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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

result the carotenoid ion signals decreased (De Rosso and Mercadante, 2007a).

The combined results of the following parameters were considered for carotenoid identification: elution order on the C_{30} column, UV/vis spectrum features (maximum absorption wavelength (λ_{max}), spectral fine structure (%III/II) and peak *cis* intensity (%A_B/A_{II})), and MS spectrum characteristics as compared to standards analyzed in the same conditions and data available in the literature (Back and Enzell, 1995; Britton, 1995; De Rosso and Mercadante, 2007a,b; Britton et al., 2004).

The carotenoids were quantified by HPLC, using external calibration curves for all-*trans*-lutein (0.9–15.7 μg/mL, $r^2 = 0.998$), all-*trans*-β-cryptoxanthin (1.1–40.8 μg/mL, $r^2 = 0.988$) and all-*trans*-β-carotene (2.0–40.9 μg/mL, $r^2 = 0.997$). Neoxanthin, violaxanthin and neochrome were estimated using the curve of lutein, the β-cryptoxanthin epoxides using the curve of β-cryptoxanthin and the *cis*-isomers of β-cryptoxanthin and β-carotene were estimated using the curve of the corresponding all-*trans* isomer. The NAS-IOM (2001) conversion factor was used to calculate the vitamin A value, with 12 μg of dietary all-*trans*-β-carotene and 24 μg of other dietary provitamin A carotenoids corresponding to 1 μg of retinol activity equivalent (RAE), and the activities used were 100% for all-*trans*-β-carotene, 50% for all-*trans*-β-cryptoxanthin (Bauerfeind, 1972) and for all *cis*- isomers of β-cryptoxanthin and of β-carotene.

The precision of the analysis was evaluated by the coefficient of variation (CV) among extractions. The CV values of the major carotenoids ranged from 0.0% to 10.0% for all-*trans*-β-carotene, from 3.0% to 6.1% for all-*trans*-β-cryptoxanthin and from 1.0% to 10.7% for 5,6:5′,6′-diepoxy-β-cryptoxanthin. These values were lower than those reported for major carotenoids from citrus fruits, 3–20%, analyzed on a C_{30} column (Fanciullino et al., 2006) and for mixed vegetable reference material, 3–30%, analyzed on a C_{18} column (Scott et al., 1996). In addition, these CV values were in the same range of those found for major carotenoids from fruits of *Lycium barbarum* Linnaeus, 1–9% (Inbaraj et al., 2008) and from the alga *Dunaliella salina*, 7–8% (Hu et al., 2008), both analyzed on a C_{30} column, and for other mixed vegetable reference material, 5–12%, analyzed on a C_{18} column (Hart and Scott, 1995). As expected, the CV values observed for minor *cis* isomers of β-carotene and β-cryptoxanthin were higher as compared to the respective *trans*, ranging from 2.9% to 19.2%.

2.6. Statistical analysis

To evaluate the differences in the carotenoid concentrations and vitamin A values between the cultivars, analyses of variance were conducted using one-way ANOVA (Sotware Origin 7.5), with Tukey test for mean differences at $P < 0.05$.

3. Results and discussion

3.1. Carotenoid identification

Figs. 1 and 2 show the chromatograms obtained by HPLC on a C_{30} column from Néctar de Cristal and Mizauto cultivars, respectively. The carotenoid profiles of Centenária, Mizuho and Mizumo cultivars were similar to those of cultivar Mizauto. The carotenoid structures are illustrated in Fig. 3.

