



# Influence of persimmon astringency type on physico-chemical changes from the green stage to commercial harvest



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## ABSTRACT

Persimmon (*Diospyros kaki*, Thunb.) cultivars are currently classified into four astringency types based on two significant characteristics, astringency of fruit at harvest and variable pollination (PCNA, PVNA, PCA, PVA). The aim of this study was to evaluate the influence of astringency type on physicochemical changes occurring during persimmon maturation. To this end, unseeded fruits of 10 persimmon cultivars of four persimmon types were evaluated in three maturity stages according to skin colour: green, colour-breaking and orange-reddish. The Principal Component Analysis showed that soluble tannins content, total antioxidant capacity, total soluble solids and the accumulation pattern of individual sugars were affected by astringency type. During maturation, all the cultivars exhibited a decline of firmness, and also increased flesh carotenoids and acetaldehyde production. Such changes were cultivar-dependent rather than type-dependent. All the cultivars also shared a similar CO<sub>2</sub> production trend.

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## 1. Introduction

Persimmon (*Diospyros kaki* Thunb.) belongs to the *Diospyros* L. gender (Ebenaceae), and is believed to have originated in China, from there it was widely distributed worldwide from tropical to temperate regions with suitable climatic conditions, such as Brazil, Italy, Spain, Israel, Iran, New Zealand or Australia (FAOSTAT, 2012; Mowat and Collins, 2000). The persimmon cultivars exhibit a singular characteristic that is not common to other fruit trees; thus fruits from some persimmon cultivars are astringent at harvest, while others produce non-astringent fruits. Accordingly, persimmon cultivars are classified into two astringency categories, non-astringent and astringent cultivars, depending on the concentration of the soluble tannins present in pulp when fruits are harvested. Based on two significant persimmon characteristics (fruit astringency at harvest and variable pollination), persimmon cultivars are currently classified into four astringency types: Pollination Variant Non-Astringent (PVNA) (non-astringent cultivars when seeds are present); Pollination Constant Non-Astringent (PCNA) (non-astringent cultivars, regardless of the presence of

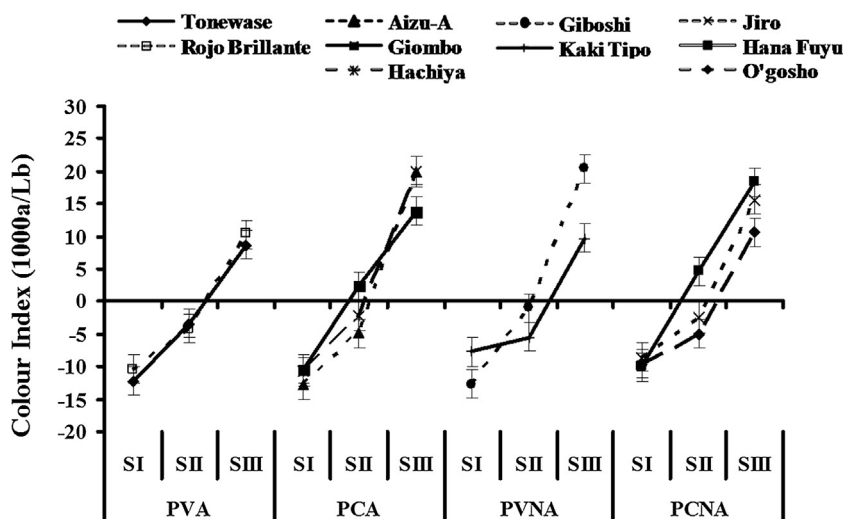
seeds); Pollination Variant Astringent (PVA) (astringent cultivars when fruit are seedless and mostly astringent when seeds are present); Pollination Constant Astringent (PCA) (astringent cultivars, regardless of the presence of seeds). 'Variant pollination' is expressed as the browning of the tissues surrounding locules when seeds are present, whereas flesh colour type is invariable with the 'pollination constant'.

As with most fruits, knowledge of the physicochemical changes associated with persimmon maturation is essential to not only determine the optimum maturity stage for harvesting, but also as a base to study postharvest behaviour. The response of persimmon fruits to different postharvest treatments has been shown to rely heavily on the fruit maturity stage at harvest (Besada et al., 2008,2010a). In line with this, some physicochemical changes, other than the evolution of tannins, that take place during the persimmon maturation process have been evaluated in specific cultivars, such as cv. Rojo Brillante, cv. Kaki Tipo or cv. Harbiye (Candir et al., 2009; Del Bubba et al., 2009; Salvador et al., 2007). These studies, which have related the evolution of external colour fruit (the non-destructive index currently used for harvesting persimmon fruit) with the internal changes during maturity, have provided useful information about these particular cultivars.

Nowadays, the impact of astringency type on changes in tannins during maturation is well-known. Yet very little information

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**Fig. 1.** External colour. Evolution of external colour (1000a/Lb) during the maturation of ten persimmon cultivars. Three maturity stages were evaluated: SI- green stage; SII-colour-break; SIII-characteristic homogeneous orange or orange-reddish tones. Vertical bars represent Tukey HSD-intervals when comparing all the cultivars and maturity stages ( $P < 0.05$ ).

is available about the influence of astringency type on the evolution of other relevant physicochemical parameters during the persimmon maturity process, especially for unseeded fruits, which are not affected by pollination type. Similarly after reviewing knowledge on persimmon composition, [Giordani et al. \(2011\)](#) claimed that the ripening stage of samples is of paramount importance in comparative studies among cultivars.

In this context, this study aimed to investigate the physicochemical changes associated with the maturity process of 10 persimmon cultivars that belong to the four persimmon types (PVNA, PCNA, PVA and PCA). To this end, the main physicochemical parameters were evaluated in unseeded fruits in three maturity stages: green coloured fruit, colour break stage and fruit exhibiting the orange-reddish commercial colour. A principal components analysis, an analysis of variance and a study of the correlations between parameters were performed to obtain as much information as possible from the data.

