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Microstructural changes while persimmon fruits mature and ripen. Comparison between astringent and non-astringent cultivars

Magda Andréia Tessmer[a,1], Cristina Besada[b,1], Isabel Hernando[c], Beatriz Appezzato-da-Glória[a], Amparo Quiles[c], Alejandra Salvador[b,*]

[a] *University of São Paulo, ESALQ, Department of Biological Sciences, 13418-900, Piracicaba, SP, Brazil*
[b] *Instituto Valenciano de Investigaciones Agrarias, Postharvest Department, 46113, Valencia, Spain*
[c] *Universidad Politécnica de Valencia, Food Technology Department, Valencia, Spain*

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ABSTRACT

Persimmon cultivars are classified into two categories, those that bear astringent fruit at harvest and those that bear non-astringent fruit. Causes of natural astringency loss that fruits of non-astringent cultivars undergo on tree are yet to be clarified. A physicochemical and microstructural study of the maturity process of two astringent cultivars ('Rojo Brillante' and 'Giombo'), and two non-astringent ones ('Fuyu' and 'Hana Fuyu') has been carried out. Light microscopy (LM) analysis allowed visualizing the higher soluble tannins content in astringent cultivars compared to non-astringent ones in early maturity stages as well as the natural tannin insolubilization that took place in both the astringent and non-astringent cultivars during maturation and ripening. In astringent cultivars this tannin insolubilization process was gradual and led to a progressive decline in soluble tannins. In non-astringent cultivars this insolubilization was much faster than in astringent cultivars. The present study also revealed that both colour evolution and fruit softening during maturation are characteristic for each cultivar, although in all of them a strong negative correlation was observed between these two parameters. Fruit softening was associated with a gradual parenchyma structure loss due to a cell wall and membrane degradation process. The total soluble solids content was found to be a good indicator of the sugars level in non-astringent cultivars, but should be ruled out in astringent varieties.

1. Introduction

Persimmon fruits are characterized for possessing high soluble tannins content when immature. Therefore, all unripe fruits are intensely astringent. However, persimmon cultivars can be classified into two general categories: those that bear astringent fruits until they are soft and ripe; those that bear non-astringent fruits from the stage when fruits turn orange in colour onwards. In both categories, there are cultivars in which fruit astringency is influenced by pollination (pollination variant) and cultivars whose fruits are not affected by pollination (pollination constant). Accordingly, persimmon fruits can be classified into four groups (Sugiura, 1983): the Pollination Constant Non-Astringent (PCNA) group, which is not astringent with or without seeds, and persimmons can be eaten at harvest when they are crisp as apples; the Pollination Variant Non-Astringent (PVNA) group, which is not astringent at harvest if fruits have seeds, and fruits are not edible when firm if they have been not pollinated; the Pollination Constant Astringent (PCA) group, which is always astringent when firm; the Pollination Variant Astringent (PVA) group, which is also astringent if pollinated, and is not astringent only around seeds, where they have dark tannin spots.

Loss of astringency that takes place during the maturation of fruits from the PVNA and PVA cultivars has been associated with the production of acetaldehyde by seeds, which must be involved in gradual tannins insolubilization. For PCA-type fruits, the production of volatile compounds by seeds is almost null (Sugiura and Tomana, 1983; Sugiura et al., 1979). In PCNA cultivars, an early drop in soluble tannins occurs even when seeds are absent. According to Yonemori and Matsushima (1985, 1987) early cessation of tannin cell development is the main cause of natural astringency loss in PCNA fruits since it leads to the dilution of tannin cells in flesh as fruits grow.

However, some questions on naturally decreasing astringency in persimmon fruits remain unresolved. Thus in non-PCNA fruits

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* Corresponding author.
E-mail address: salvador_ale@gva.es (A. Salvador).

[1] These authors contributed equally to this work.

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(PVA, PCA and PVNA), a drastic drop in soluble tannins occurs as the maturity process advances, even in non-seeded fruits. After studying the maturation of different persimmon cultivars (non-seeded fruits from three cultivars of each astringent type), Novillo et al. (2013) reported decreased soluble tannin content in non-PCNA fruits, with values as high as 2.5% in green fruits and ranging to 0.5% in firm coloured fruits. Although the soluble tannins level is still high at harvest and, therefore, fruits maintain high astringency, they completely lose astringency when they become soft with over-ripening. It is assumed that natural gradual tannins insolubilization during maturation is implied in the complete astringency loss observed in non-PCNA type fruits when the over-ripened maturity stage is reached. However, this has not yet been demonstrated.

Moreover in some PCNA cultivars, external temperature during fruit growing has been observed to influence natural astringency loss, so temperatures of around 25 °C are needed to bring about whole astringency loss on trees (Mowat et al., 1998). This fact is inconsistent with the hypothesis that tannin cells dilute as fruits grow is the main cause of astringency loss in PCNA-cultivars, as suggested by Yonemori and Matsushima (1985, 1987).

