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Physiological and structural changes during ripening and deastringency treatment of persimmon fruit cv. 'Rojo Brillante'

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Abstract

The aim of this study was to evaluate the changes in physicochemical properties together with the structural changes that occur during the period of harvesting 'Rojo Brillante' persimmon fruit, and the effect of the deastringency treatment on these properties. Fruit were harvested at different stages of maturation and the treated to remove astringency (95% CO_2 for 24 h at 20 °C, 90% R.H.). Just after harvest and following the subsequent deastringency treatment, physiological and microstructural changes were evaluated. Measurements of external colour, flesh firmness, pectinmethylesterase and polygalacturonase activity, astringency level as soluble tannins and sensory evaluation, acetaldehyde production, total soluble solids, pH and ethylene production were made. Microstructural changes were evaluated by Cryo Scanning Electron Microscopy. Firmness loss during fruit maturity, concomitant with an increase in external colour, was related to changes in cell structure. Although 'Rojo Brillante' fruit produce a small amount of ethylene during ripening, the change in ethylene production reflects the typical climacteric behaviour of this cultivar. The high concentrations of CO_2 tested to eliminate astringency proved effective for all stages of maturity and are related to the reduction in soluble tannin content and with the appearance of insoluble material inside the vacuoles of some tannic cells.

Keywords: Persimmon; Firmness; Deastringency treatment; Maturity stage; Cryo-SEM

1. Introduction

The persimmon 'Rojo Brillante' cultivar, along with cultivars including 'Triumph' and 'Hiratanenashi', belongs to the group that are astringent when harvested. This makes it necessary to apply a postharvest deastringency treatment before the fruit can be marketed. The current method used to remove astringency, while maintaining a high degree of firmness, involves holding the fruit in air-tight chambers for 24 h under 95–98% CO_2 at 20 °C and 90% R.H. (Arnal and Del Río, 2003; Salvador et al., 2005a). The effectiveness of this method lies in the fact that it triggers anaerobic respiration in the fruit, which gives rise to an accumulation of acetaldehyde and then a reaction between this acetaldehyde and the soluble tannins that are responsible for the astringency. The tannins then become insoluble (Taira et al., 1997; Matsuo and Itoo, 1982; Arnal and Del Río, 2003).

Fruit firmness is the property most commonly taken into account in order to rate the quality of 'Rojo Brillante' persimmons when marketed after astringency has been removed. Loss of firmness is an unavoidable fact that occurs sooner or later, depending on the different conditions experienced by the fruit during the postharvest period. A high degree of firmness at harvest plays a decisive role in being able to preserve the quality of the fruit during the postharvest period. Nevertheless, the external colour of the persimmon is the property used as a non-destructive index for harvesting, as is the case for many other commodities. In 'Rojo Brillante' fruit, the colour of the epidermis varies from green to a bright red when the fruit is in the last stages of ripeness. Although a correlation between changes in external colour and a reduction in firmness during storage has been reported (Salvador et al., 2006), at present no relationship has been established between the changes in the colour of the fruit and the physicochemical changes that take place during maturation. The physiological stage of fruit maturity can be an important factor when it comes to evaluating the effectiveness of astringency removal method.

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Although a number of studies have been carried out relating the degree of astringency with structural differences in the cell walls in different varieties of persimmons (Gottreich and Blumenfeld, 1991), the changes in astringency and other physiological parameters that occur due to the effect of CO_2 have not been linked with the structural changes that take place in the fruit pulp during the ripening process.

The aim of this study was to evaluate the changes in physicochemical properties, together with the structural changes that occur during the harvest period for 'Rojo Brillante' persimmons, and the effect of the deastringency treatment on these properties.

2. Material and methods

Persimmon (*Diospyros kaki* Thunb. cv. Rojo Brillante) fruit were harvested in l'Alcúdia (Spain) at different stages of commercial maturity. The maturity index used was visual observation of the external colour of the fruit, with six maturity stages defined, ranging from I (yellow-green) to VI (orange-red). After harvest, the fruit were taken to the Instituto Valenciano de Investigaciones Agrarias (IVIA), where they were maintained under ambient conditions. Approximately 24 h after harvest, they were treated to remove astringency and stored at 15 °C (R.H. 90%) for 3 days. Deastringency treatment was carried out in closed containers which contained 95% CO_2 for 24 h at 20 °C and 90% R.H. These conditions were established by passing a stream of air containing 95% CO_2 through the containers.

