH_2PO_4 pH 7.5, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol,

0.2% Triton X-100, 1% PVPP with ultraturax. The homogenate was filtered through four layers of cheese cloth and centrifuged at 18000 $\times g$ for 20 min.

The reaction mixture consisted of 0.68 ml of glicine buffer pH 9.0, 0.07 ml NAD$^+$ 11.4 mM, 0.15 ml of ethanol 2 M and 0.1 ml of enzyme extract.

The reduction of NAD$^+$ was measured by recording the increase in absorbancy at 340 nm.

1.9. Evaluation of data

The data (from three determinations of each treatment) were analysed by the method of the least squares using the GLM procedure (SAS, 1990). The statistical model for parameters included the effects of M, C and D (Model 1).

$$y_{ijkl} = \mu + M_j + C_j + D_k + e_{ijkl} \tag{1}$$

where y_{ijkl} the observation ijkl, μ: general mean, M: effect of methode i (i=1 NA, i=2 CA 0.5% CO_2–2% O_2, I=3 3% CO_2–2% O_2, i=4 vacuum), C_j = effect of cultivar j (j = 1, 2, 3, 4, 5, 6), D_k: effect of determination k (k = 1, 2, 3), and e_{ijk} = residual random term with variance σ^2_e.

In addition, in the analysis of astringency removal, the statistical model 2 included the effect of T, C and D:

$$y_{ijkl} = \mu + T_j + C_j + D_k + e_{ijkl} \tag{2}$$

where T_j = effect of time i after treatment (i = 1 beginning, i = 2 20 h, i = 3 92 h), all the other parameters have the same meaning as above.

Least square means for experimental groups were obtained using the LSM procedure (SAS, 1990) and were compared at the 5% probability level.

2. Results and discussion

2.1. Firmness

The most rapid decrease of firmness occurred in the fruit stored in NA. Both CA storage conditions had a beneficial effect on keeping fruit firmness, as compared to the NA system (Table 1). The vacuum had a more pronounced effect on fruit firmness retention; vacuum stored fruit had a four fold higher firmness compared to CA conditions, probably due to the low O_2 concentration within the fruit tissue. Persimmon fruit produces a relatively low amount of ethylene, which is permanently removed under vacuum conditions and cannot influence ripening. The CO_2 induced deastringency provoked a higher decrease of fruit firmness compared to the N_2 induced deastringency, as also observed by PESIS and BEN-ARIE (1984).

Table 1

Fruit firmness (N) of persimmon, before and after storing the fruit under different conditions

Cultivar	At harvest	After storage				F value
		1	2	3	4	
Fuji	71.6 ± 4.0	<15	21.6 ± 2.2	–	–	
Hachyia	76.5 ± 5.9	<15	30.4 ± 4.4	31.4 ± 3.2	–	0.11
Kaki Tipo	102.0 ± 9.7	<15	15.7^4 ± 1.5	17.7^4 ± 0.9	67.7 ± 5.7	221.74***
Lycopersicon	100.0 ± 9.5	<15	30.4 ± 4.0	27.5 ± 1.9	–	1.22
Thiene	102.0 ± 4.8	<15	30.4^3 ± 4.7	21.6 ± 2.0	–	8.68***

–: no data

***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$

Values represent the mean of ten fruits ±S.E.

1: NA; 2: CA 0.5% CO_2–2% O_2; 3: CA 3% CO_2–2% O_2; 4: VACUUM 70 mm Hg

The figures above averages show that they are significantly different from the treatment indicated by the figure

2.2. Tannin

The fresh parthenocarpic fruits contained from 1.6 to 4.1% soluble tannin at harvest (Table 2). A tannin concentration above 0.1% conferred an astringent taste; such fruits are not edible (SEONG, 1986). On the other hand, the fruit with seeds (at least 2) were much lower in tannin content (0.03%, Table 2), but still slightly astringent at the part most distant from the seeds. Seeds are involved in acetaldehyde production; the mesocarp located around the seed is not astringent. The fruit from all the storage conditions had a soluble tannin concentration below 50 mg/100 g after storing. CA conditions conferred a lower soluble tannins concentration because of partially anaerobic conditions, or a higher CO_2 concentration. Significant differences in tannin content were found among different storage conditions, vacuum stored fruit had the lowest tannin content, followed by CA 3% CO_2–2% O_2, CA 0.5% CO_2–2% O_2 and NA.

