Food Chemistry 119 (2010) 653–659

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Characterization and thermal lability of carotenoids and vitamin C of tamarillo fruit (*Solanum betaceum* Cav.)

C. Mertz [a], P. Brat [a,*], C. Caris-Veyrat [b], Z. Gunata [c]

[a] Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Département PERSYST, UMR Qualisud, TA B-95/16, 34398 Montpellier Cedex 5, France
[b] UMR 408, Sécurité et Qualité des Produits d'Origine Végétale, INRA, Université d'Avignon, F-84000 Avignon, France
[c] UMR Qualisud, Université Montpellier 2 Place E. Bataillon, 34095 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 17 March 2009
Received in revised form 15 May 2009
Accepted 3 July 2009

Keywords:
Tomato tree
Tamarillo
Solanum betaceum Cav.
Carotenoids
Ascorbic acid
Thermal treatment

ABSTRACT

The carotenoids from yellow tamarillo were determined by high-performance liquid chromatography-photodiode array detection/mass spectrometry (HPLC-PDA/MS). Xanthophylls were found as esterified with palmitic and myristic acids. *All-trans*-β-cryptoxanthin esters and *all-trans*-β-carotene were the major carotenoids of tamarillo. Changes in carotenoid and vitamin C contents after thermal pasteurization of degassed and not degassed tomato tree nectars were studied. Zeaxanthin esters appeared to be the less thermo-labile carotenoids. Carotenoids degradation was not significantly influenced by dissolved oxygen level. However, thermal treatment induced 5,8-epoxidation and *cis*-isomerization. Retention of ascorbic acid was total under degassed conditions while losses of dehydroascorbic acid were not affected by the initial level of dissolved oxygen.

1. Introduction

The tamarillo or tomato tree (*Solanum betaceum* Cav.) is a small tree, cultivated for its edible fruit. In the Andean region, three tamarillo types, rather than cultivars, are distinguished according to fruit colour: the red, the yellow and the purple. The fruits can be consumed in many ways such as eaten raw as a dessert fruit or in salad. The fruits are juicy, with a very attractive colour. Due to the demand of consumers and its exportation potential, the cultivation of this fruit in Ecuador increased since the 1980's.

Despite the increasing interest in the tamarillo as a potential new crop, only few data, notably on their carotenoid composition, are available in the literature. Six carotenoids were identified in the peel and the pulp of the Brazilian fruit *Cyphomandra betacea*, with β-cryptoxanthin and β-carotene being the major ones (Rodriguez-Amaya, Bobbio, & Bobbio, 1983). Recently, a more complete characterization of carotenoids in the whole tamarillo fruit from Brazil was achieved after saponification using HPLC-PDA-MS/MS techniques (De Rosso & Mercadante, 2007a). However, the origin of the fruits as well as the cultural practices and the environmental factors may influence the composition.

Carotenoids, together with ascorbic acid (AA), are well known for their antioxidant capacity (Böhm, Puspitasari-Nienhaber, Ferruzzi, & Schwartz, 2002; Gardner, White, McPhail, & Duthie, 2000). Many factors, such as oxygen, heat, pH, presence of heavy metal ions, light can be responsible for their degradation. Thus, during food processing, thermal treatment may cause losses of micronutrients. The thermal degradation of ascorbic acid has been extensively studied in different food matrices. AA is easily reversibly oxidised to dehydroascorbic acid (DHAA), which also exhibits some vitamin C activity. Afterwards, it can irreversibly be hydrolyzed to generate diketogulonic acid (DKGA) leading to a total loss of its biological activity. The degradation kinetics of AA are variable and the rate constants depend on environmental conditions (Vieira, Teixeira, & Silva, 2000). Even if oxygen is known to play a role in the ascorbic acid degradation during storage (Kennedy, Rivera, Lloyd, Warner, & Jumel, 1992; Robertson & Samaniego, 1986), only few data are available on the AA thermal degradation in food matrices containing very low levels of dissolved oxygen. Conversely, investigations into the effects of processing on carotenoids have been extensively conducted. Thermal treatment induced variable losses, depending on its severity and length (Chandler & Schwartz, 1988; Gama & De Sylos, 2007) and led to structural modifications such as *cis*-isomerization (Seybold, Fröhlich, Bitsch, Otto, & Böhm, 2004). The thermal lability of carotenoids is also influenced by their chemical structure. Thus, carotenoid esters showed an increased stability compared to the corresponding non-esterified carotenoids (Schweiggert, Kurz, Schieber, & Carle, 2007). Nevertheless, there is a lack of data in the literature about

* Corresponding author. Tel.: +33 4 67 61 65 03; fax: +33 4 67 61 44 33.
E-mail address: pierre.brat@cirad.fr (P. Brat).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.07.009

the influence of initial dissolved oxygen content on the thermal degradation of carotenoids.

The objective of our study was firstly to characterise the carotenoid composition of the yellow tamarillo from Ecuador. In addition, the influence of thermal treatment on nectars of tomato tree containing two different levels of dissolved oxygen was assessed. Losses in micronutrients (AA, DHAA, and carotenoids) were evaluated to better understand the role of oxygen. In addition, degradation products of carotenoids were tentatively identified.

2. Materials and methods

2.1. Chemicals

All solvents were of HPLC grade, purchased from Carlo Erba (Val de Reuil, France) except of methyl *tert*-butyl ether (MTBE) (Sigma–Aldrich, Steinheim, Germany). 2,6-Di-*tert*-butyl-4-methylphenol (BHT) was from Sigma (L'isle d'Abeau, France). Sodium chloride, anhydrous sodium sulphate, magnesium carbonate, potassium hydroxide, and ammonium acetate were supplied by Carlo Erba (Val de Reuil, France). Sulfuric acid, *meta*-phosphoric acid (MPA) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were from Sigma–Aldrich (Steinheim, Germany). β-Carotene, β-cryptoxanthin, lutein, and zeaxanthin were from Extrasynthese (Genay, France). *All-trans*-violaxanthin and *cis*-neoxanthin were supplied by CaroteNature (Lupsingen, Switzerland).