## 2. Materials and methods

### 2.1. Plant material and experimental design

The following cultivars were studied in the present work: 'Rojo Brillante' and 'Tonewase' (PVA-type cultivars); 'Aizumishirazu-A', 'Giombo' and 'Hachiya' (PCA-type cultivars); 'Giboshi' and 'Kaki Tipo' (PVNA-type cultivars); 'Jiro', 'O'gosho' and 'Hana Fuyu' (PCNA-type cultivars). They all form part of the persimmon germplasm collection hosted by the Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencia), Spain. Cultivars classification is according to [Naval et al. \(2010\)](#).

From August to December (2012), 30 unseeded fruits from each cultivar were harvested in three different maturity stages (maturity stage 1 (SI): fruit with a homogenous green skin; maturity stage 2 (SII): colour-break stage, fruits showing a yellowish-green skin; maturity stage 3 (SIII): commercial maturity, fruits showing no visible green background, but the characteristic orange or orange-reddish colour of each cultivar). After harvest, fruits were taken to the IVIA, where they were carefully evaluated in order to perform three homogeneous replicates of seven fruits free of damage for each cultivar and maturity stage. Then measurements of external colour, firmness, carbon dioxide and ethylene, and total soluble solids, were taken. Besides, fruit samples were frozen to

determine acetaldehyde and ethanol production, soluble tannin content, total and individual sugars, total antioxidant capacity and total carotenoids.

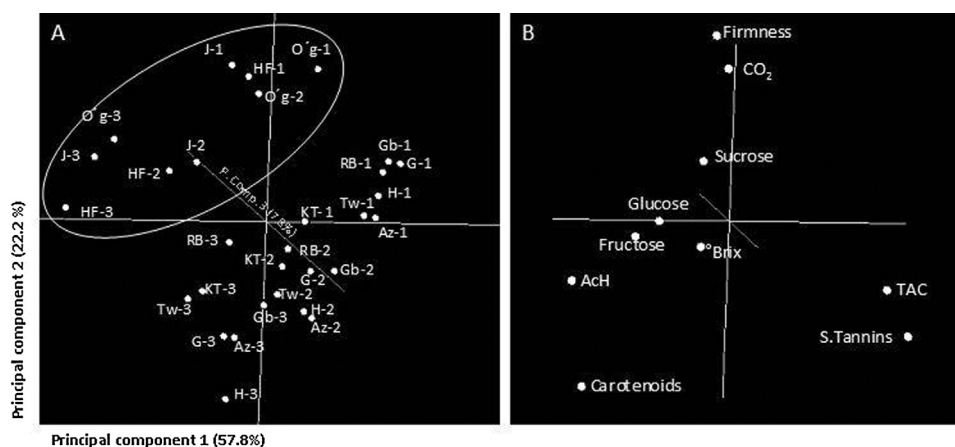
### 2.2. Evaluation of the physicochemical parameters

External colour was determined in a Minolta colorimeter (Model CR-300 Ramsey, NY, USA) on three replicates of seven fruits each by taking two measurements on opposite equatorial areas on each fruit. Hunter parameters 'L', 'a', 'b', were measured and the results were expressed as Colour Index =  $1000a/Lb$ , which accurately reflect the skin colour changes of persimmon fruits ([Salvador et al., 2007](#)). Respiration rate ( $CO_2$  production) and ethylene production were measured with three replicates of individual fruits, which were incubated in hermetic 1-l jars for 2 h at  $20^\circ C$ . One ml of air from the headspace was withdrawn with a hypodermic syringe and injected into a PerkinElmer gas chromatograph equipped with a Poropak QS 80/100 column. A thermal conductivity detector was used to determine carbon dioxide. Helium was the carrier gas used at 9.2 psi. The injector, oven and detector temperatures were  $115^\circ C$ ,  $35^\circ C$  and  $150^\circ C$ , respectively. A flame ionization detector was used to determine ethylene. Helium was the carrier gas at 8 psi. Injector, oven, and detector temperatures were 175, 75 and  $175^\circ C$ , respectively;  $CO_2$  production was expressed as  $ml\ CO_2\ kg^{-1}\ h^{-1}$  and ethylene production as  $\mu l\ C_2H_4\ kg^{-1}\ h^{-1}$ .

Flesh firmness was evaluated at harvest by a Texturometer Instron Universal Machine (model 4301, instron Corp., Canton, MA, USA) using an 8-mm plunger. The results were expressed as load in Newtons (N) to break the flesh in each fruit on  $180^\circ$  sides after removing peel (3 replicates of 6 fruits each).

Immediately after measuring firmness, each fruit was cut into four longitudinal parts. Two opposite parts were placed into an electric juice extractor and filtered juice was used to determine total soluble solids (TSS) and the acetaldehyde (AcH) concentration (3 lots of juices, each from 6 fruits). TSS were evaluated using a digital refractometer (Atagomod. PR1) and the results were expressed in  $^\circ Brix$ . The AcH concentration was analysed by headspace gas chromatography, as described by [Salvador et al. \(2004\)](#), and the results were expressed as  $mg/100\ ml$ .

The other two opposite fruit parts were sliced and frozen with liquid  $N_2$  to be ground and kept at  $-80^\circ C$  to evaluate the fol-



**Fig. 2.** Principal component analysis of the total data set. Principal component analysis of the physicochemical parameters of persimmon fruits of the total data set (10 cvs. and 3 maturity stages). The score and loading plots for the first, second and third principal components are shown. Three replicates were analysed per cultivar in each maturity stage; the mean values of the replicates are shown. (RB: Rojo Brillante; Tw: Tonewase; Az: Aizumishirazu-A; Gb: Giombo; H: Hachiya; G: Giboshi; KT: Kaki Tipo; J: Jiro; O'ig: O'goshō and HF: Hana Fuyu. The number after the initials indicates the stage of maturity).

lowing parameters: soluble tannins, antioxidant capacity, total carotenoids and sugars.