2.3. Content of acetaldehyde and ethanol

Acetaldehyde increases during the storage time (Table 3). Persimmon ripening involves acetaldehyde accumulation; this phenomenon is also known in other fruits. Significant differences in acetaldehyde content among the treatments were found except for the cultivar Lycopersicon. Aacetaldehyde concentration was lower in vacuum treatments because of the permanent removal of acetaldehyde due to its high volatility. PESIS and co-workers (1988) found the highest concentration of acetaldehyde in persimmon fruit enclosed in PE bags with CO_2 for 48 h, followed by vacuum treated fruits and N_2 treated fruits.

Table 2

Concentration of tannin in persimmon (mg/100 g f.w.) before and after storing the fruit under different storage conditions for 100 days

Cultivar	At harvest		After storage						F value
	Seedless	With seeds	1 Seedless		2 Seedless		3	4	
Fuji	4100 ± 740	–	$40.0^{2,4}$ ± 7.0		13.3^3 ± 1.7		33.3^4 ± 2.5	13.3± 1.5	39.85***
Hachyia	3400 ± 257	–	–		–		–	–	–
Kaki Tipo	2150 ± 330	17.0 ± 2.0	$33.3^{2,3,4}$ ± 4.6		20.0^4 ± 3.5		20.0^4 ± 2.6	6.7± 0.6	34.39***
Kurokuma	3250 ± 335	–	$120.0^{2,3}$ ± 9.2		26.7^3 ± 3.5		0	–	371.53***
Lycopersicon	1600 ± 122	–	$46.7^{2,3}$ ± 5.6		13.3^3 ± 1.7		26.7 ± 2.0	–	68.72***
Thiene	2600 ± 220	33.0 ± 4.0	46.7 ± 4.3		20.0 ± 6.0		0	0	38.55**

–: no data
f.w.: fresh weight
***P≤0.001; **P≤0.01; *P≤0.05
Values represent the mean of 3 fruits ±S. E.
1: NA; 2: CA 0.5% CO_2–2% O; 3: CA 3% CO_2–2% O_2; 4: VACUUM 70 mm Hg
The figures above averages show that they are significantly different from the treatment(s) indicated by the figure

Table 3

Concentration of acetaldehyde in persimmon tissue (mg/100g f.w.) before and after storing the fruit under different storage conditions for 100 days

Cultivar	At harvest	After storage				F value
		1	2	3	4	
Kaki Tipo	0.03 ± 0.01	$1.2^{3,4}$ ± 0.15	$1.2^{3,4}$ ± 0.17	1.7^4 ± 0.17	0.6± 0.06	26.40***
Lycopersicon	0.01 ± 0.005	$1.3^{2,3}$ ± 0.17	1.0 ± 0.08	0.8 ± 0.11	–	12.06**
Thiene	0.06 ± 0.008	0.8^3 ± 0.11	0.7^3 ± 0.17	1.3^4 ± 0.17	0.7± 0.17	9.42**

–: no data
Values represent the mean of 3 fruits ±S.E.
***P≤0.001; **P≤0.01; *P≤0.05
1: NA; 2: CA 0.5% CO_2–2% O_2; 3: CA 3% CO_2–2% O_2; 4: VACUUM 70 mm Hg
The figures above averages show that they are significantly different from the treatment(s) indicated by the figure

Ethanol concentration rises during the storage period, depending on the storage conditions (Table 4). The lowest ethanol content was found in fruit stored in NA, followed by CA 0.5% CO_2–2% O_2 and CA 3% CO_2–2% O_2. Additional CO_2 provoked a higher ethanol concentration than did only the CA 0.5% CO_2–2% O_2, which agrees with the observation of PESIS and BEN-ARIE (1984). Interestingly, the highest ethanol concentration was observed in fruit stored in a vacuum, probably because of a very low O_2 concentration. PESIS and co-workers (1988) also demonstrated a higher ethanol

accumulation in vacuum stored persimmon than in CO_2 treated fruit. On the other hand, PESIS and BEN-ARIE (1984) reported an inhibition of ADH with high CO_2 treatment and a consequently higher build-up of acetaldehyde.

2.4. Activity of alcohol dehydrogenase

ADH activity increased during the ripening of the control fruit or the CO_2 treated fruit (Fig. 1). No clear differences between ADH activity in the control fruit and the CO_2 treated fruit were observed. Increased ADH activity resulted in an ethanol accumulation, but there was no clear relationship between the ADH activity and ethanol accumulation.

