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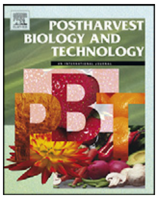

Changes in external and internal color during postharvest ripening of ‘Manila’ and ‘Ataulfo’ mango fruit and relationship with carotenoid content determined by liquid chromatography–APcI$^+$-time-of-flight mass spectrometry

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ABSTRACT

High-performance liquid chromatography–atmospheric pressure chemical ionization (APcI$^+$)-time-of-flight mass spectrometry studies revealed that all-*trans*-β-carotene and the dibutyrates of all-*trans*-violaxanthin and 9-*cis*-violaxanthin were the main carotenoids in ‘Ataulfo’ and ‘Manila’ mango fruit mesocarp. The concentration of these carotenoids in the mesocarp was measured during fruit ripening and correlated with colorimetric changes of mesocarp and epidermis. The lowest and highest concentrations of all-*trans*-β-carotene, all-*trans*-violaxanthin and 9-*cis*-violaxanthin (as dibutyrates) during the ripening of ‘Manila’ mango were 0.25×10^{-3} to 35.57×10^{-3}, 0.40×10^{-5} to 31.97×10^{-3} and 0 to 16.81×10^{-3} g kg^{-1} of fresh mesocarp, respectively. For ‘Ataulfo’ they were 2.55×10^{-3} to 39.72×10^{-3}, 0.16×10^{-3} to 15.00×10^{-3} and 0.21×10^{-3} to 7.48×10^{-3} g kg^{-1} of fresh mesocarp, respectively. The concentration of these carotenoids increased in an exponential manner during fruit ripening in ‘Ataulfo’ and in an exponential or second-order polynomial manner in ‘Manila’. The highest correlation coefficients were obtained for the relationships between the mesocarp and epidermis a^* and h° color values and the concentration of the evaluated carotenoids in both mango cultivars ($R = 0.81$–0.94). Equations to predict the concentration of the most important carotenoids in ‘Manila’ and ‘Ataulfo’ mango fruit on the basis of their mesocarp and epidermis color values were obtained.

1. Introduction

Mango (*Mangifera indica* L.) is a very popular fruit in many countries. Mexico is the fourth biggest producer and first exporter, with very high fruit consumption (Yahia et al., 2006a). Mexico produces more than 50 mango cultivars but the most important are ‘Manila’ and ‘Ataulfo’, which are also becoming important in some export markets (Yahia et al., 2006a). ‘Manila’ and ‘Ataulfo’ mango fruits are medium size (200–300 g), rich in sugars at ripe stage, and their color changes from green to yellow-orange during ripening. The epidermal color of these fruits is homogeneous and does not develop a red blush (Ornelas-Paz et al., 2007).

Mango is a climacteric fruit (Sane et al., 2005; Yahia et al., 2006a), and its ripening process occurs rapidly after harvest, depending on cultivar, stage of maturity at harvest, and postharvest conditions (Vázquez-Caicedo et al., 2004). Several biochemical changes occur during mango ripening, among which carotenoid biosynthesis is one of the most important (Vázquez-Caicedo et al., 2004, 2005). Carotenoids are lipid soluble compounds associated with protective health effects such as against some types of cancer (Bertram and Vine, 2005), age-related macular degeneration (Bruno and Medeiros, 2000), and heart disease (Palace et al., 1999). In addition, some carotenoids, such as all-*trans*-β-carotene, are precursors of vitamin A. Several carotenoids have been identified in fruit of different mango cultivars (Cano and de Ancos, 1994; Ben-Amotz and Fishler, 1998; Chen et al., 2004), but only a few of them occur in significant concentrations (Ornelas-Paz et al., 2007). Mercadante et al. (1997) quantified several carotenoids in saponified extracts of ‘Keitt’ mangoes and concluded that the predominant ones were all-*trans*-β-carotene, all-*trans*-violaxanthin and 9-*cis*-violaxanthin accounting for 27, 38 and 18% of total carotenoid content, respectively. Similar findings have been reported for crude extracts from some other mango cultivars (Mercadante and Rodríguez-Amaya, 1998; Pott et al., 2003a,b). Carotenoids are also the pigments responsible for the yellow-orange color of mango mesocarp (Vázquez-Caicedo et al., 2005).