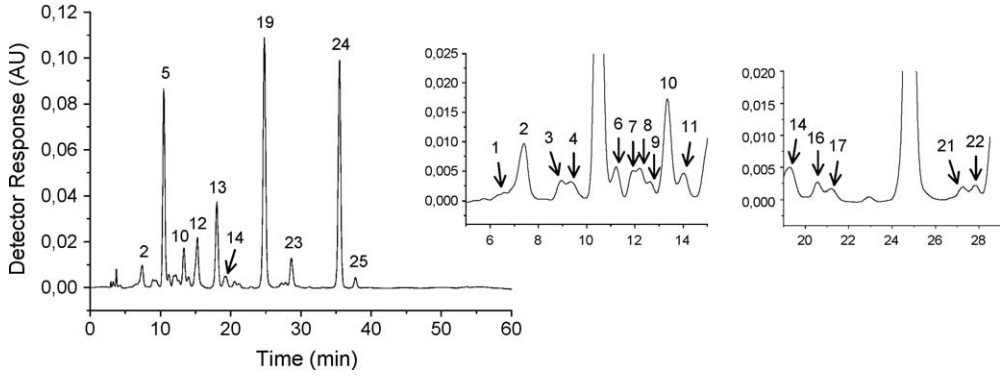

Fig. 1. Chromatogram of the carotenoids from Néctar de Cristal cultivar, obtained by HPLC-PDA. Chromatographic conditions: see text. Processed at 450 nm.

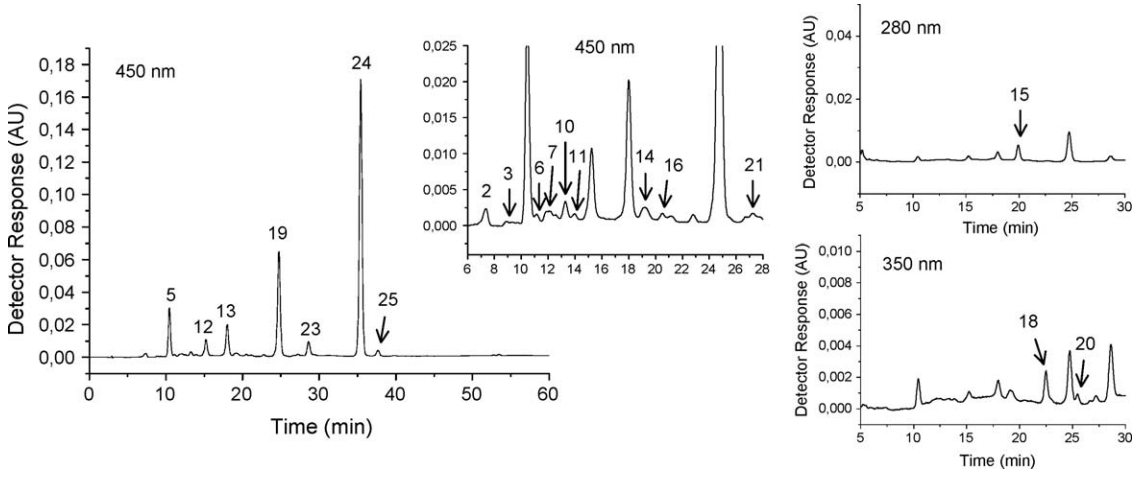

Fig. 2. Chromatogram of the carotenoids from Mizauto cultivar, obtained by HPLC-PDA. Chromatographic conditions: see text.