Table 4

Concentration of ethanol in persimmon (mg/100 g f.w.) before and after storing the fruit under different storage conditions

Cultivar	At harvest	After storage				F value
		1	2	3	4	
Kaki Tipo	3.7 ± 0.06	40.9[2,3,4] ± 4.4	62.4[4] ± 8.2	69.8[4] ± 7.2	91.1 ± 7.4	38.09***
Lycopersicon	4.1 ± 0.17	50.4[2,3] ± 2.6	80.0[3] ± 5.3	90.4 ± 5.5	–	59.52***
Thiene	3.3 ± 0.30	41.2[3,4] ± 2.9	49.6[4] ± 4.3	57.7[4] ± 0.8	82.6 ± 6.8	43.70***

–: no data
Values represent the mean of 3 fruits ±S.E.
***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$
1:NA; 2: CA 0.5% CO_2–2% O_2; 3: CA 3% CO_2–2% O_2; 4: VACUUM 70 mm Hg
The figures above averages show that they are significantly different from the treatment(s) indicated by the figure
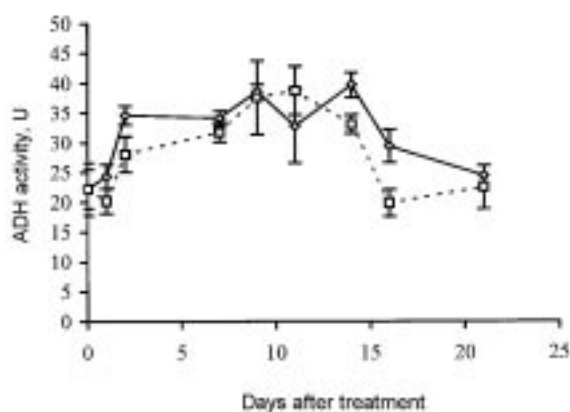

Fig. 1. Activity of alcohol dehydrogenase (U) of Fuji persimmon treated with CO_2 for 20 h (---□---) and control fruit stored in air (—◊—). Data represent means of 3 replicates ±SE

2.5. Astringency removal

The Lycopersicon and Thiene cultivars lost their astringent taste after 92 h during the astringency removal; tannin decreased to 20 mg/100 g (Table 5), the same level as during normal ripening (Table 2). The Kaki Tipo cultivar retained soluble tannins within 92 h, so it did not loose its astringent taste. This cultivar needs a longer (48 h) CO_2 exposure and additional storage at 20 °C for 72 h. A certain lag phase is required before the acetaldehyde builds up to a certain level and reacts with the tannins (GAZIT & ADATO, 1972). Acetaldehyde is formed in an enzymatic system from pyruvate and accumulates in cytosol; soluble tannins are present in vacuoles. Thus, it is impossible for the acetaldehyde to react directly with tannins (MATSUO & ITOO, 1977).

Table 5

The amount of soluble tannin in persimmon (mg/100 g f.w.) before treatment and after astringency removal (92 h from the beginning)

Cultivar	Before treatment	After 92 h	F value
Kaki Tipo	2350 ± 366	2350 ± 360	0.00
Lycopersicon	2600 ± 265	20 ± 2.6	285.25***
Thiene	2600 ± 300	20 ± 2.6	221.86***

Values represent the mean of 3 fruits.
***P≤0.001; **P≤0.01; *P≤0.05

Table 6

Concentration of acetaldehyde in persimmon (mg/100 g f.w.) before treatment, after 20 h of CO_2 treatment and after additional 72 h of storage at 20 °C

Cultivar	1	2	3	F value
Kaki Tipo	0.03[2,3] ± 0.005	2.9 ± 0.20	2.6 ± 0.20	258.04***
Lycopersicon	0.01[2,3] ± 0.005	1.4[3] ± 0.15	2.3 ± 0.20	184.61***
Thiene	0.06[2,3] ± 0.01	1.9 ± 0.20	1.7 ± 0.30	69.64***

Values represent the mean of 3 fruits ±S.E.
***P≤0.001; **P≤0.01; *P≤0.05
1: before treatment; 2: after 20 h in CO_2; 3: after 72 h in air (92 h from the beginning)
The figures above averages show that they are significantly different from the treatment(s) indicated by the figure

Table 7

Concentration of ethanol in persimmon (mg/100 g f.w.) before treatment, after 20 h of CO_2 treatment and after additional 72 h of storage at 20 °C