Just after harvest and following the subsequent deastringency treatment, physiological and microstructural changes were evaluated. Measurements of external colour, flesh firmness, astringency level as soluble tannins and sensory evaluation, acetaldehyde production, total soluble solids, pH and, ethylene production were made. Microstructural changes were evaluated by Cryo Scanning Electron Microscopy (Cryo-SEM). Pectinmethylesterase (PME) and polygalacturonase (PG) activity were measured only after deastringency treatment.

2.1. Physiological measurements

Skin colour was evaluated using a Minolta Colorimeter (Model CR-300, Ramsey, NY, USA), on samples of 20 fruit. '*L*', '*a*', '*b*' Hunter parameters were measured and results were expressed as a skin colour index, as described by Jiménez-Cuesta et al. (1981). Colour index = $(1000a)/(Lb)$.

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 are an average from 20 fruit. Results are expressed as the load in newtons (N) required for breaking the flesh of the fruit on opposite sides after peel removal.

Lots of 15 fruit were divided into three samples and cut into four longitudinal parts. Two of the opposite parts were sliced and frozen at −20 °C or flash frozen in liquid N_2 at −70 °C to determine soluble tannins or enzymes, respectively. The other opposite parts of the fruit were placed in an electric juice extractor (model 753, Moulinex, Spain) and filtered through cheese cloth; the juice obtained was then used to determine acetaldehyde production, total soluble solids (TSS) and pH.

Soluble tannins were evaluated using the Folin-Denis method (Taira, 1995), as described by Arnal and Del Río (2004b); the results are expressed in % (FW).

The astringency level of the fruit was also estimated by sensory evaluation. This evaluation was performed using composite samples of five (peeled and sliced) fruit from each replicate. A trained panel of 8–10 people who were familiar with this cultivar was asked to evaluate astringency. A 4-point scale was used, where 1 = very high astringency and 4 = no astringency. Samples were presented to members of the panel in trays labelled with random 3-digit codes and served at room temperature (25 ± 1 °C). The judges had to taste several segments of each sample in order to compensate, as far as possible, for biological variation of the material. Milk was provided for palate-rinsing between samples.

Activity of pectinmethylesterase and polygalacturonase was determined from 5 g of flesh samples frozen in liquid N_2 at −75 °C. For PME extraction, samples were homogenized with a Polytron (model PT-2100, Kinemática, AG Inc., Lucerne. Switzerland.) with addition of 30 mL 1.5 M NaCl buffer, pH 7.5, allowed to stand for 1 h, centrifuged (15,000 rpm, 10 min), and the pellet discarded and supernatant filtered through cheese cloth and brought to 50 mL with 1.5 M NaCl. All extraction procedures were carried out at 4 °C. PME activity, as meq-g/min g, was determined by titration of the carboxylic groups generated by PME during the hydrolysis of a pectin solution at pH 7.5 and 30 °C, modified from the method of Stoforos et al. (2002).

PG was extracted by homogenization of flesh samples in 1 M NaCl, pH 4.6 containing 0.1 M citric acid, 13 mM EDTA, 10 mM β-mercaptoethanol, and 1% polyvinylpyrrolidone-10. The tissue was homogenized for 1 min with a Polytron (model PT-2100, Kinemática, AG Inc., Lucerne. Switzerland). The homogenate was stirred for 1 h and then centrifuged (10,000 rpm, 15 min.). The supernatant was filtered through cheesecloth and brought to 25 mL with the same buffer. Aliquots of 1.5 mL were desalted on a Sephadex G-25 column. All extraction procedures were carried out at 4 °C. PG activity as nmol/mg min was assayed by measuring the appearance of reducing groups accompanying the cleavage of a polygalacturonic acid substrate by a spectrophotometric method using 2-cyanoacedamide, modified from the method of Gross (1982).

Acetaldehyde production was measured in three replicates per juice sample, and analysed by headspace gas chromatography (Ke and Kader, 1990), as described by Salvador et al. (2005a). Results were expressed as mg/100 mL. Total soluble solids of each sample were measured twice using a digital refractometer (model PR1, Atago, Tokyo, Japan) and results were expressed as %. Measurements of pH were taken with a pH-meter (model C231, Consort, Belgium). Ethylene production rates were measured in 10 replicates of two fruit per maturity stage and were analysed by injecting 1 mL of headspace gas into a Perkin Elmer gas chromatograph, as described by Salvador et al. (2005a). Ethylene production was given as $\mu L\, C_2H_4\, kg^{-1}\, h^{-1}$.