Soluble tannins were evaluated by the Folin-Denis method described by Taira (1995), modified by Arnal and Del Rio (2004). The results were expressed as a percentage of fresh weight (fw).

Antioxidant capacity was determined as the antiradical activity of methanolic extracts, where 20 mg of dried sample were homogenised with 2 ml of methanol (80% v/v). It was spectrophotometrically tested by measuring absorbance at 515 nm of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), adapted from Novillo et al. (2014). The obtained values were compared to the concentration-response curve of the standard Trolox solution, expressed as micromoles of Trolox equivalents (TE) per 100 mg of dry weight (dw).

Total carotenoid content was determined according to Koca et al. (2007), where 50 mg of dried sample were homogenised with 2 ml of a hexane:acetone mixture (7:3). Carotenoid content was determined spectrophotometrically at 450 nm as  $\beta$ -carotene equivalents ( $\beta$ CE) from the standard curve.

Extraction and determination of sugars were conducted from 50 mg of dried sample, extracted with 1 ml of twice-distilled water. The extracted sample was centrifuged twice at 14,000 rpm for 15 min at 4 °C. The supernatant was filtered through 0.45  $\mu$ m filters and purified through a Sep-Pak C18 column. The sugars analysis was performed on a Thermo Separation Products HPLC. Separation of sugars was performed isocratically with Millipore water as the mobile phase, which flowed at 0.5 ml min<sup>-1</sup> in a fast carbohydrate column (Aminex HPX87-C column), 100  $\times$  7.8 mm i.d. (Bio Rad Laboratories, Hercules, CA, USA), preceded by a micro-guard cartridge, Carbo-C 30  $\times$  4.6 mm i.d. (Bio Rad Laboratories, Hercules, CA, USA), maintained at 75 °C and attached to a refractive index detector (RID). Concentrations were calculated with the corresponding external standards and expressed as  $\mu$ g/mg dw.

The following standards were used to determine sugars, carotenoids and antioxidant capacity: sucrose, fructose and glucose;  $\beta$ -carotene and trolox solution from Sigma-Aldrich Chemie (Steinheim, Germany).

### 2.3. Statistical analysis

A principal components analysis (PCA), an analysis of variance (ANOVA) and a study of the correlations between parameters were performed.

The ANOVA was carried out using the Statgraphics Plus 5.1 software application (Manugistics Inc., Rockville, MD, USA), and multiple comparisons were made between means by the LSD test and Tukey test ( $P \leq 0.05$ ).

A PCA was run and included the normalised data (log 2-transformed) from the 10 studied cultivars, with the following parameters: firmness, acetaldehyde, carbon dioxide production, soluble tannins content, total antioxidant capacity, concentrations of fructose, sucrose and glucose, total soluble solids and total carotenoids. Ethylene production was not included because it remained at undetectable levels for all the cultivars and maturity stages. The Acuity 4.0 program (Axon Instruments) was used with distance measures based on Pearson's correlation to perform both the PCA and the study of correlations.

## 3. Results and discussion

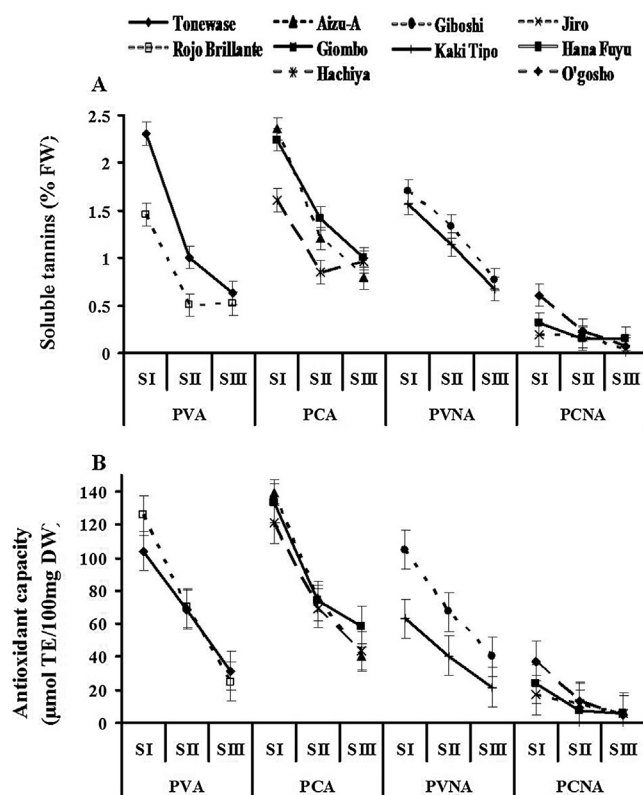
### 3.1. External colour

Evolution of external colour from green to green-yellow, and later to orange or orange-redish tones, depending on the cultivar, is the most obviously change during persimmon maturation (Zhou et al., 2011). Fig. 1 presents the skin colour index exhibited by the 10 selected cultivars in the three maturity stages under study: completely green fruits (SI), fruits in the colour-break stage (SII) and fruits with the homogeneous commercial colour.

In some cultivars, such as 'Tonewase', 'Rojo Brillante', 'Kaki Tipo' and 'O'igoshō', the change noted from green to oranges tones occurs homogeneously all around the fruit and therefore, persimmons are considered to obtain the commercial colour (stage SIII) when showing homogeneous orange tones (colour index value close to +10). However in other cultivars ('Aizumishirazu-A', 'Giombo', 'Hachiya', 'Jiro', 'Hana Fuyu' and 'Giboshi') colour change is irregular and most of the surface depict reddish-orange tones when the green tones are been lost. Thus the colour index at commercial maturity (SIII) is high (close to +20).