Cultivar	1	2	3	F value
Kaki Tipo	$3.7^{2,3} \pm 0.15$	$27.0^3 \pm 4.1$	46.0 ± 5.0	96.21***
Lycopersicon	$4.1^{2,3} \pm 0.20$	$15.4^3 \pm 1.1$	70.3 ± 6.0	302.43***
Thiene	$3.3^{2,3} \pm 0.40$	$22.3^3 \pm 0.7$	45.6 ± 4.2	218.00***

Values represent the mean of 3 fruits ±S.E.
***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$
1: before treatment; 2: after 20 h in CO_2; 3: after 72 h in air (92 h from the beginning)

Table 8

Sensory evaluation of Fuji cultivar carried out by 15 trained panellists. Ciphers represent the numbers of panellists who chose the given parameter

	Compared to the reference[a] sample, CO_2 treated fruit is						General
	sweet	sour	firm	juicy	aromatic	astringent	impression
much more	1		11	1	2		6
more	4	1	4	2	2		4
slightly more	4	5		1	2	4	1
equal	1	5		2	5	6	
slightly less	4	4		4	2	3	3
less	1			4	1	2	1
much less				1	1		

[a]: naturally ripened

Acetaldehyde becomes a substrate for alcohol dehydrogenase and is converted to ethanol, which builds in cytosol. Sodium bisulfite and dimedon, which are acetaldehyde-binding agents, inhibit astringency removal (MATSUO & ITOO, 1977). MATSUO and ITOO (1977) proposed a two stage deastringency process. In the first step, the fruit produces acetaldehyde via glycolysis. In the second step, acetaldehyde affects soluble tannins in the vacuoles and causes their polycondensation.

The acetaldehyde concentration rose within 20 h during the deastringency treatment, but did not change significantly afterwards with the exception of cultivar Lycopersicon (Table 6). Acetaldehyde accumulated during the anaerobic or high CO_2 treatment only, then remained at the same level or with few changes. Acetaldehyde also accumulates in persimmons treated with warm water (MATSUO & ITOO, 1977), or during chilling injuries when the temperature goes below 0 °C. Both hot water treated or chilled fruits are known to be non astringent. The fruit responded to stress caused by

chilling or high temperatures with an accumulation of acetaldehyde and the consequent polymerisation of tannins. More acetaldehyde accumulates during high CO_2 treatment than during normal ripening. PESIS and BEN-ARIE (1984) demonstrated that CO_2 and N_2 affect the acetaldehyde accumulation differently; CO_2 always produces more acetaldehyde, but may provoke internal browning when the fruit is exposed to high CO_2 for too long.

The ethanol increased after 20 h of CO_2 treatment, but its concentration increased significantly (nearly twofold) in the next 72 h (Table 7). Ethanol accumulation is the fruit tissue response to convert the toxic acetaldehyde into ethanol. More ethanol accumulated during normal ripening as compared to the high CO_2 treatment. The acetaldehyde accumulation was much slower during natural ripening, compared to the high CO_2 treatment; the higher acetaldehyde content caused tannin polymerisation in a much faster and efficient way. This agrees with the observation of PESIS and BEN-ARIE (1984) that CO_2 inactivates ADH, and thus prevents the conversion of acetaldehyde into ethanol.

2.6. Sensorial properties

The high CO_2 treated fruits were rather firm at the edible stage and preferred by the panelists to the naturally ripened fruits (Table 8). The CO_2 treated fruit were also considered less juicy compared to the control fruit. Such fruit can be stored at 0 °C to prolong their storage life with no risk of astringency reoccurring.

3. Conclusion

Natural ripening, storing or astringency removal by high CO_2 treatment provoke an increase of acetaldehyde, ethanol and a decrease of soluble tannins. Astringency removal is usually brought to an end in 92 h by means of high CO_2 treatment. The natural ripening takes much longer – about 30 days depending on the storage conditions to confer a non astringent taste. Some cultivars like Kaki Tipo may require longer CO_2 exposure to remove astringency efficiently. Anaerobic metabolism provoked by high CO_2 and/or low O_2 enable the accumulation of acetaldehyde, a substance responsible for tannin insolubilisation. Fruit firmness, an important attribute to pome fruits quality, does not change during astringency removal, although some authors have found a partial fruit softening. On the other hand, naturally ripened persimmon is very soft at the edible stage, and thus susceptible to mechanical damage, and later on, to microbiological spoilage.

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