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Changes during the last ripening stage in pomological and biochemical parameters of the 'Redhaven' peach cultivar grafted on different rootstocks

Primoz Orazem, Maja Mikulic-Petkovsek, Franci Stampar, Metka Hudina *

University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

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ABSTRACT

The development of the 'Redhaven' peach [*Prunus persica* (L.) Basch.] fruit grafted on six rootstocks (Adesoto, GF 677, Monegro, Ishtara, Penta, Tetra) was evaluated in 2011. Several quality indices (fresh weight, flesh firmness, ground, blush color and flesh color measurements, and soluble solids content) were measured. The chlorophyll *a* content and total carotenoid content were measured using a spectrophotometer, and HPLC analyses were performed for numerous chemical parameters (quantification of individual phenolic compounds and carotenoids in the skin and flesh). Sampling was carried out on three dates (94, 100 and 107 days after full bloom – DAFB) during the last 15 days of fruit development, to determine changes in fruit quality. Fruit weight as well as the a^* value of ground, blush color and flesh color increased, while fruit firmness decreased with the progress of ripening. This indicates a transition from green to red in the case of ground and blush color and a gradual absence of green tones in the case of flesh color. All metabolites and pigments were more concentrated in the skin than in the flesh tissues regardless of the development stage or rootstock. In general, contents of metabolites followed similar patterns during ripening in both tissues. Chlorophyll *a* decreased, while anthocyanins, total carotenoids, lutein and β-carotene increased with ripening. Phenolics generally presented the highest content levels at 94 DAFB, which then decreased by 100 DAFB and again increased by 107 DAFB. Rootstocks had no influence on chlorophyll *a* level in the last 15 days of fruit development, but did influence individual carotenoid and phenolic compound levels in peach skin in ripe fruit.

1. Introduction

Peaches are appreciated for their visual, nutritional and organoleptic properties (Brandi et al., 2011). Previous studies have confirmed the versatile carotenoid (Di Vaio et al., 2008; Gil et al., 2002; Tourjee et al., 1998; Wright and Kader, 1997) and phenolic (Gil et al., 2002; Leontowicz et al., 2002; Orazem et al., 2011a,b; Remorini et al., 2008; Tomás-Barberán and Espín, 2001; Tomás-Barberán et al., 2001) profile of peach fruit. The comparison of phenolic and carotenoid contents in peach fruit (with the exception of β-carotene) compared to other fruit species reveals that peaches are not among the richest sources of these metabolites (Dragović-Uzelac et al., 2009). Nevertheless, peaches are widely produced and consumed (Derail et al., 1999) and are therefore an important source of these metabolites in the human diet.

No member of the animal kingdom can synthetize carotenoids (Britton et al., 2009). Plants, fungi, algae and bacteria are the only organisms capable of synthesizing these pigments (Stahl and Sies, 2003). Fruit and vegetables, by estimation, represent from 70 to 90% of the carotenoid intake in the human diet in developing countries (Britton et al., 2009; Hulshof et al., 2003). After ingestion, plants provide carotenoids that are converted to vitamin A, which is essential for normal growth, reproduction and resistance to infection (Tee and Lee, 1992). The analysis of human blood revealed significant carotenoid content, associated with good health and lower risk of disease (Britton et al., 2009). As with carotenoids, numerous studies report potent antioxidant capabilities of phenolics in vitro (Sakihama et al., 2002). However, the potential positive effects of phenolics on human health are the subject of ongoing debate and research (Halliwell, 2007).

An early study revealed that carotenoids in peach fruit remain in similar concentrations until maturity (Katayama et al., 1971). On the other hand, a recent genetic study by Brandi et al. (2011) reported maximal accumulation of carotenoids in the 'Redhaven' peach at the end of maturity (122 days after pollination). However, another study (Andreotti et al., 2008) showed that phenolics decreased during ripening.

In previous studies we proved a significant effect of rootstocks on maturity (Orazem et al., 2011b). In the current study

* Corresponding author. Tel.: +386 13203142; fax: +386 14231088.
E-mail address: metka.hudina@bf.uni-lj.si (M. Hudina).

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we focus on the final stages of fruit development, where several pomological and biochemical parameters were monitored. The aim was to follow the evolution of quality-related metabolites and pigments to its endpoint (the harvest) and the effects of rootstock of different genetic origin on this process. We also tried to determine the relation between pomological and biochemical parameters, which would in time enable a non-destructive evaluation of biochemical characteristics on the basis of pomological properties.

2. Materials and methods

2.1. Experimental design

Our experiment was set up at the Fruit Growing Centre Bilje near Nova Gorica (UTM coordinates: N 45°53′528″; E 0130°38′606″). The 'Redhaven' peach cultivar was studied in combination with six rootstocks: GF 677, Monegro, Tetra, Penta, Adesoto and Ishtara. GF 677 (*Prunus persica* × *Prunus amygdalus* L.) is currently the predominant rootstock in Slovenia and was used as the standard. The Monegro rootstock is of the same genetic origin as GF 677 and also produces trees of similar size (Felipe, 2009). Tetra and Penta are both rootstocks originating from *Prunus domestica* L. that are known to result in smaller peach tree size (Orazem et al., 2011b). Adesoto (*Prunus insititia*) and Ishtara (*Prunus cerasifera* × *Prunus salicina*) × (*Prunus cerasifera* × *P. persica*) proved successful in previous preliminary studies in our pedoclimatic conditions (Orazem et al., 2011a). The field on which we carried out our trial had previously supported two generations of peach orchard. The first generation was grafted on peach seedling and the second on GF 677 rootstocks. The rootstocks were planted in a permanent place in spring 2005, with 4 m × 2 m tree spacing. Grafting was performed in August 2005. The trees were trained to a free spindle (Loreti and Pisani, 1990). The entire orchard was covered with hail net to protect the trees and yield from possibly damaging weather conditions. The same pest and disease protection was maintained in the entire orchard and was managed according to the standard integrated pest management prescribed by the Ministry of Agriculture and the Environment (2012). Twelve trees from each rootstock/cultivar combination were selected on the basis of similar trunk circumference and canopy volume.