2.2. Sample preparation

Yellow tamarillo fruits at a ripe state were frozen and supplied by the Escuela Politecnica nacional (EPN, Ecuador). The fruits (about 1 kg) were peeled, ground with an ultra turax in a dark room and the seeds were removed by sieving. The resulting puree was diluted with de-ionised water in a 1/3 ratio (puree/water, w/v) and homogenised to afford a nectar which was divided in two parts. All these treatments were conducted rapidly to minimise the changes in targeted compounds. One of them was immediately degassed under argon until dissolved oxygen content was below 1 ppm and stored at −20 °C before treatment. The other part was immediately analysed for the vitamin C content.

2.3. Thermal treatment

The tamarillo nectar was heated in hermetically sealed amber vials immersed in an oil bath with a temperature control (AM 3001 K, Fischer Bioblock Scientific, Illkirch, France). A digital temperature probe fitted to an amber vial was used to measure the nectar temperature during heating. Temperatures applied were of 80, 90, and 95 °C, respectively. When the nectar temperature reached the set up temperature, the vial was kept 10 min in the bath at this temperature and then cooled in iced water. The temperature was monitored each minute. This treatment was applied to both degassed (<1 ppm) and not degassed nectars (~8 ppm). Final levels of dissolved oxygen were measured in the not degassed nectar with a dissolved oxygen probe (Cell Ox 325, WTV France, Limonest). The vials were stored at −20 °C before analysis of carotenoids. The vitamin C content was immediately determined.

2.4. Extraction of carotenoids

Carotenoid extraction was adapted from that described by Taungbodhitham, Jones, Walhlqvist, and Briggs (1998). Three grams of puree or 5 g of nectar was added with 80 mg of $MgCO_3$, 15 ml of extraction solvent (ethanol/hexane, 4:3 v/v, containing 0.1% of BHT as antioxidant) and stirred for 5 min. The residue was separated from the liquid phase by filtration with a filter funnel (porosity No. 2) and washed successively with 15 ml of the above solvent, 15 ml of ethanol and 15 ml of hexane. Organic phases were transferred in a separatory funnel and successively washed with 40 ml of 10% sodium chloride and 2 × 40 ml of distilled water. The aqueous layer was removed. The hexanic phase was dried under anhydrous sodium sulphate, filtered and evaporated to dryness at 40 °C in a rotary evaporator. The residue was dissolved in 500 µl dichloromethane and 500 µl MTBE/methanol (80:20, v/v). Samples were placed in amber vials before chromatographic analysis.

2.5. Saponification

The saponification was carried out in 10% methanolic KOH, according to the method described by Fanciullino et al. (2006). Analyses were carried out under red light to avoid carotenoid degradation during extraction and saponification.

2.6. HPLC-MS analysis of carotenoids and related fatty acid esters

The HPLC apparatus was a Surveyor plus model equipped of an autosampler, a PDA detector and LC pumps (Thermo Electron Corporation, San Jose, CA, USA). Carotenoids and related fatty acid esters were analysed according to the previously published method of Dhuique-Mayer, Caris-Veyrat, Ollitraut, Curk, and Amiot (2005). Carotenoids were separated along a C_{30} column (250 × 4.6 mm, 5 µm particle size), YMC (EUROP, GmbH). The mobile phases were water/20 mM ammonium acetate as eluent A, methanol/20 mM ammonium acetate as eluent B and MTBE as eluent C. Flow rate was fixed at 1 ml/min and the column temperature was set at 25 °C. A gradient program was performed: 0–2 min, 40% A/60% B, isocratic elution; 2–5 min, 20% A/80% B; 5–10 min, 4% A/81% B/15% C; 10–60 min, 4% A/11% B/85% C; 60–71 min, 100% B; 71–72 min, back to the initial conditions for reequilibration. The injection volume was 10 µl and the detection was monitored from 250 to 600 nm. After passing through the flow cell of the diode array detector the column eluate was split and 0.5 ml was directed to the ion trap of a LCQ mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, California, USA). Experiments were performed in positive ion mode. Scan range was 100–2000, scan rate: 1 scan/s. The desolvation temperature was set at 250 °C.

2.7. High-performance liquid chromatography analysis of carotenoids and related fatty acid esters

Carotenoids and related fatty acid esters were analysed by HPLC using an Agilent 1100 System (Massy, France). The column and gradient conditions were the same as used in mass spectrometry analysis. The injection volume was 20 µl. Absorbance was followed at 290, 350, 400, 450 and 470 nm using an Agilent 1100 photodiode array detector. An Agilent Chemstation plus software was used for data analysis. For carotenol esters, only the areas corresponding to peaks that disappeared after alkaline treatment were integrated at 450 nm. Phytofluene was integrated at 350 nm. Peak areas were compared before and after thermal treatment. Each analysis was made in triplicate.

2.8. Extraction of vitamin C

Five grams of nectar were added with 3 ml of 4% MPA (pH 1.5), centrifuged in the dark at 4 °C (5 min, 10,000 rpm). The supernatant

was filtered through a 0.45 μm filter (Millipore) and transferred to amber vials before analysis. Each analysis was done in triplicate.

2.9. Kinetics reduction of DHAA

The used method was adapted and optimised from that described by Wechtersbach and Cigić (2007). Three solutions of TCEP at 2, 5 and 10 mM in MPA were prepared and 1 ml of the filtered supernatant was added with 1 ml of TCEP (2, 5 or 10 mM) and stored at 30 °C. The kinetic reduction of DHAA to AA was followed by HPLC. The DHAA content was calculated by difference between the peak area of AA with and without reduction. TCEP at 5 mM and a reaction time of 60 min were used to study the reduction of DHAA to AA. Each analysis was made in triplicate.