Color is an important food quality parameter. It affects consumer acceptance (Crisosto et al., 2003), the perception of sweetness and flavor (Bayarri et al., 2001), and can even evoke emotional feelings

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in humans (Ou et al., 2004). In the case of mango, color of the epidermis plays an important role in the perception of overall quality (González-Aguilar et al., 2001), and can be an important tool for determining the appropriate maturity for harvest (Cocozza et al., 2004; Jha et al., 2007), processing (Mahayothee et al., 2004) and consumption (Cocozza et al., 2004; Jha et al., 2007). In addition, mango color can be used to estimate the content of all-*trans*-β-carotene (Vázquez-Caicedo et al., 2005), the most important provitamin A carotenoid (Wolf, 1984).

Color measurements have been used for carotenoid estimation in sweet potatoes (Lauber et al., 1967; Takahata et al., 1993; Ameny and Wilson, 1997), cassava (Sánchez et al., 2006), and some mango cultivars (Vázquez-Caicedo et al., 2005). Thus, it might be feasible to apply color measurements for rapid estimation of carotenoid content in foods (Lauber et al., 1967; McGuire, 1992; Takahata et al., 1993; Camire et al., 1994) where sophisticated equipments for carotenoid quantification may not be available.

Therefore, the objective of the present work was to identify the most important carotenoids in the mesocarp of ‘Ataulfo’ and ‘Manila’ mangoes, and to quantify the relationship between carotenoid concentration and the color changes of fruit mesocarp and epidermis during fruit ripening.

2. Materials and methods

2.1. Chemicals and solvents

High-performance liquid chromatography (HPLC) grade methanol, acetone, *n*-hexane, 2-propanol, and reactive grade benzene, anhydrous granular sodium sulfate, calcium carbonate and $Na_2S_2O_3$ were purchased from Mallinckrodt Baker (Mexico). 2,6-Di-*tert*-butyl-4-methyl phenol (BHT) was obtained from Merck KGaA (Darmstadt, Germany). Diethyl ether, *tert*-butyl-methyl ether (MTBE) HPLC grade and, all-*trans*-β-carotene (purity = 97%) were from Sigma–Aldrich (St. Louis, MO). All-*trans*-violaxanthin (purity = 95%) was from CaroteNature GmbH (Lupsingen, Switzerland). HPLC grade water was prepared by a Milli-Qplus purification system (Millipore Corp., Bedford, MA).

2.2. Fruit selection and sampling

Fresh mango fruit (*Mangifera indica* L. cvs. Manila and Ataulfo) were bought in the local market of Querétaro, Mexico. Fruit of both cultivars were selected for uniform size and freedom from blemishes and defects, but with different ripening stages, as judged subjectively based on epidermal color. Fruit were then stored for up to 16 days at 28 ± 0.5 °C and 60% relative humidity and eight samplings were performed during the storage period. During each sampling period, four fruit of each cultivar were taken randomly and each fruit was individually evaluated for color and carotenoid concentration. The experiment was repeated twice.

2.3. Color measurements

Color was measured with a Minolta colorimeter (Minolta Co. Ltd., Osaka, Japan) on the basis of the CIELAB color system (L^*, a^*, b^*, C^*, and h°). In this system L^*, a^* and b^* describe a three-dimensional space, where L^* is the vertical axis and its value varies from 100, for perfect white, to zero, for black. Values of a^* and b^* specify the green-red and blue-yellow axis, respectively, ranging from −60 to +60 or from $-a$ (green) to $+a$ (red) and from $-b$ (blue) to $+b$ (yellow). C^* and h° values are calculated based on a^* and b^* values according to the following equations: $C^* = [(a^*)^2 + (b^*)^2]^{0.5}$ and $h^\circ = 180\,\pi \tan^{-1} (b^*/a^*)$. C^* describes the length of the color vector in the plane formed by the values of a^* and b^*, while h° determines the position of such vector. The colorimeter was calibrated with the white pattern during each sampling time. Epidermis color was longitudinally determined on three points of each flat side of the fruit (six points for each fruit). For mesocarp color, a big slice from a flat side of each fruit was obtained and color was determined longitudinally at three equidistant points.

