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Original Article

Cultivar influence on carotenoid composition of loquats from Brazil

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ABSTRACT

Cultivar, growing conditions and geographical origin are factors that influence the carotenoid composition in fruits. Because the loquat cultivars evaluated in this study, Centenária, Mizauto, Mizuho, Mizumo and Néctar de Cristal, have not previously been investigated, the present work was carried out to determine and compare the carotenoid composition of these five loquat cultivars, by applying high-performance liquid chromatography connected to a photodiode array and mass spectrometry detectors (HPLC-PDA-MS/MS). Twenty-five carotenoids were separated on a C_{30} column, and 23 of them were identified. All-*trans*-β-carotene (19–55%), all-*trans*-β-cryptoxanthin (18–28%), 5,6:5′,6′-diepoxy-β-cryptoxanthin (9–18%) and 5,6-epoxy-β-cryptoxanthin (7–10%) were the main carotenoids. The total carotenoid content ranged from 196 μg/100 g (cv. Néctar de Cristal) to 3020 μg/100 g (cv. Mizumo). The carotenoid profile of cv. Néctar de Cristal was different from the other cultivars, which was in agreement with its cream pulp colour, in contrast to the other four cultivars with orange pulp colour. Cultivars Mizauto, Mizuho, Mizumo and Centenária showed provitamin A values between 89 and 162 μg RAE/100 g, and can be considered good source of this provitamin.

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a fruit that belongs to the Rosaceae family. This fruit is native to China and it has been widely cultivated for commercial purposes since the 19th century. The major worldwide producers of loquat fruits are China and Spain, with production reaching 200,000 and 41,487 tons/year, respectively. In Brazil, loquat production is centralized in São Paulo State, mainly in Mogi das Cruzes region, and the annual production was about 2400 tons of fruits in 2001 (Caballero and Fernández, 2003).

The loquat cultivars are commercially classified as orange-pulped and white-pulped. In Brazil, Mizuho and Precoce de Itaquera are the cultivars with major economic importance (Ojima et al., 1999). Other cultivars that have been developed in Brazil by the Agronomic Institute of Campinas (IAC) are Centenária (IAC 1567-420), Mizumo (IAC 1567-411), Mizauto (IAC 167-4) and Néctar de Cristal (IAC 866-7).

When fully ripe, the loquat fruits show yellow to orange colour due to the presence of carotenoids. Apart from their colorant properties, the carotenoids are related to important functions and physiological actions, provitamin A activity being the most well known. In addition, a positive correlation has been observed between ingestion of vegetables and fruits containing carotenoids and prevention of several chronic-degenerative diseases, such as cancer, inflammation, cardiovascular diseases, cataract, age-related macular degeneration, among others (Coyne et al., 2005; Krinsky et al., 2003; Stahl and Sies, 2005).

The carotenoid composition of certain plant species is influenced by genetic, agricultural and/or geographical factors, since these factors are known to affect the biosynthetic pathway of these pigments (Britton, 1998). In fact, studies with different cultivars of citrus (Dhuique-Mayer et al., 2005), *Capsicum annuum* (Collera-Zúñiga et al., 2005) and seabuckthorn, *Hippophae rhamnoides* L. (Raffo et al., 2004), for example, showed the influence of this genetic factor on carotenoid composition.

The carotenoid composition of some loquat cultivars has been studied by other researchers. Kon and Shimba (1988) evaluated the carotenoid composition in Toi and Tanaka cultivars using thin-layer chromatography (TLC). These pigments were also analyzed in

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cv. Golden Nugget using open-column chromatography (OCC), TLC and mass spectrometry (Gross et al., 1973). Godoy and Rodriguez-Amaya (1995) used OCC and TLC for the evaluation of carotenoids from an unspecified cultivar of loquat from Brazil. The carotenoids from a non-saponified extract of loquat were also determined by high-performance liquid chromatography (HPLC) (Breithaupt et al., 2002). In all these cultivars, the major carotenoids were reported to be β-cryptoxanthin and β-carotene.

Since the carotenoid composition of the loquat cultivars evaluated in the present study, Mizauto, Mizumo, Centenária, Mizuho and Néctar de Cristal, has not previously been determined, the objective of this work was to apply HPLC coupled to photodiode array and mass spectrometry detectors (HPLC-PDA-MS/MS) to identify and quantify the carotenoids from different loquat cultivars.

2. Materials and methods

2.1. Material

Methanol (MeOH) and methyl *tert*-butyl ether (MTBE) for HPLC were obtained from Merck (Darmstadt, Germany) or from Mallinckrodt Baker (Philipsburg, USA). The other reagents were all of analytical grade from Labsynth (Diadema, Brazil). The samples and solvents were filtered through Millipore (Billerica, USA) membranes (0.22 and 0.45 μm) prior to HPLC analysis.

Standards of all-*trans*-lutein, all-*trans*-β-cryptoxanthin, all-*trans*-β-carotene, 15-*cis*-β-carotene, 13-*cis*-β-carotene, and 9-*cis*-β-carotene were provided by DSM Nutritional Products (Basel, Switzerland), showing 93–99% purity by HPLC-PDA.