2.10. Chromatographic analysis of AA

AA was analysed using a Dionex liquid chromatograph equipped with model P680 pumps, an ASI 100 autosampler and a UVD 340U diode array detector coupled to a HP ChemStation (Dionex, France). The elution was performed on a 250 mm × 4.6 mm, 5 μm particle size, endcapped reversed-phase Lichrospher ODS-2 column (Interchim, Montluçon, France), in the isocratic mode with 0.01% H_2SO_4 (pH 2.6) as mobile phase. The flow was 0.8 ml/min, the oven temperature was set at 25 °C and the detection was performed at 245 nm. Each analysis was made in triplicate.

2.11. Statistical analysis

Values were given as means ± standard deviations. Analysis of variance (ANOVA) was used to determine significant differences

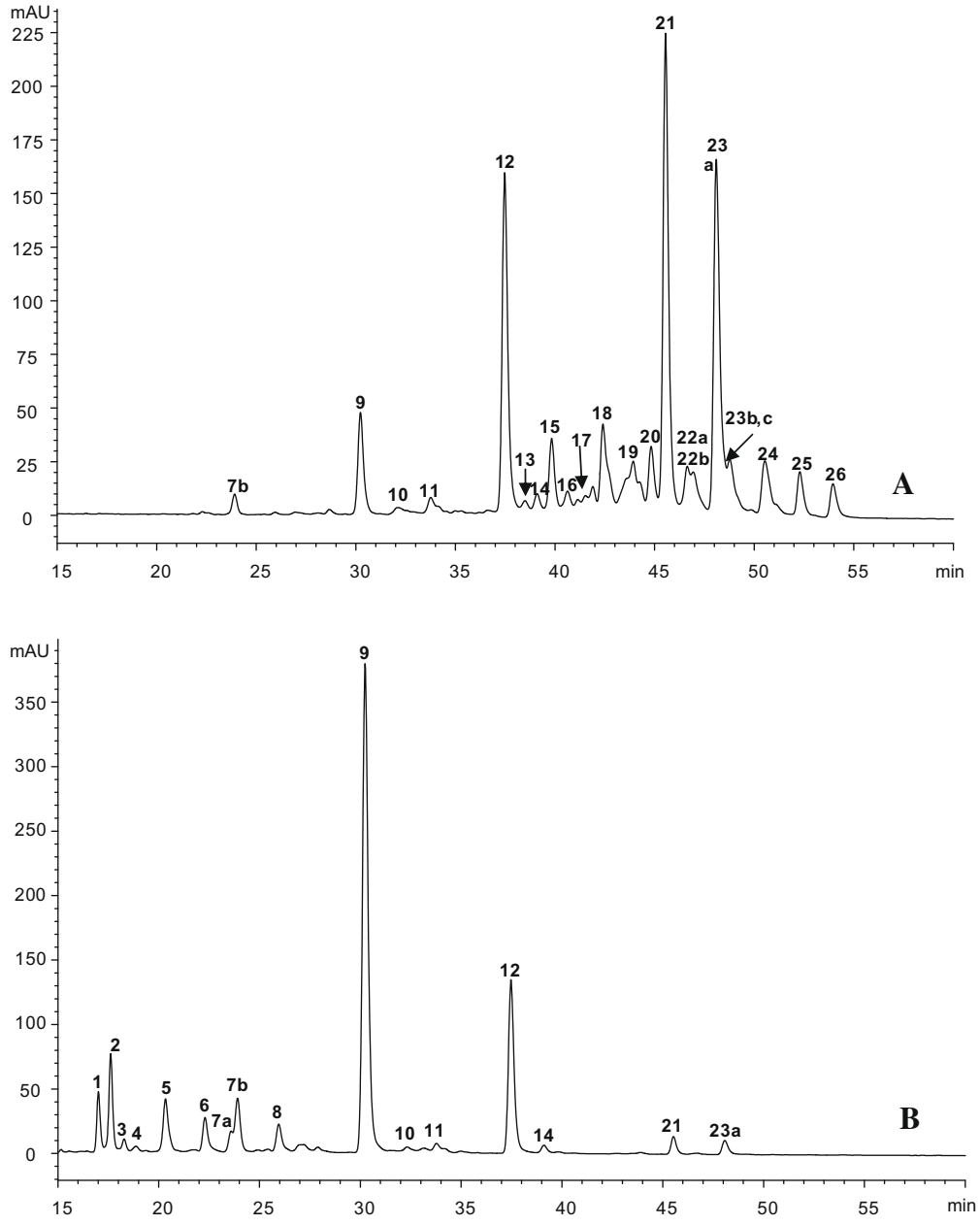

Fig. 1. HPLC chromatograms (15–60 min segments at 450 nm) of a crude (A) and a saponified (B) extract of tomato tree. Peak assignment refers to Table 1.

($P < 0.05$) between thermal treatments. Means were further compared, with Newman–Keuls's test, and differences were considered significant when $P < 0.05$. Statistical software was Statbox, V6.2.

3. Results and discussion

3.1. Carotenoid and carotenoid fatty acid ester identification

As shown in Fig. 1, the carotenoids in tamarillo are mainly present as esterified forms (peaks assigned from 15 to 26) since they disappeared after saponification. Free carotenoid peaks were assigned from 1 to 14. The identifications were tentatively made using the elution order, the co-chromatography with authentic standards as well as the UV–visible characteristics (λ_{max}, spectral fine structure, *cis* peak intensity), and MS data (Table 1).

Peak 12 was identified as *all-trans*-*β*-carotene, on the basis of its UV–visible spectrum, MS data and co-elution with standard. Peak 11a was tentatively identified as its 13-*cis* isomer, according to the UV characteristics (hypsochromic shift, lower spectral fine structure value) and the intensity of the *cis* peak (De Rosso & Mercadante, 2007b). Peaks 9a and 10a had identical wavelength of absorption, both characteristic of phytofluene. This identification was confirmed by mass spectrometry (*m/z* 543). Considering the spectral fine structure of peak 9a (III/II = 65%) and literature data (De Rosso & Mercadante, 2007b) this compound may be assumed as a *cis* isomer of phytofluene. Peak 11b was tentatively identified as *cis*-ζ-carotene, according to the UV spectrum, MS data and *cis* peak intensity.