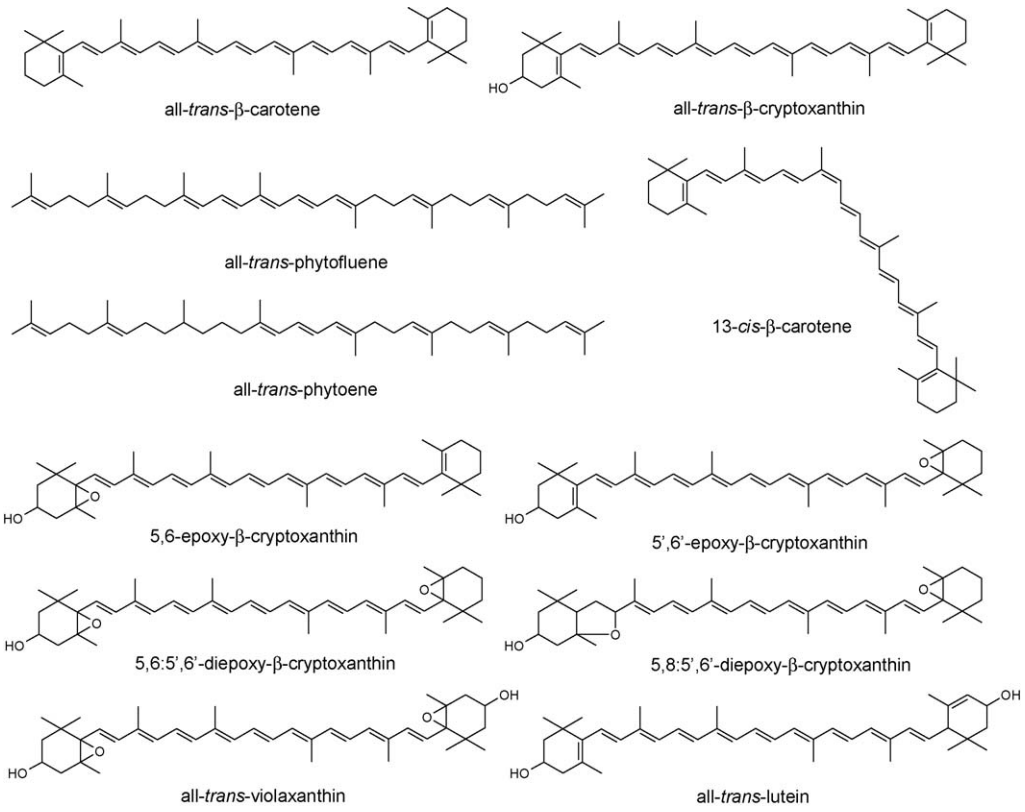

Fig. 3. Structures of some of the carotenoids detected in loquat.

The carotenoids separated in the five loquat cultivars analyzed in the present study were identified based on the combined information obtained from chromatographic elution, UV/vis and mass spectra characteristics (Table 1). The MS/MS experiments confirmed the assignment of the protonated molecule ($[M+H]^+$) of all identified peaks, since expected fragments from both polyene chain and functional groups were obtained when the protonated molecule was further ionized (Table 1). Although in-source fragmentation occurred in the APCI interface, the second order MS unambiguously allowed the assignments of some functional groups due to the specific losses from the protonated molecule. Since a detailed description of carotenoid identification using the above information was already reported by De Rosso and Mercadante (2007a,b), only considerations regarding the carotenoids not identified in these previous reports are discussed below.

The carotenoids with at least two hydroxyl groups in the structure, all-*trans*-neoxanthin (peak 1), 9-*cis*-neoxanthin + all-*trans*-violaxanthin (peak 2), all-*trans*-neochrome (peak 3), 9-*cis*-violaxanthin (peak 6) and all-*trans*-lutein (peak 10), showed UV–vis and mass spectra features similar to data reported in the literature (De Rosso and Mercadante, 2007a; Britton et al., 2004). The presence of two carotenoids coeluting in peak 2 was verified by injecting carotenoid extracts from kale and mango, containing respectively 9-*cis*-neoxanthin (Strand et al., 2000) and all-*trans*-violaxanthin (Mercadante et al., 1997; Ornelas-Paz et al., 2007; Pott et al., 2003). The location of the *cis* double in 9-*cis*-violaxanthin (peak 6) was assigned by comparison of the UV–vis spectrum characteristics with those described by Meléndez-Martínez et al. (2007) in a study involving many violaxanthin isomers. These xanthophylls were found in all five loquat cultivars, with the exception of all-*trans*-neoxanthin which was detected only in cv. Néctar de Cristal.