2.4. Extraction of carotenoids

The extraction procedure of carotenoids was carried out as described by Pott et al. (2003a), with some modifications (Yahia et al., 2006b; Yahia et al., 2007). Fresh mango mesocarp from each fruit (6 g) was ground in a homogenizer (Ika Works Inc., Wilmington, NC) in the presence of calcium carbonate (0.2 g) and methanol (15 mL). The homogenate was filtered through a filter paper by adding methanol until retained solids became colorless. The methanolic extract was mixed with 50 mL of a mixture of hexane–acetone (1:1, v/v) containing 0.1% of BHT. After vigorous stirring, 40 mL of 10% sodium sulfate were added for phase separation. The upper layer was separated, washed several times with water, and evaporated in a Rotovapor® (Büchi Labortechnik AG, Flawil, Switzerland) at 35 °C. For saponification, the residue was dissolved in diethyl ether (30 mL) and 0.2 mL of 40% KOH in methanol were added. The mixture was left for 16 h in the dark at room temperature. After completion of saponification the extract was washed with water and evaporated as described above. Saponified and unsaponified residues were dissolved in 2-propanol (2 mL), filtered through a polyethylene membrane of 0.45 μm of pore size (Millipore Corp., Bedford, MA), and injected into the HPLC system (25 μL).

2.5. I_2-catalized photoisomerization of carotenoids

The 9-*cis* isomer of violaxanthin was generated for identification purposes according to Molnár et al. (2004). A quantity of 0.11 mg of all-*trans*-violaxanthin was dissolved in 1 mL of benzene containing 0.002 mg of I_2. The solution was exposed to daylight until equilibrium was reached, which occurred within 40 min. The reaction mixture was then washed with 5% Na_2SO_3 (50 mL), evaporated at reduced pressure (12 kPa) at 35 °C and re-dissolved in 2-propanol (2 mL) prior to HPLC analysis.

2.6. HPLC–MS analytical conditions

Samples containing carotenoids were automatically injected into an HP 1100 series HPLC system (Hewlett-Packard, GmbH, Germany) equipped with a diode array detector. Absorption spectra for the main peaks were recorded between 200 and 500 nm (each 2 nm). Individual signals for 9-*cis*-violaxanthin, all-*trans*-violaxanthin and all-*trans*-β-carotene were monitored at 436, 439 and 452 nm, respectively. The HPLC system was equipped with a C_{30} reversed-phase column (4.6 mm × 150 mm) with a spherical particle size of 3 μm (YMC Inc., Wilmington, NC), which was kept at 15 °C. The mobile phase was composed of water (A), methanol (B) and MTBE (C) with the following gradient program: 4% A, 95.2% B and 0.8% C at 0 min, decreasing to 4% A, 55.3% B and 40.7% C within 78 min at a flow rate of 1.25×10^{-2} mL s^{-1}.

Mass spectra of the main carotenoids were obtained using the chromatographic system described above but with the addition of a 6210 model time-of-flight mass spectrometer (Agilent Technologies Inc., Palo Alto, CA) equipped with an atmospheric pressure chemical ionization (APcI$^+$) interface and MassHunter manager software (Version A.02.01). The APcI$^+$–MS system was operated in positive ion mode. High purity nitrogen (99.999%) was used as nebulizing (130 kPa) and drying gas (83.33 mL s^{-1}). Other APcI$^+$–MS parameters were as follows: gas and vaporizer temperatures were