Major peaks, 21**,** 23a**,** showed a UV–visible spectrum similar to those from β-cryptoxanthin and zeaxanthin, with molecular masses of 762 and 790, respectively. Considering their high intensity, these compounds seemed to be both esters from peak 9b, which is consistent with their similar UV–spectra, since the fatty acid moiety has no influence on the UV–visible spectrum of carotenoids (Camara & Moneger, 1978). Peak 9b was identified as *all-trans*-*β*-cryptoxanthin, according to these UV characteristics, MS data and co-chromatography with a commercial standard. No additional *cis*-peaks were detected in the spectra of peaks 21 and 23a, which, consequently, were identified as *all-trans*-*β*-cryptoxanthin myristate ($m/z = 762 = 552 + 210$) and palmitate ($m/z = 790 = 552 + 238$), respectively, which is in accordance with literature data (Breithaupt & Bamedi, 2001).

Peaks 23c and 24c had a UV–visible spectrum similar to those of peaks 5 and 6. The molecular masses, detected at 988 and 1016,

Table 1
Characteristics of carotenoids from yellow tamarillo.

Peak	Tr (min)	λ_{max}	% III/II	% A_B/II	*M*	Compound[a]
1	16.8	416, 442, 470	85	0	600	*all-trans*-Neoxanthin
2	17.5	416, 440, 468	86	0	600	*all-trans*-Violaxanthin
3	18.1	412, 436, 464	82	0	600	*cis*-Neoxanthin
4	18.8	398, 422, 448	100	0	600	*all-trans*-Luteoxanthin
5	20.1	420, 446, 474	48	0	584	*all-trans*-Antheraxanthin
6	22.1	420, 446, 474	50	0	568	*all-trans*-Lutein (std)
7a	23.5	420, 444, 472	47	0	568	Not identified
7b	23.7	422, 450, 476	21	0	568	*all-trans*-Zeaxanthin (std)
8	25.8	420, 446, 472	46	0	568	*all-trans*-5,6-epoxy-*β*-cryptoxanthin (5′,6′)
9a	30	332, 348, 368	65	n.d.[b]	542	*cis*-Phytofluene
9b	30.2	426, 452, 478	20	0	552	*all-trans*-*β*-Cryptoxanthin (std)
10a	32.2	332, 348, 368	n.c.[c]	n.d.	542	Phytofluene
10b	32.2	424, 446, 470	n.c.	n.c.	n.d.	Not identified
11a	33.7	338, 420, 444, 468	7	47	536	13-*cis*-*β*-carotene
11b	34	296, 380, 400, 424	73	28	540	*cis*-ζ-Carotene
12	37.2	426, 452, 478	18	0	536	*all-trans*-*β*-Carotene (std)
13a	38	380, 400, 426	n.c.	0	536	Not identified
13b	38.2	420, 446, 470	n.c.	n.c.	n.d.	Not identified
14	39.1	420, 444, 470	38–45	0	536	Not identified
15a	39.4–39.8	380, 400, 426	105	0	n.d.	Not identified
15b		418, 442, 470	82	0	1020	*all-trans*-Neoxanthin dimyristate
15c		420, 444, 472	n.c.	0	778	7a monomyristate
16	40.6	420, 444, 472	47	0	778	*all-trans*-5,6-epoxy-β-cryptoxanthin monomyristate
17a	41.1	418, 442, 470	85	0	n.d.	Not identified
17b	41.4	416, 438, 466	82	0	1020	*cis*-Neoxanthin dimyristate
17c	41.8	420, 440, 470	84	0	1048	*all-trans*-Neoxanthin myristate palmitate
18a	42.3	418, 440, 470	89	0	1020	*all-trans*-Violaxanthin dimyristate
18b	42.6	420, 444, 472	42	0	806	7a monopalmitate
19a	43.4	416, 438, 466	81	0	1048	*cis*-Neoxanthin myristate palmitate
19b	43.7	420, 446, 472	48	0	806	*all-trans*-5,6-epoxy-β-cryptoxanthin monopalmitate
19c	44.2	420, 442, 470	85	0	1076	*all-trans*-Neoxanthin dipalmitate
20	44.8	418, 440, 470	90	0	1048	*all-trans*-Violaxanthin myristate palmitate
21	45.5	452, 478	16	0	762	*all-trans*-*β*-Cryptoxanthin myristate
22a	46.6	420, 446, 474	56	0	1004	*all-trans*-Antheraxanthin dimyristate
22b	46.9	420, 443, 471	90	0	1076	*all-trans*-Violaxanthin dipalmitate
23a	48	452, 478	16	0	790	*all-trans*-*β*-Cryptoxanthin palmitate
23b	48.7–48.9	420, 446, 474	50–55	0	1032	*all-trans*-Antheraxanthin myristate palmitate
23c		420, 446, 474	50–55	0	988	*all-trans*-Lutein dimyristate
24a	50.3–50.5	424, 451, 476	22	0	988	*all-trans*-Zeaxanthin dimyristate
24b		420, 446, 474	n.c.	0	1060	*all-trans*-Antheraxanthin dipalmitate
24c	50.8	420, 446, 473	52	0	1016	*all-trans*-Lutein myristate palmitate
25	52.2	424, 451, 476	30	0	1016	*all-trans*-Zeaxanthin myristate palmitate
26	53.8	426, 452, 478	24	0	1044	*all-trans*-Zeaxanthin dipalmitate

[a] Tentative identification.
[b] Not determined.
[c] Not calculated.