All β-cryptoxanthin diepoxides (peaks 4, 5, 7, 9 and 11) showed the $[M+H]^+$ at *m*/*z* 585 and, as expected, identical fragmentation pattern obtained in the MS/MS experiments since it is not possible to distinguish by MS between the 5,6-epoxide and 5,8-furanoid end groups. The second order MS experiments showed successive losses of water due to the elimination of hydroxyl and/or epoxide groups, elimination of toluene from the polyene chain ($[M+H-92]^+$), alone or also combined with loss of water ($[M+H-92-18]^+$). Another interesting feature was the simultaneous presence of the fragments at *m*/*z* 205 and 221, both characteristic of epoxide groups, but one located in an unsubstituted ring (*m*/*z* 205) whilst another in a ring with a hydroxyl group (*m*/*z* 221) (Back and Enzell, 1995). Therefore, the identification of these peaks was based on the UV–vis spectra characteristics. Peak 5 was identified as 5,6:5′,6′-diepoxy-β-cryptoxanthin due to 10 nm hypsochromic shift compared to the λ_{max} of β-cryptoxanthin, indicating the presence of two epoxide groups at 5,6- and 5′,6′- positions (Britton, 1995; Britton et al., 2004). The UV–vis spectra of peaks 4 and 11 showed 5–8 nm hypsochromic shift, presence of *cis* peak at 327–328 nm, decreased spectral fine structure and increased *cis* peak intensity as compared to that of 5,6:5′,6′-diepoxy-β-cryptoxanthin (peak 5), indicating that these carotenoids were *cis* isomers of 5,6:5′,6′-diepoxy-β-cryptoxanthin, most probably at 13- or 15- (peak 4) and 9- (peak 11) *cis* positions according to their elution order. The UV–vis spectrum of peak 7 showed an hypsochromic shift of 28 nm as compared to β-cryptoxanthin, indicating the presence of one furanoid and one epoxide group; however, it was not possible to distinguish between the 5,8:5′,6′- or 5,6:5′,8′-diepoxy-β-cryptoxanthin. Peak 9 was characterized as a *cis*-isomer of 5,8:5′,6′- or 5,6:5′,8′-diepoxy-β-cryptoxanthin considering its lower λ_{max} values, appearance of a *cis* peak at 315 nm, decreased spectral fine structure and increased *cis* peak intensity as compared to peak 7.

The monoepoxides of β-cryptoxanthin (peaks 12, 13 and 16) were identified considering the UV–vis and MS characteristics discussed above and by the comparison with literature data (De

Table 1
Chromatographic, UV–vis and mass spectrometry characteristics of carotenoids from loquat cultivars, obtained by HPLC-PDA-MS/MS.

Peak[a]	Carotenoid	t_R (min)[b]	λ_{max} (nm)[c]	%III/II	$\%A_B/A_{II}$	$[M+H]^+$ (m/z)	MS/MS fragment ions (m/z)
1	All-*trans*-neoxanthin	6.4	415, 440, 469	97	0	601	583[M+H-18], 565[M+H-18-18], 547[M+H-18-18-18], 491[M+H-18-92], 393, 221
2	All-*trans*-violaxanthin + 9-*cis*-neoxanthin	7.4	328, 414, 440, 469	94	9	601	583[M+H-18][f], 565[M+H-18-18], 547[M+H-18-18-18], 491[M+H-18-92], 221
3	All-*trans*-neochrome	8.8–9.0	397, 420, 448	100	0	601	583[M+H-18], 565[M+H-18-18], 547[M+H-18-18-18], 509[M+H-92], 491[M+H-18-92], 221
4	*cis*-5,6:5′,6′-Diepoxy-β-cryptoxanthin	9.3–9.4	327, 410, 432, 460	55	40	585	567[M+H-18], 549[M+H-18-18], 493[M+H-92], 475[M+H-18-92], 221, 205
5	5,6:5′,6′-Diepoxy-β-cryptoxanthin	10.4–10.5	415, 440, 469	89	0	585	567[M+H-18], 549[M+H-18-18], 493[M+H-92], 475[M+H-18-92], 221, 205
6	9-*cis*-Violaxanthin	11.1–11.2	325, 410, 435, 464	87	13	601	583[M+H-18], 565[M+H-18-18], 547[M+H-18-18-18], 509[M+H-92], 491[M+H-18-92], 221
7	5,8:5′,6′- or 5,6:5′,8′-diepoxy-β-cryptoxanthin	11.9–12.1	396, 422, 448	97	0	585	567[M+H-18], 549[M+H-18-18], 493[M+H-92], 475[M+H-18-92], 221, 205
8	Not identified 1	12.2	399, 422, 448	98	0	601	583[M+H-18], 565[M+H-18-18], 221
9	*cis*-5,8:5′,6′- or *cis*-5,6:5′,8′-diepoxy-β-cryptoxanthin	12.6	315, 397, 420, 448	89	35	585	567[M+H-18], 549[M+H-18-18], 493[[M+H-92], 475[M+H-18-92], 221, 205
10	All-*trans*-lutein	13.3	420, 445, 472	62	0	569	551[M+H-18][f], 533[M+H-18-18], 495[M+H-18-56], 459[M+H-18-92], 477[M+H-18-18-56]
11	*cis*-5,6:5′,6′-Diepoxy-β-cryptoxanthin	14.0	328, 410, 435, 464	78	26	585	567[M+H-18], 549[M+H-18-18], 493[[M+H-92], 475[M+H-18-92], 221, 205
12	5′,6′-Epoxy-β-cryptoxanthin	15.2–15.3	420, 445, 472	60	0	569	551[M+H-18], 533[M+H-18-18], 205
13	5,6-Epoxy-β-cryptoxanthin	18.0–18.1	420, 446, 474	55	0	569	551[M+H-18], 533[M+H-18-18], 477[M+H-92], 221
14	13- or 13′-*cis*-β-cryptoxanthin	19.2–19.3	337, 419, 443, 470	7	50	553	535[M+H-18]
15	Phytoene	19.9	272, 285, 294	0	0	545	489, 435, 395, 339[M-205]
16	5,8-Epoxy-β-cryptoxanthin	20.5–20.6	404, 426, 453	54	0	569	551[M+H-18], 221
17	Not identified 2	21.2	442	0	n.c.[d]	n.d.[e]	n.d.[e]
18	*cis*-Phytofluene	22.5	331, 347, 365	55	0	543	461, 406[M+H-137], 337[M-205]
19	All-*trans*-β-cryptoxanthin	24.7–24.8	424, 451, 477	32	0	553	535[M+H-18], 497[M+H-56], 461[M+H-92]
20	All-*trans*-phytofluene	25.4–25.5	331, 347, 365	76	0	543	461, 406[M+H-137], 337[M-205]
21	15-*cis*-β-Carotene	27.2–27.3	335, 421, 448, 475	0	57	537	444[M-92]
22	9- or 9′-*cis*-β-cryptoxanthin	27.8	337, 420, 445, 472	30	15	553	535[M+H-18][f], 479[M+H-18-56]
23	13-*cis*-β-Carotene	28.6	338, 420, 445, 470	11	47	537	444[M-92]
24	All-*trans*-β-carotene	35.4–35.5	425, 452, 479	27	0	537	444[M-92]
25	9-*cis*-β-Carotene	37.6–37.8	337, 421, 447, 474	23	17	537	444[M-92]