### 3.2. Soluble tannin content and total antioxidant capacity, the main parameters that differentiate the maturation of astringent and non-astringent cultivars

The Principal Components Analysis, which was done using the complete data set (all the cultivars, maturity stages and physicochemical parameters), revealed that 80% of total variance was



**Fig. 3.** Soluble tannin content and total antioxidant capacity. Evolution of soluble tannin content and total antioxidant capacity during the maturation of ten persimmon cultivars. Three maturity stages were evaluated: SI- green stage; SII-colour-break; SIII-characteristic homogeneous orange or orange-reddish tones. Vertical bars represent Tukey HSD-intervals when comparing all the cultivars and maturity stages ( $P < 0.05$ ).

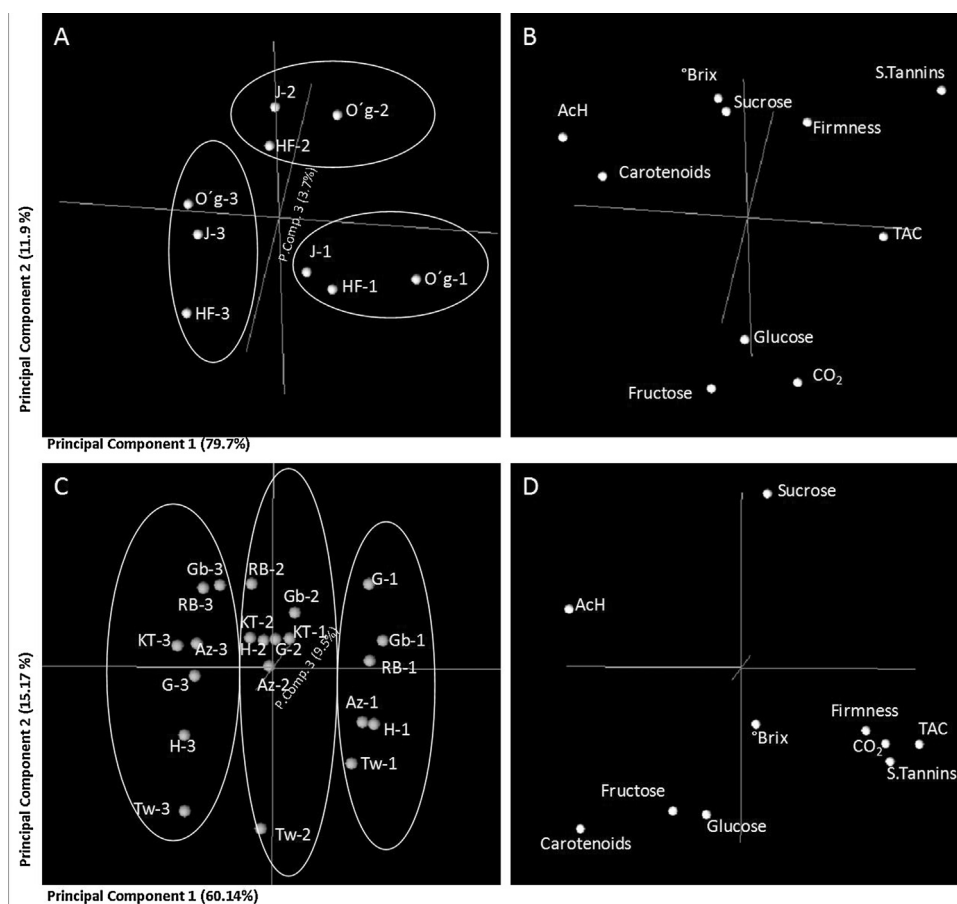
explained by the first two principal components (Fig. 2). However, the third component, that which explained 7.8% of variance, clearly separated the persimmon samples into two groups (Fig. 2A): a first group of fruits from naturally non-astringent cultivars (PCNA-type cultivars: 'Jiro', 'O'gosho' and 'Hana Fuyu'); a second group of cultivars that were astringent at harvest, which includes the PCA- ('Aizumishirazu-A', 'Giombo' and 'Hachiya'), PVA- ('Tonewase' and 'Rojo Brillante') and PVNA-type ('Giboshi' and 'Kaki Tipo') cultivars. In both these groups (non-astringent and astringent), the first and second components, which explained most variability, were associated with maturity stage. So the samples in maturity stage SI were located in the right and upper spaces in relation to those in stage SIII (located to the left and downwards). Stage SII was located in the middle of stages SI and SIII.

The loading plots revealed the main parameters responsible for separation between the groups (astringent and non-astringent cultivars; Fig. 2B). As expected, soluble tannins (ST) content was the most relevant parameter for separating these two cultivar groups. Besides soluble tannins, total antioxidant capacity greatly contributed to differentiate them. Hence at the three evaluated maturity stages, the level of both ST and total antioxidant capacity was higher in the astringent cultivars compared with the non-astringent ones. According to Yonemori and Matsushima (1985,1987), tannin cell development in astringent cultivars continues until late fruit growth stages, while tannin cell development stops in early fruit growing stages in non-astringent cultivars (PCNA-type). Early cessation of tannin cell development is thought to be the main cause of natural astringency loss in PCNA-type fruits as it results in a diluted tannins concentration in flesh as fruits grow. The proximity to the location of soluble tannins content and antioxidant capacity in the loading plot space can be explained by the high radical scavenging activity that soluble tannins possess (Gu et al.,

2008). In fact a strong positive correlation between soluble tannins content and total antioxidant capacity was detected ( $r = 0.93$ ).