respectively, seemed to correspond to a free carotenoid of mass 568 bound, respectively with fatty acid moieties of mass 420 and 448. The free carotenoïd corresponds to peak 6, which was identified as *all-trans*-lutein, according to its UV–visible and MS data. This was confirmed by co-elution with the authentic standard. Thus, peaks 23c and 24c were assumed as being *all-trans*-lutein dimyristate and *all-trans*-lutein myristate palmitate. It was not possible to determine if peak 24c was a mixture of regioisomers of mixed lutein diesters. In fact, it was reported, in a previous study using a similar chromatographic system, that these regioisomers were not separated as well (Breithaupt, Wirt, & Bamedi, 2002). On the same way, peaks 22a, 23b and 24b seemed to be esters of peak 5 (*m/z* 585), which was tentatively identified as *all-trans*-antheraxanthin, according to its UV–visible, MS and literature data (De Rosso & Mercadante, 2007a; Lee, 2001). Therefore, peaks 22a, 23b and 24b were tentatively identified as *all-trans*-antheraxanthin dimyristate, myristate palmitate and dipalmitate, respectively. Note that *all-trans*-lutein dipalmitate was not detected in our extracts. The increase of the intensity of peak 7b in chromatogram B seemed to be due to the saponification of peaks 24a, 25 and 26, considering the similarity of their UV–visible characteristics. Peak 7b was identified as *all-trans*-zeaxanthin on the basis of the UV–visible and MS data, and this was confirmed by co-elution with the authentic standard. The protonated molecules at *m/z* 989, 1017 and 1045 allowed us to tentatively identify peaks 24a, 25 and 26 as being *all-trans*-zeaxanthin dimyristate, myristate palmitate and dipalmitate, respectively. As described for lutein diesters, no structural elucidation of the regioisomers of mixed esters of peaks 5 and 7b was performed.

The same reasoning using UV–visible characteristics, MS data and chromatographic retention, was applied to the structural elucidation of other esters. Briefly, peaks 18a, 20, 22b were esters of *all-trans*-violaxanthin (peak 2). *All-trans*-neoxanthin (peak 1) and *cis*-neoxanthin (peak 3) showed characteristic UV–visible spectra, with a slightly decreased spectral fine structure and a hypsochromic shift of 4 nm in peak 3 compared to the *all-trans*-isomer. Their carotenoid ester parents are listed Table 1 (peaks 15b, 17b, 17c, 19a, 19c). The identity of peaks 2 and 3 was supported by co-elution with authentic standards. *cis*-Neoxanthin dipalmitate was not detected in our extracts, probably due to a low quantity and/or co-elution with other compounds. On the same way, esters of peak 4 (tentatively identified as *all-trans*-luteoxanthin) were not detected in our extracts.

On the basis of the chromatographic retention and MS data, peaks 15c, 18b and 16, 19b were assumed to be monoesters of peaks 7a and 8, respectively, which were detected at *m/z* 569. Since in our extracts all xanthophylls possessing two free hydroxyl groups were detected under diester conjugates, the presence of these monoesters seemed to indicate that only one hydroxyl group is implicated in the structures of 7a and 8. Peak 8 was tentatively identified as *all-trans*-5,6-epoxy-β-cryptoxanthin (or 5′, 6′) according to its characteristics similar to the data from the literature and the lack of *cis*-peak in the UV–visible spectrum. Peak 7a and other peaks (10b, 13a, 13b, 14, 15a, 17a) remained unidentified.

3.2. Impact of thermal treatment on carotenoids

The impact of thermal treatment, in degassed and not degassed nectars, on the quantitative evolution of carotenoids is given in Table 2. Only carotenoids which could be unambiguously quantified among those previously identified were mentioned. Whatever the oxidative conditions, the thermal treatment induced various losses, depending on the nature of the carotenoid. Increasing temperatures led to significantly higher losses in some carotenoids, such as zeaxanthin and β-carotene as shown by ANOVA treatment. Esters of zeaxanthin and β-cryptoxanthin were less affected than the corresponding free xanthophylls. Similar observations were reported by Schweiggert, Kurz, Schieber, and Carle (2007) showing an increased processing stability of diesters compared to their non-esterified counterparts. Zeaxanthin esters were the least degraded, while violaxanthin and 5,6-epoxy-β-cryptoxanthin esters being the more thermo-labile. These results are in agreement with Lee and Coates (2003) who reported that the highest thermal losses were from 5,6-epoxide carotenoids. The losses for zeaxanthin are similar to those of provitamin A carotenoids, i.e., *trans*-β-cryptoxanthin and *trans*-β-carotene. Dhuique-Mayer et al. (2007) reported that provitamin A carotenoids had a higher heat stability than other xanthophylls. On the contrary Gama et al. (2007) found smaller losses for zeaxanthin than for β-carotene and ζ-carotene. Therefore thermal lability seems to be influenced by both experimental conditions and nature of the food matrix. The *cis*-isomerization of *trans*-β-carotene was responsible of the increase in concentration of 13-*cis*-β-carotene (peak 11a). It was reported that thermal treatment of *all-trans*-β-carotene at temperatures below 100 °C induced formation of 13- and 15-*cis*-β-carotene whereas 9-*cis*-β-carotene was formed above 100 °C (Von Doering, Sotiriou-Leventis, & Roth, 1995). However, Chandler et al. (1988) did not detect 15-*cis*-β-carotene after heat processing and 13-*cis*-β-carotene was the only isomer detected in pasteurised carrot juices by Marx, Stuparic, Schieber, and Carle (2003). In our case, only the 13-*cis*-isomer was observed.

Table 2
Impact of thermal treatment on the quantitative evolution of yellow tamarillo carotenoids.