[a] Numbered according to Figs. 1 and 2.
[b] Range of retention time on the C_{30} column.
[c] Linear gradient of methanol/MTBE.
[d] n.c.: not calculated.
[e] n.d.: not detected.
[f] In-source fragments.

Rosso and Mercadante, 2007b). The 5,6-epoxide or 5,8-furanoid groups that could be located in one of the end groups of β-cryptoxanthin (Fig. 3) was assigned as 5,6-epoxy-β-cryptoxanthin (peak 13) and 5,8-epoxy-β-cryptoxanthin (peak 16) due to the presence of the mass fragment at m/z 221 that corresponds to the location of the epoxide/furanoid group in the 3-hydroxy-β-ring, whereas the fragment at m/z 205 detected in peak 12 allowed its identification as 5′,6′-epoxy-β-cryptoxanthin. The exact location of the epoxide group is important to the vitamin A value, since 5′,6′-epoxy-β-cryptoxanthin does not have provitamin activity, whereas both 5,6- and 5,8-epoxy-β-cryptoxanthin shows 50% activity. For example, in the case of the Mizuho cultivar, if 5′,6′-epoxy-β-cryptoxanthin was identified as 5,6-epoxy, and 5,6- and 5,8-epoxy-β-cryptoxanthin were identified as 5′,6′- and 5′,8′-epoxy, respectively, the vitamin A value for this cultivar would be 122 mg RAE/100 g, whereas using the correct identification this value was 127 mg RAE/100 g, implying in an error of 4%.

Among the eight epoxides and diepoxides of β-cryptoxanthin separated in loquat cultivars, six of them were found in all cultivars, whereas *cis*-5,6:5′,6′-diepoxy-β-cryptoxanthin (peak 4) was detected only in cultivars Centenária and Néctar de Cristal, and *cis*-5,8:5′,6′-(or 5,6:5′,8′)-diepoxy-β-cryptoxanthin was found in cultivars Mizuho and Néctar de Cristal.