The ANOVA corroborated the importance of these two factors for differentiating the astringent cultivars from the non-astringent ones (Fig. 3A and B). In both cultivar groups, a decline in soluble tannins content with advanced maturation was observed (Fig. 3A). Yet while the soluble tannins content ranged from 0.6% in SIII to 2.3% in SI in the non-astringent cultivars, the tannins concentration in the astringent cultivars was higher and ranged from 0.5% in SIII to 2.3% in SI (Fig. 3A). Thus in the three studied maturity stages, the soluble tannins concentration was clearly higher in the astringent vs. non-astringent cultivars. The antioxidant capacity analysis revealed a similar trend to that observed for soluble tannins content (Fig. 3B). Antioxidant capacity decreased in all the cultivars and in parallel to the decline in soluble tannins associated with maturation. The antioxidant capacity of the astringent cultivars ranged from 130  $\mu\text{mol TE}/100 \text{ mg}$  in SI to 20  $\mu\text{mol TE}/100 \text{ mg}$  in SIII, while these values ranged from 40  $\mu\text{mol TE}/100 \text{ mg}$  to 10  $\mu\text{mol TE}/100 \text{ mg}$  for the non-astringent group.

It is worth highlighting that in both groups (astringent and non-astringent cvs.) relevant differences in the profile of soluble tannins and total antioxidant capacity were detected among cultivars (Fig. 3A and B). In maturity stage SI, cultivars 'Tonewase', 'Aizumishirazu-A' and 'Giombo' obtained a ST concentration close to 2.5%, while the other astringent cultivars displayed a significantly lower content, around 1.5%. Significant differences among cultivars were also observed in the commercial maturity stage (SIII) since 'Rojo Brillante' exhibited a 0.4% content, while that of 'Aizumishirazu-A' came close to 1%. Among the non-astringent cultivars, the main differences in the ST concentration were observed in SI, when cv. O'gosho showed a 2-fold higher soluble tannins content (0.6%) than cultivars 'Fuyu' and 'Jiro' (0.3%). Yet in maturity



**Fig. 4.** Principal component analysis of the non-astringent and astringent cultivars. Principal component analysis of the physiological and physicochemical parameters of persimmon fruits. The score and loading plots for the first, second and third principal components are shown. a, b Data set (PCNA-cultivars, three maturity stages). c, d Data set (PCA, PVA and PVNA-cultivars, three maturity stages). Three replicates were analysed per cultivar in each maturity stage; the mean values of the replicates are shown. (RB: Rojo Brillante; Tw: Tonewase; Az: Aizumishirazu-A; Gb: Giombo; H: Hachiya; G: Giboshi; KT: Kaki Tipo; J: Jiro; O'g: O'goshi' and HF: Hana Fuyu. The number after the initials indicates the stage of maturity).

stage SIII, all the non-astringent cultivars gave an ST concentration value close to 0.06%.

Our results for antioxidant capacity (Fig. 3B) shows that despite its high correlation with ST content, other compounds must provide antioxidant capacity to persimmon fruits. This fact became particularly clear when comparing cultivars 'Giboshi' and 'Kaki Tipo' since they showed the same ST content throughout maturation. However, the total antioxidant capacity of 'Kaki Tipo' was significant lower than that of 'Giboshi', and was also lower than that of all the other cultivars.

### 3.3. Evolution of the physicochemical parameters not responsible for astringency during the maturation process

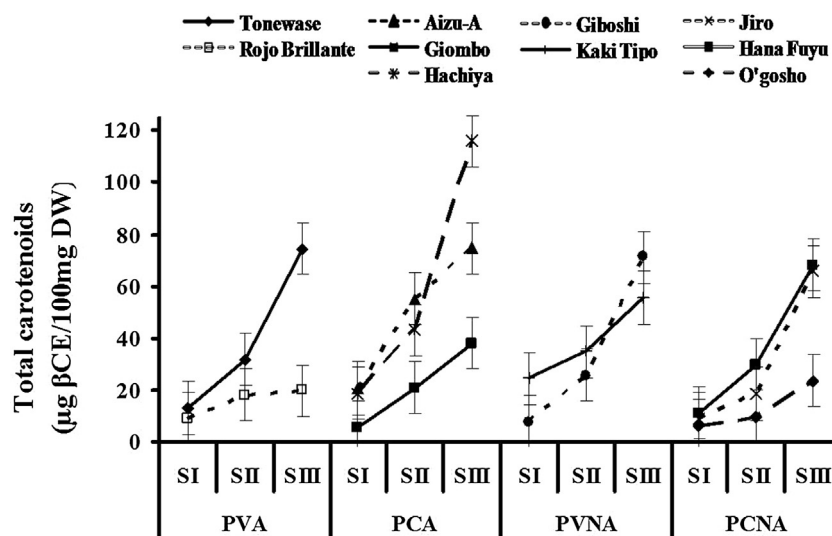
In order to further explore the differences among maturity stages, Principal Components Analyses were performed independently for the non-astringent (Fig. 4A and B) and astringent cultivars (Fig. 4C and D). In both cultivar groups, this analysis allowed us to group samples according to the maturity stage of fruits.

The samples from the non-astringent cultivar group were separated according to maturity stage by the first two principal components (Fig. 4A). Component 1 (79% of variance) separated SI and SII (located on the right) from SIII (located on the left), while component 2 (11% of variance) separated SII (located in the upper space) from SI and SIII, situated at the bottom of the space.

With the astringent cultivars (Fig. 4C), the three maturity stages were separated by the component 1, which explained 60% of variability. SI was located on the right, SII in the central space and SIII on the left of the space. However, one exception was observed since the fruits of cultivar 'Kaki Tipo' in stage SI were closer to the samples from stage SII of the other cultivars (in the central area of the space) than to those in stage SI. It is also worth mentioning that the cultivar 'Tonewase' samples were separated from the other cultivars by component 2, while the samples of this cultivar in the three maturity stages were located at the bottom of the space.