In this context, the aim of this study was to provide new information on the natural astringency loss process in persimmons. To this end, we approached the study of the maturity process of non-seeded persimmon fruits from two astringent cultivars (‘Rojo Brillante’ and ‘Giombo’ (PVA)) and two non-astringent cultivars (‘Fuyu’ and ‘Hana Fuyu’ (PCNA)) by paying attention to the decline in soluble tannins that takes place as maturity advances. For this purpose, physiological and microstuctural studies were carried out in seven maturity stages from small and immature fruits until the over-ripened stage.

2. Material and methods

2.1. Plant material and experimental design

Persimmon (*Diospyros kaki* Thunb.) unseeded fruit cvs. Rojo Brillante (RB) and Giombo (Gi) (PVA-group) and cvs. Fuyu (Fu) and Hana Fuyu (HF) (PCNA-group) were harvested in Valencia (Spain) in seven maturity and ripening stages (from stage S1-green fruits to stage S7-over-ripening). Harvesting took place between 19 September 2012 and 29 January 2013, with average intervals of 15 days. The criterion for harvesting each maturity stage was the visual evolution of skin colour: S1: green; S2: light green; S3: light yellow; S4: yellow; S5: orange; S6: intense orange and S7: orange-red.

After harvest, six fruits were taken to the Instituto Valenciano de Investigaciones Agrarias (IVIA) for physiological analyses, and two fruits were taken to the Microstructure Laboratory of the Universitat Politècnica de València. The following physiological analyses were carried out: equatorial diameter, weight, external colour, flesh firmness, total soluble solids, content of soluble tannins. A sensory evaluation of astringency level was also made. Microstructural analyses of flesh were performed by light microscopic techniques (LM) and Cryo Scanning Electron Microscopy (Cryo-SEM).

2.2. Physiological analysis

Fruit weight was determined on an analytical balance and fruit diameter was measured with an Absolute Digimatic caliper (Mitutoyo 500-171-20).

Fruit skin colour was evaluated with a Minolta Colorimeter (Model CR-300, Ramsey, NY, USA). The L, a, b Hunter parameters were measured and the results were expressed as an external colour index (CI) = (1000a)/(Lb) (Salvador et al., 2007), which reflects the colour evolution of persimmon fruits. Flesh firmness was determined with a Texturometer Instron Universal Machine, model 4301 (Instron Corp., Canton, MA, USA), using an 8-mm flat plunger. Fruit firmness values were taken from an average of six fruits. The results are expressed as the load in Newtons (N) required breaking the flesh of fruit on opposite sides after removing peel.

After the firmness measurements, fruits were longitudinally cut into four. Two opposite quarters were used to determine total soluble solids, and the other two to collect samples, which were frozen at −20 °C to later analyze soluble tannins content. Three replicates of two fruits each were analyzed.

To determine total soluble solids, fruit juice was extracted with an electric juice extractor and filtered. Measurements were recorded with a refractometer (Atagomod. PR1) and the results were expressed as% of soluble solids.

Soluble tannins were determined by the Folin-Denis method (Taira, 1995), as described by Arnal and Del Río (Arnal and Del Río, 2004), and the results were expressed as% of fresh weight.

The astringency level of the fruit was sensory evaluated by a semitrained panel of 6–8 people who were familiar with astringency of persimmon. A 4-point scale was used, where 1 = no astringency and 4 = intense astringency. Samples were presented to members of the panel in trays labeled with random 3-digit codes and served at room temperature (25 ± 1 °C). Milk was provided for palate-rinsing between samples

2.3. Analysis of light microscopic (LM) and cryo scanning electron microscopy (Cryo-SEM)

For the light microscopic analysis, tissue sections were taken from the equatorial portion of fresh fruitsusing a stainless blade. Sections were placed on histological slides and stained with vanillin-HCl (1:1, v/v) to identify tannins (Vázquez-Gutiérrez et al., 2011). Tannins react with hydrochloric vanillin to give a red colour. Cutting promotes the extravasate of tannins from tannin cells when they come in a soluble form. For the control, sections without coloring were used. Images were captured under a light microscope (Nikon Eclipse E800 V-PS100E, Tokyo, Japan).

For the Cryo-SEM analyses, cubes (3 mm^3) were cut from the equatorial area perpendicularly to the main axis of the persimmon flesh with a stainless steel cutter. These cubes were then immersed into slush nitrogen (−210 °C) and were transferred to a cryo-trans (CT 15000C from Oxford Instruments, Oxford, England) linked to a scanning electron microscope, JEOLJSM 5410 (JEOL, Tokyo, Japan), which operated at a temperature below −130 °C. Samples were cryofractured at −180 °C and etched at −90 °C. Observations under the microscope were made at 15 kV and at a working distance of 15 mm.

2.4. Statistical analysis

Data were subjected to analyses of variance (ANOVAs), and the multiple comparisons between means were determined using the least significant difference ($P = 0.05$) with the Statgraphics Plus 5.1 software application (Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Morphological characteristics of the studied persimmons cultivars

Persimmon fruits have a double sigmoidal growth curve for three phases: two phases of rapid growth (phase I and III) separated by a slow growth period (phase II) (Sugiura et al., 1991; Yakushiji and Nakatsuka, 2007). The maturity and ripening stages