The *cis* isomers of β-cryptoxanthin (peaks 14 and 22) were identified considering that the spectral fine structure (%III/II) decreases and intensity of the *cis*-peak ($\%A_B/A_{II}$) increases as the *cis*-double bond is getting closer to the centre of the molecule (Zechmeister, 1944) according to the results of those isomers formed by acid-catalyzed isomerization of β-cryptoxanthin standard (De Rosso and Mercadante, 2007b). However, it was not possible to distinguish between the 13-*cis* or 13′-*cis*- and 9-*cis* or 9′-*cis* isomers since there is no data available in the literature using nuclear magnetic resonance for exact determination of the *cis*-double bond positions in the β-cryptoxanthin structure for these compounds eluted on a C_{30} column. The 13-*cis* or 13′-*cis*-β-cryptoxanthin was found in all loquat cultivars, whilst the 9-*cis* or 9′-*cis* isomer was detected only in cv. Néctar de Cristal. Similar results for fresh and pasteurized orange juice and fresh and canned peach samples, with detection of 13-*cis*-β-cryptoxanthin but not 9-*cis*-β-cryptoxanthin, were reported by Lessin et al. (1997). These results denote that the relative abundance of the *cis*-isomers of β-cryptoxanthin shows a trend similar to those for β-carotene, since

the contents of 13-*cis*-β-carotene were usually higher than those of 9-*cis*-isomer in fruits (De Rosso and Mercadante, 2007a,b).

All the other carotenoids, phytoene (peak 15), *cis*-phytofluene (peak 18), all-*trans*-phytofluene (peak 20), all-*trans*-β-cryptoxanthin (peak 19), all-*trans*-β-carotene (peak 24), 9-*cis*-β-carotene (peak 25), 13-*cis*-β-carotene (peak 23) and 15-*cis*-β-carotene (peak 21) were identified by elution order on C_{30} column, UV–vis and mass spectra characteristics as compared to standards and to the literature (Dachtler et al., 2001; De Rosso and Mercadante, 2007a,b; Ornelas-Paz et al., 2007; Strohschein et al., 1999). The non-coloured carotenes (peaks 15, 18, 20) were not found in cv. Néctar de Cristal.

The main carotenoids found in the present study, 5,6:5′,6′-diepoxy-β-cryptoxanthin, 5,6-epoxy-β-cryptoxanthin, all-*trans*-β-cryptoxanthin and all-*trans*-β-carotene, were previously found by Gross et al. (1973) in cv. Golden Nugget. In a non-specified cultivar of loquat cultivated in Brazil, all the carotenoids cited above were detected, but 5,6:5′,6′-diepoxy-β-cryptoxanthin (Godoy and Rodriguez-Amaya, 1995). In addition, all-*trans*-β-carotene was the major carotenoid in non-saponified extract of loquat, accompanied by 9-*cis*- and 13-*cis*-β-carotene, as well as by free all-*trans*-β-cryptoxanthin and its esters (Breithaupt et al., 2002). On the other hand, the *cis* isomers of 5,6:5′,6′-diepoxy-β-cryptoxanthin, 9-*cis*-violaxanthin, *cis*-5,8:5′6′(or 5,6:5′,8′)-diepoxy-β-cryptoxanthin, 5,8-epoxy-β-cryptoxanthin and 15-*cis*-β-carotene are being reported for first time in loquat cultivars.

3.2. Quantitative composition

Table 2 shows the carotenoid concentrations obtained for the five loquat cultivars. In all cultivars the major carotenoids were all-*trans*-β-carotene (peak 24), varying from 19% (cv. Néctar de Cristal) to 55% (cv. Centenária) of the total carotenoid content, and all-*trans*-β-cryptoxanthin (peak 19), ranging between 18% (cv. Centenária) and 28% (cv. Néctar de Cristal). This characteristic was already observed for all different loquat cultivars evaluated by other researchers (Breithaupt et al., 2002; Godoy and Rodriguez-Amaya, 1995; Gross et al., 1973; Kon and Shimba, 1988).