The observation made of the loading plot of both the non-astringent and astringent cultivar groups (Fig. 4B and D) revealed that the concentrations of acetaldehyde and carotenoids were the parameters that helped separate the samples in stage SIII from those in earlier maturity stages. Thus the highest levels of both acetaldehyde and carotenoids were associated with the most advanced maturity stage (SIII). Meanwhile, the earliest maturity stage (SI) was characterised by showing, besides the highest levels of soluble tannins and antioxidant capacity, the greatest firmness and the highest CO<sub>2</sub> production values.

The lowest antioxidant capacity level shown by 'Kaki Tipo' if compared to the other above-mentioned astringent cultivars (Fig. 3B), explains why its samples in stage I are located in the PCA space close to the samples in stage II of the other cultivars. Thus the 'Kaki Tipo' fruits in stage I showed a similar antioxidant capacity level (60 μmol TE/100 mg) to that shown by the other astringent cultivars in stage II (Fig. 3B).



**Fig. 5.** Carotenoids flesh content. Evolution of the total carotenoids flesh content during the maturation of ten persimmon cultivars. Three maturity stages were evaluated: SI-green stage; SII-colour-break; SIII-characteristic homogeneous orange or orange-reddish tones. Vertical bars represent Tukey HSD-intervals when comparing all the cultivars and maturity stages ( $P < 0.05$ ).

As regards the particular location of cultivar 'Tonewase', according to the loading plot (Fig. 4D), the separation of this cultivar from the other astringent ones for all three maturity stages took place because it had a higher content of carotenoids and sugars.

In order to further understand the changes associated with the maturation of the different cultivars, an ANOVA was performed for each individual parameter.

### 3.3.1. Flesh carotenoids accumulation and softening during maturation are affected mainly by the specific cultivar

Similarly to the changes in skin colour exhibited by persimmon fruits during maturation, flesh colour also evolves from white to orange tones. Like that observed in loquat cultivars, in which red-fleshed and white-fleshed fruits can be distinguished, some persimmon cultivars depicted a much more intense orange coloured flesh upon commercial harvest than others (Zhou et al., 2011). Carotenoids compounds are known to be mainly responsible for the flesh colour of persimmons (Ebert and Gross, 1985; Yuan et al., 2006), and  $\beta$ -Cryptoxanthin and Zeaxanthin have been reported as the most abundant carotenoids (Zhou et al., 2011).

In the present study, the 10 evaluated cultivars gradually accumulated carotenoids in flesh during maturation (Fig. 5). For all the cultivars, the largest increment of carotenoid content was observed between stages SII and SIII; that is, from the colour-break stage to the stage when fruits exhibited a homogeneous external colour. This suggests that accumulation of carotenoids in flesh and skin may occur in parallel. However, the relationship between internal and external colours was cultivar-dependent. 'Tonewase' and 'Rojo Brillante' showed not significant differences in external colour throughout maturation (Fig. 1), but carotenoids accumulated considerably more in the flesh of cultivar 'Tonewase' (75  $\beta$ CE  $\mu$ g/100 mg) than in 'Rojo Brillante' (20  $\beta$ CE  $\mu$ g/100 mg) (Fig. 5). Such differences in carotenoids accumulation were related to the visual flesh colour. Thus cultivars 'Rojo Brillante', 'Giombo' and 'O'goshi', whose flesh was the lightest in colour, showed a 2-fold lower total carotenoids content (20–40  $\mu$ g/100 mg) than the other cultivars (80–120  $\mu$ g/100 mg).

An astringent effect category was observed for fruit firmness in the earliest maturity stage (SI) as all the non-astringent cultivars presented greater firmness (close to 100N) than the astringent ones (close to 80N) (data not shown). Yet these differences disappeared as the maturity process advanced. A gradual decline in firmness was

noted in all the cultivars associated with fruit maturation, which was characteristic for each cultivar. In stage III, firmness values differed, and ranged from 47 N (cv. Giombo) to 14 N (cv. Tonewase) among the astringent cultivars. Similarly the firmness values of the non-astringent cultivars fell between 65 N (cv. O'goshi) and 24 N (cv. Fuyu). Therefore intensity of softening, which took place during maturation, was associated with neither astringency category nor astringency type, but was cultivar-dependent. The study of the correlations found between external colour and firmness revealed a strong negative correlation ( $r = -0.9$ ) between these two parameters in most cultivars, excluding cultivar 'Tonewase', which showed a poorer correlation ( $r = -0.6$ ). The 'Tonewase' fruits did not exhibit any significant decrease in firmness in relation to external colour evolution between stages SI and SII. Nevertheless, a major drop in the firmness values was recorded between maturity stages II and III.

Loss of firmness that persimmon fruits underwent during maturation has been associated by Salvador et al. (2007) to microstructural changes in flesh. A progressive degradation of the parenchyma, which gradually shows less swollen and more deformed cells, leads to generalised intercellular adhesion loss.

### 3.3.2. All the studied cultivars share a similar pattern of CO<sub>2</sub>, ethylene and acetaldehyde production

All the studied cultivars showed a similar pattern of CO<sub>2</sub> production (data not shown). The highest values were observed in the green maturity stage (SI), when both the astringent and non-astringent cultivars gave CO<sub>2</sub> values which ranged from 9 to 15 ml kg<sup>-1</sup> h<sup>-1</sup>. Upon colour-break (SII), production gave lower values within the 2–4 ml kg<sup>-1</sup> h<sup>-1</sup> range, which then slightly increased to values of around 4–8 ml kg<sup>-1</sup> h<sup>-1</sup> when fruits presented a homogeneous colour (SIII).