2.2. Sampling

Fruit samples were picked in three ripening stages (5 July, 2011 – 94 DAFB, 11 July, 2011 – 100 DAFB, 18 July, 2011 – 107 DAFB). At each sampling, six fruit samples were randomly collected from each tree (from each side of the tree, two fruit from the outer part of the crown and one fruit from the inner part of the crown) and transported to the laboratory facilities for further analysis. Three dimensions (length, width, and height) of each fruit were measured. Each fruit was weighed on a precision scale to 0.01 g confidence level. Ground and blush skin color, and flesh color were measured (L^*, a^*, b^*, values) using a digital CR 300 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan). The skin was removed on four sides of each fruit, and flesh firmness was measured four times on each fruit using a digital penetrometer (TR, Turini, Italy) with an 8 mm tip. Soluble solids content was measured by crushing the flesh and transferring the intact juice to a digital refractometer, model WM-7 (Atago, Tokyo, Japan).

The extraction of chlorophylls, total carotenoids and individual carotenoids was performed immediately after measurement to prevent pigment degradation. The rest of the fruit was peeled, and then the skin and flesh were immediately frozen in liquid nitrogen and kept separately at −20 °C until the extraction of phenolics.

2.3. Chemicals

The following standards were used for quantification of single phenolic compounds: chlorogenic acid (5-caffeoylquinic acid) from Sigma (St. Louis, MO), (+)-catechin from Roth (Karlsruhe, Germany), procyanidin B1 and procyanidin B2 from Fluka Chemie GmbH (Buchs, Switzerland), chlorogenic acid from Sigma (St. Louis, MO). For quantification of flavonols, rutin (quercetin 3-*O*-rutinoside) was obtained from Sigma (St. Louis, MO), and quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside from Fluka Chemie GmbH (Buchs, Switzerland). To quantify anthocyanins, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside were purchased from Sigma–Aldrich (Steinheim, Germany). Methanol was acquired from Sigma. Water was bidistilled and purified with the Milli-Q system (Millipore, Bedford, MA). For the chlorophyll *a* and total carotenes, dimethyl sulfoxide and magnesium hydroxide carbonate were purchased from Merck (Darmstadt, Germany). For the individual carotenoid analysis, ethanol, hexane and sodium chloride were purchased from Sigma–Aldrich (Steinheim, Germany), while potassium hydroxide from Merck (Darmstadt, Germany) was used. To quantify carotenoids, β-carotene from Sigma–Aldrich (Steinheim, Germany) and lutein from DHI (Hørsholm Denmark) were used. In the mobile phase for determination of carotenes, ammonium acetate was purchased from Fluka Chemie GmbH (Buchs, Switzerland).

2.4. Chlorophyll a and total carotenoids analysis

The determination of chlorophyll *a*, and total carotenoids was carried out only in the skin, using the method by Wellburn (1994). A disk was taken from each fruit (4 mm in diameter). Dimethyl sulfoxide (DMSO) was used for the extraction of chlorophyll and carotenoids. First, 0.5 mL DMSO was poured into a microcentrifuge tube, and a disk was added. Magnesium hydroxide carbonate crystals were added to ensure a sufficient concentration of Mg^{2+} ions in the solution. The disk was macerated for better extraction using a special plunger. Then another 0.5 mL DMSO was added. Samples were left in a water bath (65 °C) in the dark for 2 h. The samples were then left at room temperature for 20 min to cool and transferred into cuvettes. Immediately the absorbances were measured using a spectrophotometer (Lambda Bio 20, PerkinElmer) at wavelengths of 480 nm (carotenoids) and 665 nm (chlorophyll *a*). Concentrations of chlorophyll *a* and carotenoids in the extract were calculated according to equations described by Wellburn (1994).

2.5. Extraction of carotenoids

For the extraction of carotenoids, the method previously described by Wright and Kader (1997) was used. One gram of skin or three grams of flesh tissue was added to a round-bottomed centrifuge tube, which was wrapped in aluminum foil to prevent light access, and homogenized with 10 mL of cold ethanol for 3 minutes using the T-25 Ultra-Turax (IKA® – Labortechnik, Staufen, Germany) at 8400 rpm. Eight milliliters of hexane was added, and the sample was again homogenized for 2 min at 8400 rpm. The homogenate was then centrifuged for 4 min at 10,000 rpm and 4 °C. The hexane layer was transferred to an Erlenmeyer flask with a pipette, flushed with nitrogen, sealed and kept in the absence of light. We added 5 mL of saturated sodium chloride to the contents of the centrifuge tube. The mixture was stirred gently until homogenized. Afterwards another 8 mL of hexane was added to the mixture and then homogenized for 2 min with the Ultra-Turax at 3800 rpm. The centrifugation step was repeated, and the hexane layer was added to the first extract in the Erlenmeyer flask. Another 8 mL were added to the contents of the centrifuge tube, homogenized at 3800 rpm with the Ultra-Turax for 2 min, and