However, the cream pulp cv. Néctar de Cristal showed both qualitative and quantitative differences as compared to the other four cultivars with orange pulp. The total carotenoid content in this cultivar was eight and fifteen times significantly lower, respectively, than those found in cv. Centenária and cv. Mizumo. In addition, differently from the other cultivars, the all-*trans*-β-cryptoxanthin level was higher than that of all-*trans*-β-carotene in cv. Néctar. Another difference concerns the proportion of lutein in relation to total carotenoid content, 3.3% in cv. Néctar de Cristal and from 0.2% to 0.6% in the other cultivars. These characteristics are probably because cv. Néctar de Cristal was derived from different cultivars, whereas all the other cultivars analyzed in this study, Mizuho, Centenária, Mizumo and Mizauto, had similar origins. Other loquat with whitish-yellow pulp, cv. Toi, also showed β-cryptoxanthin content higher than that of β-carotene and lutein proportion about 4.2% (Kon and Shimba, 1988).

Despite the fact that cv. Mizuho showed total carotenoid content significantly higher ($P < 0.05$) than that found in cv. Mizauto, the relative proportion of the major carotenoids from these cultivars were similar: 22% and 24% of all-*trans*-β-cryptoxanthin, and 43% and 49% of all-*trans*-β-carotene, respectively. In fact, the all-*trans*-β-cryptoxanthin content in cv. Mizuho was significantly higher compared to Mizauto, but the all-*trans*-β-carotene levels were not statistically different ($P > 0.05$). These similarities can be expected since cv. Mizauto was derived from cv. Mizuho. However, cv. Tanaka whose crossing with cv. Kusunoki gave origin to cv. Mizuho, showed 3850 μg/100 g of total carotenoid level, with 55% for β-carotene (2110 μg/100 g) and 25% for β-cryptoxanthin (960 μg/100 g) (Kon and Shimba, 1988), values higher than those found in the present study for cv. Mizuho.

The concentrations of all carotenoids, except *cis*-5,6:5′,6′-diepoxy-β-cryptoxanthin, were significantly higher ($P < 0.05$) in cv. Mizumo compared to those found in cv. Centenária, despite both cultivars having been obtained from crossing Mizuho with

Table 2
Concentration (μg/100 g) of carotenoids and vitamin A values (μg RAE/100 g) of loquat cultivars.

Carotenoids	cv. Centenária[a]	cv. Mizauto[a]	cv. Mizuho[a]	cv. Mizumo[a]	cv. Néctar de Cristal[a]
All-*trans*-neoxanthin	Not detected	Not detected	Not detected	Not detected	0.2
All-*trans*-violaxanthin + 9-*cis*-neoxanthin	9.3 ab	12.9 a	22.7 c	28.2 c	4.4 b
All-*trans*-neochrome	0.3 a	1.5 bc	1.9 b	6.4 d	1.2 c
cis-5,6:5′,6′-Diepoxy-β-cryptoxanthin	3.5 a	Not detected	Not detected	Not detected	1.8 b
5,6:5′,6′-Diepoxy-β-cryptoxanthin	161.0 a	180.3 a	339.5 b	324.9 b	35.0 c
9-*cis*-Violaxanthin	2.4 a	5.4 b	7.1 b	12.8 c	2.3 a
5,8:5′,6′- or 5,6:5′,8′-diepoxy-β-cryptoxanthin	3.1 a	6.4 a	34.8 b	7.1 a	1.8 a
Not identified 1	3.1 a	Not detected	Not detected	8.4 b	1.9 c
cis-5,8:5′,6′- or 5,6:5′,8′-diepoxy-β-cryptoxanthin	Not detected	Not detected	10.9 a	Not detected	1.1 b
All-*trans*-lutein	3.9 a	12.5 b	13.5 b	7.9 c	6.4 c
cis-5,6:5′,6′-Diepoxy-β-cryptoxanthin	4.6 a	4.5 a	12.1 b	10.4 c	1.9 d
5′,6′-Epoxy-β-cryptoxanthin	40.3 a	67.9 b	104.1 c	109.4 c	11.5 d
5,6-Epoxy-β-cryptoxanthin	102.4 a	137.9 b	213.9 c	207.2 c	19.0 d
13- or 13′-*cis*-β-cryptoxanthin	5.8 a	15.1 b	20.1 b	16.6 b	4.0 a
Phytoene	25.3 a	22.0 b	22.1 b	34.0 c	Not detected
5,8-Epoxy-β-cryptoxanthin	4.6 a	3.8 a	13.1 b	15.3 b	1.6 a
Not identified 2	Not detected	Not detected	Not detected	Not detected	1.1
cis-Phytofluene	12.5 a	8.5 b	10.1 b	19.3 c	Not detected
All-*trans*-β-cryptoxanthin	278.4 a	480.2 b	557.6 c	715.2 d	54.8 e
All-*trans*-phytofluene	1.7 a	1.9 a	3.3 b	3.4 b	Not detected
15-*cis*-β-Carotene	2.0 a	4.8 a	3.0 a	3.4 a	0.7 a
9- or 9′-*cis*-β-cryptoxanthin	Not detected	Not detected	Not detected	Not detected	0.8
13-*cis*-β-Carotene	19.4 a	42.0 b	45.9 b	36.2 b	5.0 c
All-*trans*-β-carotene	858.5 a	980.9 ab	1090.7 b	1441.5 c	38.1 d
9-*cis*-β-Carotene	6.7 a	17.3 bc	18.0 b	12.3 c	1.6 a
Total carotenoids	1548.6 a	2005.7 b	2544.4 c	3019.5 d	196.2 e
Vitamin A value	89.0 a	111.0 b	127.2 b	162.0 c	6.8 d