Our study revealed that ethylene production remained at undetectable levels in all the cultivars throughout the three studied maturity stages (data not shown). Although persimmon fruits are considered climacteric fruit (Takata, 1983), it is well-known that they produce very low levels of ethylene, and are highly sensitive to the presence of exogenous ethylene (Besada et al., 2010b; Salvador et al., 2007).

A common pattern was observed for acetaldehyde production in the four persimmon types. Thus a gradual increase in acetaldehyde production was associated with maturation in all the cultivars.

**Table 1**

Total soluble solids and individual sugar concentrations of ten persimmon cultivars in three maturity stages. SI-green stage, SII-colour-break and SIII-characteristic homogeneous orange or orange-reddish tones. Different letters in the same column indicate significant differences among the cultivars in each maturity stage (Tukey test,  $P < 0.05$ ). Different letters (capital letters) in the same row indicate significant differences among the maturity stages for each cultivar (LSD test,  $P < 0.05$ ).

GROUP. Cultivar	TSS (°Brix)			Sucrose ( $\mu\text{g}/\text{mg dw}$ )			Glucose ( $\mu\text{g}/\text{mg dw}$ )			Fructose ( $\mu\text{g}/\text{mg dw}$ )		
	SI	SII	SIII	SI	SII	SIII	SI	SII	SIII	SI	SII	SIII
PVA												
Tonewase	17.3e <sup>A</sup>	18.7d <sup>B</sup>	18.9d <sup>B</sup>	116.6a <sup>B</sup>	38.3a <sup>A</sup>	74.7a <sup>AB</sup>	269.6c <sup>A</sup>	268.7c <sup>A</sup>	509.9e <sup>B</sup>	198.1c <sup>A</sup>	206.3c <sup>A</sup>	412.1e <sup>B</sup>
Rojo Brillante	13.5c <sup>A</sup>	16.5c <sup>B</sup>	17.5c <sup>C</sup>	290.4d <sup>A</sup>	357.6c <sup>B</sup>	396.9d <sup>B</sup>	203.3b <sup>A</sup>	203.2b <sup>C</sup>	317.7 cd <sup>B</sup>	140.7b <sup>A</sup>	162.1bc <sup>A</sup>	264.0 cd <sup>B</sup>
PCA												
Aizu-A	16.6de <sup>A</sup>	18.7d <sup>B</sup>	19.6d <sup>C</sup>	153.9ab <sup>A</sup>	236.4bc <sup>B</sup>	241.2bc <sup>B</sup>	180.9b <sup>A</sup>	139.9ab <sup>A</sup>	194.5abc <sup>B</sup>	116.3ab <sup>A</sup>	104.6ab <sup>A</sup>	171.9abc <sup>B</sup>
Giombo	16.0d <sup>A</sup>	16.6c <sup>B</sup>	17.7c <sup>C</sup>	199.6bc <sup>A</sup>	248.7bc <sup>A</sup>	401.9d <sup>B</sup>	169.2b <sup>A</sup>	131.4ab <sup>A</sup>	240.2abc <sup>B</sup>	124.8b <sup>A</sup>	96.4ab <sup>A</sup>	201.7abc <sup>B</sup>
Hachiya	16.6de <sup>A</sup>	17.7 cd <sup>B</sup>	17.9c <sup>B</sup>	195.3bc <sup>AB</sup>	277.1bc <sup>B</sup>	147.6ab <sup>A</sup>	197.3b <sup>AB</sup>	153.7ab <sup>A</sup>	281.5bcd <sup>B</sup>	98.1ab <sup>A</sup>	105.1ab <sup>A</sup>	232.6bcd <sup>B</sup>
PVNA												
Giboshi	16.8de <sup>A</sup>	18.4d <sup>B</sup>	19.1d <sup>C</sup>	238.0 cd <sup>A</sup>	203.6b <sup>A</sup>	143.3ab <sup>A</sup>	114.5a <sup>A</sup>	167.6abc <sup>A</sup>	204.3abc <sup>B</sup>	64.1a <sup>A</sup>	125.4ab <sup>B</sup>	183.7abc <sup>B</sup>
Kaki Tipo	17.2e <sup>A</sup>	18.6d <sup>B</sup>	19.0d <sup>B</sup>	298.4d <sup>AB</sup>	204.8b <sup>A</sup>	356.5 cd <sup>B</sup>	193.7b <sup>A</sup>	198.6abc <sup>A</sup>	394.4de <sup>B</sup>	150.9bc <sup>A</sup>	147.8bc <sup>A</sup>	335.7de <sup>B</sup>
PCNA												
Jiro	9.7b <sup>A</sup>	12.9b <sup>B</sup>	15.2b <sup>C</sup>	299.7d <sup>A</sup>	284.5bc <sup>A</sup>	310.8 cd <sup>A</sup>	164.4b <sup>B</sup>	121.3ab <sup>A</sup>	152.2ab <sup>AB</sup>	131.7b <sup>B</sup>	99.9ab <sup>A</sup>	146.5ab <sup>B</sup>
Hana Fuyu	8.2a <sup>A</sup>	12.5ab <sup>B</sup>	13.3a <sup>C</sup>	200.1bc <sup>A</sup>	185.5b <sup>A</sup>	199.0bc <sup>A</sup>	196.3b <sup>B</sup>	133.9ab <sup>A</sup>	295.9 cd <sup>B</sup>	142.7b <sup>B</sup>	102.8ab <sup>A</sup>	277.2 cd <sup>C</sup>
O'goshō	9.0ab <sup>A</sup>	11.3a <sup>B</sup>	13.5a <sup>C</sup>	182.6b <sup>A</sup>	237.7bc <sup>A</sup>	224.8bc <sup>A</sup>	209.3b <sup>C</sup>	99.4a <sup>A</sup>	120.8a <sup>B</sup>	143.8b <sup>C</sup>	55.6a <sup>A</sup>	97.9a <sup>B</sup>

In fact a positive correlation ( $r = 0.79$ ) was observed between skin colour evolution and acetaldehyde production. Note that this study was performed with seedless fruits, so acetaldehyde production was unrelated to presence of fruit seeds. All the cultivars showed acetaldehyde production to be lower than 1 mg/100 ml in the commercial maturity stage (data not shown).