Compound (peak)	80 °C[A]	90 °C[A]	95 °C[A]	80 °C[B]	90 °C[B]	95° C[B]
Zeaxanthin (7b)	86.4 ± 5.3^{a}	64.1 ± 0.8^{b}	68.5 ± 9.5^{b}	89.1 ± 5.2^{a}	67.4 ± 4.2^{b}	72.7 ± 2.3^{b}
β-Cryptoxanthin (9b)	69.6 ± 8.7^{a}	52.7 ± 7.7^{b}	52.0 ± 9.7^{b}	79.1 ± 11.3^{c}	63.9 ± 5.0^{a}	67.5 ± 9.4^{a}
13-*cis*-β-carotène (11a)	284.2 ± 12.2^{a}	483.5 ± 14.6^{b}	544.1 ± 11.1^{c}	298.2 ± 12.5^{a}	456.1 ± 4.3^{b}	518.6 ± 12.3^{c}
cis-ζ-Carotene (11b)	65.9 ± 6.7^{a}	48.7 ± 6.3^{b}	46.5 ± 4.5^{b}	83.9 ± 9.5^{c}	77.7 ± 10.6^{c}	80.5 ± 9.8^{c}
β-Carotene (12)	79.2 ± 1.8^{a}	64.9 ± 3.0^{b,c}	61.1 ± 5.4^{c}	85.5 ± 11.9^{a}	70.1 ± 11.5^{b,d}	73.4 ± 7.5^{d}
5,6-epoxy-β-cryptoxanthin myristate (16)	66.8 ± 10.0^{a,b}	60.0 ± 4.9^{b}	n.c.	70.2 ± 4,8^{a,b}	62.3 ± 4.9^{b}	n.c.
Violaxanthin dimyristate (18a)	73.3 ± 3.9^{a}	n.c.	n.c.	73.3 ± 8.0^{a}	n.c.	n.c.
Violaxanthin myristate palmitate (20)	72.1 ± 6.6^{a}	54.5 ± 4.2^{b,c}	47.9 ± 3.2^{c}	78.0 ± 4.1^{a}	59.1 ± 4.6^{b}	54.4 ± 11.6^{b,c}
β-Cryptoxanthin myristate (21)	86.7 ± 1.8^{a,b}	76.6 ± 3.6^{c,d}	73.6 ± 3.3^{d}	94.7 ± 9.3^{a}	82.8 ± 9.1^{b,c}	83.5 ± 6.5^{b,c}
β-Cryptoxanthin palmitate (23a)	85.8 ± 2.0^{a}	73.6 ± 2.9^{b,c}	70.1 ± 2.9^{c}	95.4 ± 9.5^{d}	80.6 ± 9.4^{a,b}	81.2 ± 5.9^{a,b}
Lutein myristate palmitate (24c)	89.0 ± 5.1^{a,b}	77.4 ± 8.4^{b,c}	70.4 ± 7.2^{c}	86.1 ± 9.0^{a,b}	66.7 ± 7.3^{c}	70.7 ± 10.9^{c}
Zeaxanthin myristate palmitate (25)	98.8 ± 4.8^{a,b}	93.8 ± 10.1^{a,b,c}	85.2 ± 10.1^{c,d}	96.9 ± 4.5^{a,b,c}	81.3 ± 10.4^{d}	86.0 ± 7.3^{b,c,d}
Zeaxanthin dipalmitate (26)	98.8 ± 4.3^{a}	98.6 ± 11.7^{a}	92.6 ± 12.0^{a,b}	103.9 ± 6.7^{a}	84.6 ± 7.7^{b}	91.4 ± 7.1^{a,b}

Values are means ± SD of three independent determinations. Values are expressed as% residual carotenoid. n.c.: not calculated. Values within a row with similar letters are not significantly different (Newman–Keuls, $P < 0.05$).

[A] Not degassed nectar.

[B] Degassed nectar.

The low level of dissolved oxygen seemed to have none effect on the thermal degradation of carotenoids, except maybe for *cis*-ζ-carotene. This needed to be confirmed by a statistical analysis using a higher number of samples.

3.3. Tentative identification of degradation products

In order to facilitate the identifications, some extracts of heated purees were saponified and their HPLC chromatograms (data not shown) were compared to the initial profile. Table 3 summarises the chromatographic, spectral and mass data of the newly formed compounds obtained by HPLC-DAD-MS analysis. It is well known that heating under slightly acidic conditions lead to a furanoid rearrangement of 5,6-epoxides. We observed the formation of neochrome, auroxanthin and mutatoxanthin, tentatively identified on the basis of their UV characteristics, spectral fine structure, and mass data. Two isomers were found for each compound. In addition, the epoxide test was carried out with diluted HCl on neoxanthin and violaxanthin standards. The resulting 5,8-epoxy derived products (neochrome and auroxanthin, respectively) exhibited identical retention times and UV characteristics similar to the corresponding presumed compounds in our extracts. This supported the identifications of neochrome and auroxanthin as well as the decrease in concentration in the extracts of neoxanthin (not shown) and violaxanthin (Table 3). As we did not have antheraxanthin standard in our lab, the epoxide test with diluted HCl was performed on the saponified tamarillo extract. The compounds tentatively identified as isomers of mutatoxanthin (formed after thermal treatment) were also detected after the epoxide test. This was corresponding with the decrease of antheraxanthin (data not shown). In the same way, the 5,8-epoxide of peak 7a and cryptoflavin (derived from 5,6-epoxy-β-cryptoxanthin) were tentatively identified.

We also observed an increase of peak 4, tentatively identified as luteoxanthin on the basis of the UV–visible and MS data. This compound is known as an intermediate in the acid-catalysed rearrangement of violaxanthin to auroxanthin (Eugster, 1995). The concentration of luteoxanthin was higher in extracts heated at 80 °C. At 95 °C, its decrease matched with the increase of auroxanthin. Three other new peaks, eluted at 25.8, 26.8 and 27.1 min, were identified as *cis* isomers of β-cryptoxanthin. It was not possible to distinguish between the 13 or 13′ *cis* isomers (e.g. 15 or 15′).