Different letters (a, b, c, d, e) in the same row indicate mean difference at significant level of 5%.
[a] Average of duplicated analysis.

Mogi. For example, cv. Mizumo showed total carotenoid and all-*trans*-β-carotene contents about two times significantly higher than those found in Centenária. Furthermore, these cultivars showed other differences, in respiratory activity, ethylene production and chemical composition, according to results not published (Hasegawa et al.,2009).

The total carotenoid concentration (2000–2500 μg/100 g) and the β-cryptoxanthin proportion (22%) from cv. Golden Nugget (Gross et al., 1973) were in the same range as those found for Centenária, Mizauto, Mizuho and Mizumo cultivars (total carotenoid 1549–3020 μg/100 g and 18–28% of β-cryptoxanthin). In addition, the contents of total carotenoid (1760 μg/100 g), β-carotene (780 μg/100 g) and β-cryptoxanthin (480 μg/100 g) from an unspecified cultivar from Brazil (Godoy and Rodriguez-Amaya, 1995) were in the same range as those found for cultivars Centenária, Mizauto and Mizuho evaluated in the present study.

3.3. Provitamin A activity

Among the 23 carotenoids identified, only nine show vitamin A activity. Table 2 shows the provitamin A values calculated for all loquat cultivars. The provitamin A values of Centenária and Mizauto cultivars were in the range of 175 ± 12 μg RE/100 g already reported for loquat (non-specified cultivar) also harvested in Brazil (Godoy and Rodriguez-Amaya, 1995).

Cultivars Mizauto, Mizuho and Mizumo can be considered good sources of provitamin A, when compared to moderate or rich sources of this nutrient, such as mango cultivars Tommy Atkins and Keitt (48–126 μg RAE/100 g) (Mercadante and Rodriguez-Amaya, 1998), caja (45–61 μg RAE/100 g) (Hamano and Mercadante, 2001), cashew-apple (5–9 μg RAE/100 g) (Assunção and Mercadante, 2003), camu–camu (7–12 μg RAE/100 g) (Zanatta and Mercadante, 2007) and acerola cultivar Olivier (74–142 μg RAE/100 g) (De Rosso and Mercadante, 2005).

In summary, in the present study, the carotenoid composition of all loquat cultivars, as well as cultivar differences, was successfully determined by HPLC-PDA-MS/MS technique. Among the analyzed cultivars, the most significant difference concerned the quantitative composition of carotenoids. The vitamin A values indicated that loquat fruits, mainly those belonging to Mizauto, Mizuho, and Mizumo cultivars, represent good source of provitamin A.

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