### 3.3.3. Total soluble solids and sugars accumulation seem influenced by astringency type

Table 1 shows the total soluble solids (TSS) and the content of individual sugars for each cultivar and maturity stage. TSS, expressed as °Brix, increased in all the cultivars as maturation progressed, although a persimmon category effect was seen as the PCNA-type cultivars obtained lower TSS values (8.2–15.2 Bx) than the astringent types (PCA, PVA, PVNA) (13.5–19.6 Bx) for all three maturity stages. It is noteworthy that the TSS content in persimmon fruit includes not only sugars, but also soluble tannins. So this parameter could reflect both an increase in sugars and a decrease in soluble tannins, which happen throughout maturation. Therefore the TSS measurement could be considered a good indicator of fruit maturity in non-astringent cultivars (Ullio, 2003) since the level of soluble tannins was very low in the maturity stages, which came close to the commercial stage and did not interfere with TSS measurements. Yet for the astringent cultivars, in which soluble tannin contents remained high, TSS had to be ruled out as an indicator of fruit sweetness.

The main sugars found in persimmon fruit flesh were sucrose, glucose and fructose (Table 1), which agrees with the work by Del Bubba et al. (2009). The non-astringent cultivars (PCNA-type) shared a similar sugar accumulation profile, which differentiated them from the astringent ones. In the PCNA-type cultivars, the concentrations of glucose and fructose lowered from SI to SII, and then increased again in SIII, while sucrose concentrations remained at relatively constant values. In commercial maturity stage SIII, sucrose was the major sugar in cv. 'Jiro' and 'O'goshō' (310.8 and 224.8  $\mu\text{g}/\text{mg}$ , respectively), while glucose and fructose were the main sugars in cv. Hana Fuyu (295.9 and 277.2  $\mu\text{g}/\text{mg}$ , respectively).

Different sugar evolution trends were observed among the astringent cultivars. The glucose and fructose content of cultivars 'Tonewase', 'Hachiya', 'Giboshi' and 'Kaki Tipo' rose as maturation evolved, while sucrose content exhibited slight changes (Table 1). This trend was similar to that reported by Del Bubba et al. (2009), who observed increasing glucose and fructose contents and an increasing-decreasing parabolic-like sucrose concentration. It must be noted the sugars content observed in cultivar 'Tonewase' had the highest contents of glucose (from 269.6 in SI to 509.9  $\mu\text{g}/\text{mg}$  in SIII)

and fructose (from 198.1 in SI to 412.1  $\mu\text{g}/\text{mg}$  in SIII), along with the lowest sucrose values (116.6 in SI, 38.3 at SII and 74.7  $\mu\text{g}/\text{mg}$  in SIII) throughout maturation; this fact explains the separation of this cultivar from the other astringent cultivars in the Principal Components Analysis space (Fig. 4C). It must also be mentioned that cultivar 'Kaki Tipo' fruits stand out for their high and similar content of all three sugars in the commercial stage (sucrose: 356.5, glucose: 394.4, fructose: 335.7  $\mu\text{g}/\text{mg}$ ).

The second trend was observed in cultivars 'Aizumihirazu-A', 'Rojo Brillante' and 'Giombo', which showed increased sucrose, fructose and glucose throughout maturation; this pattern agrees with that observed by Senter et al. (1991) and Zheng and Sugiura (1990), who all reported rising sucrose and reducing sugars. Among these cultivars, 'Rojo Brillante' obtained the highest accumulation of sugars in stage SIII.

Giordani et al. (2011) attributed the different trends they observed in sugars accumulation to invertase enzyme activity, which suggests that this enzyme activity may be influenced by soluble tannins content. Moreover, inhibition of invertase activity by gallic and tannin acid has been reported by Chen et al. (2003). The results reported herein revealed that the non-astringent cultivars showed similarly low and slightly changed levels of soluble tannins throughout maturation, while the astringent cultivars displayed a highly marked changing level of ST during this process. These tannins evolution trends noted in the two astringency cultivar categories may be key to explain how PCNA-cultivars shared similar invertase activity and, therefore, the same sugars accumulation pattern, while depending on the specific cultivar in the astringent group. Besides, the enzymatic systems responsible for glucose production in persimmon fruits, such as gluconeogenesis pathway enzymes, fructose isomerase and cellulose, have been reported to also be key factors for free sugars composition (Ittah 1993).

## 4. Conclusion

This study of physicochemical changes associated with the maturity process of 10 persimmon cultivars belonging to the four persimmon types (PVNA, PCNA, PVA and PCA) revealed that astringency type does not determine the decline in flesh firmness, carotenoids accumulation and acetaldehyde production, which occur in parallel with external colour evolution, and that these changes are cultivar-dependent. Nevertheless, astringency type clearly influenced not only soluble tannins content and antioxidant capacity, but also the accumulation of individual sugars. Therefore, despite all the cultivars presenting a declining antiox-

idant capacity through maturation, which has been related with a drop in soluble tannins, both these parameters were higher in the astringent cultivars in all the maturity stages. Moreover, the non astringent-cultivars shared a common sugar accumulation pattern, while different trends were observed in the astringent cultivars. All the cultivars shared a similar CO<sub>2</sub> production pattern.

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