2.2. Samples

Five loquat cultivars, cultivated and selected by the Agronomic Institute of Campinas (IAC), were analyzed: Centenária, Mizumo, Néctar de Cristal, Mizauto and Mizuho. The fruits were produced in the APTA Fruits Center (IAC), located in Jundiaí region (São Paulo State, Brazil, 23°8′S; 46°55′W; 700 m altitude), and they were harvested in September 2005, that is, in the spring of the Southern Hemisphere. The loquat trees of each cultivar were grown in an orchard distributed in blocks. There were four blocks for each cultivar, spaced 7 m × 5 m and one tree per block. The fruits were harvested on the same day, during the morning, in the medium portions of each plant tree directed to the main cardinal points (North, South, East and West). The harvested fruits, 1.5 kg of each cultivar, were transported to the laboratory, where they were immediately selected according to size and colour uniformity. Those fruits with apparent physical injuries were discarded, and the chosen samples were peeled, diced to 0.5–1.0 cm pieces with a stainless-steel blade and immediately frozen in liquid nitrogen. The samples were kept in the dark at −80 °C.

2.3. Brief description of cultivar characteristics

Mizuho: This cultivar was derived by crossing cv. Tanaka with cv. Kusunoki. It bears the largest fruits (60–80 g), with yellow-orange colour and sweet-sour flavour.

Centenária (IAC 1567-420) and *Mizumo* (IAC 1567-411): Both cultivars were derived by crossing cv. Mizuho with cv. Mogi, which was done by IAC. The fruits from cv. Centenária were big (60 g) with uniform soft orange colour. The loquats cv. Mizumo were also big (65 g), with round shape and orange colour. The pulp of both cultivars was soft and succulent.

Mizauto (IAC 167-4): This cultivar was derived from the Mizuho self-fertilization, which was also done by IAC. The fruits were big (60 g) with orange colour, succulent with sweet-sour flavour pulp.

Néctar de Cristal (IAC 866-7): This cultivar was developed by IAC and was derived from the free pollination of the Togoshi cultivar, which originated in Japan. The fruits were of moderate size (30–40 g), round shape and yellow colour. In contrast to the other IAC-Brazilian cultivars, the pulp of this cultivar was soft cream in colour.

2.4. Carotenoid extraction

Before extraction, all pieces of each sample were homogenized, obtaining a unique pulp of each cultivar, from which two samples of 10–15 g were weighed and set aside for extraction. The carotenoids were exhaustively extracted from the loquat pulps with acetone, transferred to petroleum ether (30–70 °C):diethyl ether (2:1) and saponified overnight at room temperature with 10% methanolic KOH. Alkali was removed after being washed with water, and then the solvent was evaporated in a rotary evaporator ($T < 40$ °C).

In order to confirm that extraction was exhaustive, after six extractions, the sample was extracted again, filtered and the absence of carotenoids in the last extract was verified at 450 nm, using an Agilent spectrophotometer, model 8453 (Santa Clara, USA).

The extracts were dried and stored under dark and nitrogen atmosphere (99.9% purity) at −35 °C until HPLC analysis. In order to avoid carotenoid degradation during analyses, the manipulation of samples and extracts was carried out in a dark ambient and at controlled room temperature of 22 °C.

2.5. HPLC-PDA and HPLC-PDA-MS/MS analysis

The quantitative analysis was carried out in a Waters HPLC (Milford, Massachusetts, USA) equipped with quaternary pumps (model 600), on-line degasser, a Rheodyne injection valve (Rheodyne LCC, Rohnert Park, USA) with a 20 μL loop, an external oven with temperature control and a photodiode array detector (PDA) (Waters, model 996). Data acquisition and processing were done with a Millennium Waters software. The UV–vis spectra were obtained between 250 and 650 nm and the chromatograms were processed at 450, 350 and 280 nm.

For the MS analysis, a Shimadzu HPLC (Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), on-line degasser and a Rheodyne injection valve with a 20 μL loop was used. The equipment included a PDA detector (Shimadzu, model SPD-M20A) and a mass spectrometer with APCI ionization source, and an ion-trap analyser from Bruker Daltonics, model Esquire 4000 (Bremen, Germany). The MS parameters were set as follows: positive mode, current corona: 4000 nA, source temperature: 450 °C, dry gas N_2—temperature: 350 °C, flow: 5 L/min, nebulizer: 60 psi, MS/MS fragmentation energy: 1.4 V. The mass spectra were acquired with scan range of m/z from 100 to 700 (De Rosso and Mercadante, 2007a).

In both Waters and Shimadzu HPLC equipments, carotenoid separation was carried out on a C_{30} YMC column (5 μm, 250 mm × 4.6 mm i.d.) using mobile phase as a linear gradient of MeOH with 0.1% triethylamine (TEA)/MTBE from 95:5 to 70:30 in 30 min, followed by 50:50 in 20 min. The flow rate was 1.0 mL/min and column temperature was set at 29 °C when the analysis was carried out in the Waters HPLC. TEA was added to the mobile phase since recovery on a C_{30} column enhanced 26% for lutein, 42% for zeaxanthin, 55% for β-cryptoxanthin and 64% for β-carotene (Emenhiser et al., 1996). On the other hand, TEA was excluded from the mobile phase, the flow rate and temperature were, respectively, decreased to 0.9 mL/min and 22 °C when analysis was carried out by the MS detector because TEA shows high proton affinity, being more easily ionized in the APCI source, and as a