All these newly formed compounds identified after saponification should be present initially as esters. Esters of *cis*-β-cryptoxanthin were detected at 43.6, 46.6, and 46.9 min and an ester of neochrome was eluted at 44.2 min (data not shown). Other esters (e.g. auroxanthin, mutatoxanthin, cryptoflavin, etc.) were not clearly detected probably because of co-eluting compounds. These overlapping peaks did not allow us to quantitatively estimate some compounds. For example, esters of *cis*-β-cryptoxanthin detected at 46.6, and 46.9 min co-eluted with peaks 22a and 22b, ester of neochrome (44.2 min) with peak 19c. Similarly, esters of *cis* and *trans*-neoxanthin (17b, 17c, and 19a) could not be quantified but the decrease of neoxanthin after saponification indicated that these esters were partially degraded by the thermal treatment.

Table 3
Tentative identification of degradation products.

Compound	T_r (min)	λ_{max}	% III/II	% A_B/II	*M*
Neochrome	17.3	400, 422, 448	90	0	600
Neochrome	17.9	400, 422, 448	91	0	600
Auroxanthin	19.7	380, 400, 426	96	0	600
Auroxanthin	21.4	380, 400, 426	96	0	600
Mutatoxanthin	21.7	402, 428, 452	55–67	0	584
Mutatoxanthin	22.4	402, 428, 452	54–62	0	584
5,8 epoxide of peak 7a	25.3	404, 428, 452	50–60	0	568
15-*cis*-β-cryptoxanthin (15′)	25.8	336, 422, 444, 470	n.c.[a]	>52	552
13-*cis*-β-cryptoxanthin (13′)	26.8	338, 422, 444, 470	n.c.	46	552
13-*cis*-β-cryptoxanthin (13′)	27.1	338, 423, 444, 470	n.c.	45	552
Cryptoflavin	28.1	402, 428, 450	50	0	568

[a] Not calculated.

3.4. Impact of thermal treatment on AA and DHAA

Table 4 summarises the initial percentages of ascorbic and dehydroascorbic acids in degassed and not degassed nectars and the residual percentages detected in these nectars after thermal treatments. These percentages were calculated in reference to the initial AA + DHAA content. In degassed nectars, the retention of AA was total and independent of the applied temperature as confirmed by ANOVA, whereas ascorbic acid was entirely degraded in the not degassed nectars after thermal treatment. The influence of the dissolved oxygen level in the thermal degradation of AA was already mentioned (Eison-Perchonok & Downes, 1982; Dhuique-Mayer et al., 2007). However, the kinetic parameters established by these authors depended on several factors. In our case, the easy oxidation of AA to DHAA was not observed in degassed nectars. On the other hand, DHAA was thermo-labile even in degassed nectars and the losses are similar than in not degassed nectars. It has been reported that the degradation of DHAA, responsible of the browning of fruit juices, proceeded both under non-oxidative and oxidative conditions (Li, Sawamura, & Yano, 1989).

Note that during the experiment time, AA was partly degraded in not degassed nectars before thermal treatment whereas DHAA content increased. After reduction, the AA + DHAA content were similar to that observed in degassed nectars. This proved a rapid conversion of AA to DHAA during the time of analysis. In both degassed and not degassed nectars, the irreversible degradation of DHAA was responsible for the decrease of the AA + DHAA content. It is known that degradation of ascorbic acid leads to several products via DHAA such as ketogulonic acid and furfural (Kurata & Sakurai 1967).

In conclusion, pasteurization of tomato tree nectar in presence of low levels of dissolved oxygen does not cause losses of ascorbic acid. Consequently the vitamin C content (AA + DHAA) was significantly higher in degassed nectars than in not degassed ones. In order to preserve the ascorbic acid content, tamarillo processing should be performed in nectars previously degassed.

On the contrary the level of dissolved oxygen did not affect significantly the carotenoids under thermal treatments applied. Thermal treatment induced 5,8-epoxidation and *cis*-isomerization. Important data about the influence of the strength of the thermal

Table 4
Residual ascorbic and dehydroascorbic acids percentages after thermal treatment.

	Degassed nectar		Not degassed nectar	
	AA	DHAA	AA	DHAA
% Initial	69.7 ± 2.0[a]	30.2 ± 1.4	9.7 ± 0.6	90.2 ± 3.1
80 °C	69.2 ± 2.6[a]	19.0 ± 2.6	nd[b]	58.6 ± 2.5
90 °C	69.0 ± 3.1[a]	10.6 ± 1.6	nd[b]	27.6 ± 1.5
95 °C	66.9 ± 2.4[a]	7.5 ± 1.0	nd[b]	18.4 ± 0.7

Values are means ± SD of three independent determinations. Percentages are expressed in reference to the initial [AA + DHAA] content. nd: not detected. Values within a column with similar letters are not significantly different (Newman–Keuls, $P < 0.05$).

treatment applied on the preservation of the vitamin A activity were obtained. Total carotenoid content did not decrease significantly.

Acknowledgements

We express our thanks to the EPN (Ecuador) for fruits supply, Michel Carail and Manuel Dornier for their help and advices, Gilles Morel (CIRAD) for help in this study. This work was financially supported by the European Union (PAVUC project, INCO n° 015279).

References

Böhm, V., Puspitasari-Nienhaber, N. I., Ferruzzi, M. G., & Schwartz, S. J. (2002). Trolox equivalent antioxidant capacity of different geometrical isomers of α-carotene, β-carotene, lycopene, and zeaxanthin. *Journal of Agricultural and Food Chemistry, 50*(1), 221–226.

Breithaupt, D. E., & Bamedi, A. (2001). Carotenoid esters in vegetables and fruits: A screening with emphasis on β-cryptoxanthin esters. *Journal of Agricultural and Food Chemistry, 49*(4), 2064–2070.

Breithaupt, D. E., Wirt, U., & Bamedi, A. (2002). Differentiation between lutein monoester regioisomers and detection of lutein diesters from Marigold flowers (*Tagetes erecta* L.) and several fruits by liquid chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry, 50*(1), 66–70.

Camara, B., & Moneger, R. (1978). Free and esterified carotenoids in green and red fruits of *Capsicum annuum*. *Phytochemistry, 17*(1), 91–93.

Chandler, L. A., & Schwartz, S. J. (1988). Isomerization and losses of *trans-β*-carotene in sweet potatoes as affected by processing treatments. *Journal of Agricultural and Food Chemistry, 36*(1), 129–133.

De Rosso, V. V., & Mercadante, A. Z. (2007a). HPLC-PDA-MS/MS of anthocyanins and carotenoids from Dovyalis and tamarillo fruits. *Journal of Agricultural and Food Chemistry, 55*(22), 9135–9141.

De Rosso, V. V., & Mercadante, A. Z. (2007b). Identification and quantification of carotenoids, by HPLC-DAD-MS/MS, from Amazonian fruits. *Journal of Agricultural and Food Chemistry, 55*(13), 5062–5072.

Dhuique-Mayer, C., Caris-Veyrat, C., Ollitraut, P., Curk, F., & Amiot, M. J. (2005). Varietal and interspecific influence on micronutrient contents in citrus from the mediterranean area. *Journal of Agricultural and Food Chemistry, 53*(6), 2140–2145.

Dhuique-Mayer, C., Tbatou, M., Carail, M., Caris-Veyrat, C., Dornier, M., & Amiot, M. J. (2007). Thermal degradation of antioxidant micronutrients in Citrus juice: Kinetics and newly formed compounds. *Journal of Agricultural and Food Chemistry, 55*(10), 4209–4216.

Eison-Perchonok, M. H., & Downes, T. W. (1982). Kinetics of ascorbic acid autoxidation as a function of dissolved oxygen concentration and temperature. *Journal of Food Science, 47*(3), 765–767.

Eugster, C. H. (1995). Chemical derivatization: Microscale tests for the presence of common functional groups in carotenoids. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Vol. 1A: Isolation and analysis* (pp. 71–80). Basel: Birkhäuser.

Fanciullino, A. L., Dhuique-Mayer, C., Luro, F., Casanova, J., Morillon, R., & Ollitrault, P. (2006). Carotenoid diversity in cultivated Citrus is highly influenced by genetic factors. *Journal of Agricultural and Food Chemistry, 54*(12), 4397–4406.

Gama, J. J. T., & De Sylos, C. M. (2007). Effect of thermal pasteurization and concentration on carotenoid composition of Brazilian Valencia orange juice. *Food Chemistry, 100*(4), 1686–1690.

Gardner, P. T., White, T. A. C., McPhail, DB., & Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry, 68*(44), 471–474.

Kennedy, J. F., Rivera, Z. S., Lloyd, L. L., Warner, F. P., & Jumel, K. (1992). L-Ascorbic acid stability in aseptically processed orange juice in TetraBrik cartons and the effect of oxygen. *Food Chemistry, 45*(5), 327–331.

Kurata, T., & Sakurai, Y. (1967). Degradation of L-ascorbic acid and mechanism of nonenzymic browning reaction. Part II, Non oxidative degradation of L-ascorbic acid including the formation of 3-deoxy-L-pentosone. *Agricultural and Biological Chemistry, 31*(2), 170–176.

Lee, H. S. (2001). Characterization of carotenoids in juice of red navel orange (Cara Cara). *Journal of Agricultural and Food Chemistry, 49*(5), 2563–2568.

Lee, H. S., & Coates, G. A. (2003). Effect of thermal pasteurization on Valencia orange juice color and pigments. *Lebensmittel-Wissenschaft und Technologie, 36*(1), 153–156.

Li, Z.-F., Sawamura, M., & Yano, H. (1989). Effect of oxygen on the browning and formation of furfural in *yuzu* juice. *Agricultural and Biological Chemistry, 53*(7), 1979–1981.

Marx, M., Stuparic, M., Schieber, A., & Carle, R. (2003). Effects of thermal processing on *trans–cis*-isomerization of β-carotene in carrot juices and carotene-containing preparations. *Food Chemistry, 83*(4), 609–617.

Robertson, G. L., & Samaniego, C. M. L. (1986). Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *Journal of Food Science, 51*(1), 184–187.

Rodriguez-Amaya, D. B., Bobbio, P. A., & Bobbio, F. O. (1983). Carotenoid composition and vitamin A value of the Brazilian fruit *Cyphomandra betacea*. *Food Chemistry, 12*(1), 61–65.

Schweiggert, U., Kurz, C., Schieber, A., & Carle, R. (2007). Effects of processing and storage on the stability of free and esterified carotenoids of red peppers (*Capsicum annuum* L.) and hot chilli peppers (*Capsicum frutescens* L.). *European Food Research and Technology A, 225*(2), 261–270.

Seybold, C., Fröhlich, K., Bitsch, R., Otto, K., & Böhm, V. (2004). Changes in contents of carotenoids and vitamin E during tomato processing. *Journal of Agricultural and Food Chemistry, 52*(23), 7005–7010.

Taungbodhitham, A. K., Jones, G. P., Walhlqvist, M. L., & Briggs, D. R. (1998). Evaluation of method for the analysis of carotenoids in fruits and vegetables. *Food Chemistry, 63*(4), 577–584.

Vieira, M. C., Teixeira, A. A., & Silva, C. L. M. (2000). Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (*Theobroma grandiflorum*) nectar. *Journal of Food Engineering, 43*(1), 1–7.

Von Doering, W. E., Sotiriou-Leventis, C., & Roth, W. R. (1995). Thermal interconversions among 15-*cis*-, 13-*cis*-, and all-*trans*-*β*-carotene: Kinetics, Arrhenius parameters, thermochemistry, and potential relevance to anticarcinogenity of all-*trans*- *β*-carotene. *Journal of the American Chemical Society, 117*(10), 2747–2757.

Wechtersbach, L., & Cigić, B. (2007). Reduction of dehydroascorbic acid at low pH. *Journal of Biochemical and Biophysical Methods, 70*(5), 767–772.