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The complex carotenoid pattern of orange juices from concentrate

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Abstract

Around 30 carotenoids were detected in samples of orange juices from concentrated (OJFC) when 25 ml aliquots were analyzed, which indicated that such foodstuff is one of the most intricate sources of those pigments. The main features of the carotenoid profile were the absence or occurrence at low levels of certain isomers of the 5,6-epoxycarotenoids antheraxanthin and violaxanthin, which are major carotenoids in fresh or slightly processed juices. This fact is connected with the higher quantitative importance of their 5,8-epoxy derivatives mutatoxanthin and auroxanthin, from which it has been suggested that a rough estimation of the age of the juices can be made by considering their epoxycarotenoids content, the absence of antheraxanthin and (9*Z*)-violaxanthin indicating that the juice has been stored for a relatively long period. In addition, the presence, in some of the samples surveyed, of geometrical isomers of zeaxanthin and β-cryptoxanthin, which were not detectable in UFOJ, was also observed. As for the quantitative analysis, the total carotenoid contents of the OJFC analyzed fell within the bracket 1.37–5.89 mg/l, the levels of individual carotenoids being lower in comparison with those reported in the literature for slightly processed orange juices.

Keywords: Auroxanthin; C_{30}; Carotenoids; Citrus; Epoxycarotenoids; Stereoisomers; Orange juice from concentrate; Pigments

1. Introduction

Citrus are one of the chief fruit products, being a food commodity in much of the world due to their appealing organoleptic characteristics and their nourishing value. Thus, the citrus industry enjoys great importance in America (Brazil, the United States and Mexico being the major producers), as well as in the Mediterranean basin, where Spain is the main producer, and in China (Johnson, 2001; Señoráns et al., 2001). Although the nutritional interest of these products has lain in their ascorbic acid content for years, their importance as sources of other nutritionally important species such as carotenoids (Fanciullino et al., 2006; Meléndez-Martínez, Britton, Vicario, & Heredia, 2005c, 2007a; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003) has increased in recent years due to the likely health benefits they provide (Meléndez-Martínez, Vicario, & Heredia, 2004b; Olson, 1999). In a sense, this renewed interest has also favoured the undertaking of studies designed to shed light on the processes involved in their biosynthesis in such fruits (Alós, Cercós, Rodrigo, Zacarías, & Talón, 2006; Rodrigo, Marcos, & Zacarías, 2004).

Focussing on orange juice, which stands out among citrus products, it can be seen that its carotenoid pattern is one of the most complex among foods, hence it has been the subject of a myriad of studies for decades (Meléndez-Martínez, Vicario, & Heredia, 2007b). In the relevant literature it is observed that the carotenoid profiles of juices obtained from different orange varieties (Gross, Gabai, & Lifshitz, 1972; Lee & Castle, 2001; Pascual, Mallent, & Cuñat, 1993) and geographical areas (Gama & Sylos, 2005; Meléndez-Martínez, Britton, Vicario, & Heredia, 2005a; Mouly, Gaydou, Lapierre, & Corsetti, 1999) have been widely studied; yet the great majority of these studies were carried out on either fresh or slightly processed samples. In this regard, the information on the carotenoid

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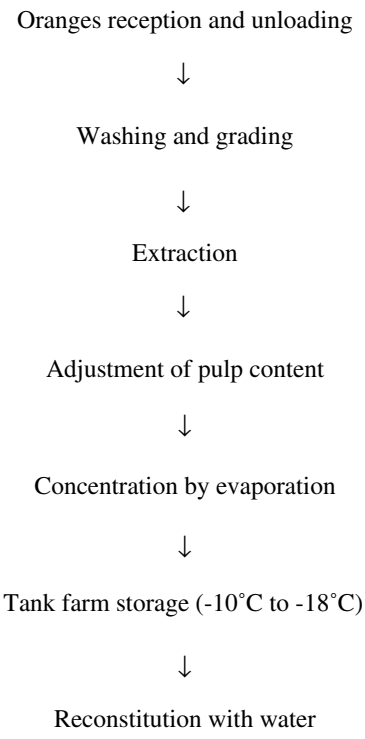


Fig. 1. Scheme of the production of orange juice from concentrate (OJFC) in brief.

pattern of orange juices from concentrate (OJFC) is still scarce and vague, despite that they account for the largest proportion of the global juice market and of the human consumption of citrus products, as reported by The Food and Agriculture Organization of the United Nations (FAO), the United States Department of Agriculture (USDA) or the United Nations Conference on Trade and Development (UNCTAD). Industrially, OJFC are normally obtained in two steps, namely production of frozen concentrated orange juice (FCOJ) and reconstitution (Johnson, 2001) (Fig. 1), such that their final characteristics differ greatly from those of the fresh juices. In relation to all these pieces of evidence, this work was undertaken to gain insight into the carotenoid pattern of OJFC, by providing up-to-date qualitative and quantitative compositional data stemming from non-tentative identifications.

2. Materials and methods

2.1. Orange juice samples

Thirty-three OJFC, corresponding to common brands marketed in Spain, were purchased in Seville from different retailers and kept following the manufacturer's recommendations up to their analyses. More specifically, 25 of the juice samples were kept at room temperature, whereas the remaining 8, which had a shorter shelf-life probably due do them having been subjected to less severe thermal treatments, were maintained in a refrigerator.

2.2. Carotenoid analysis

2.2.1. Pigment extraction from orange juice and saponification

The extraction and saponification procedures used are explained in detail elsewhere (Meléndez-Martínez et al., 2005c). In brief, 25 ml aliquots of the OJFC samples were extracted with 50 ml of the mixture methanol/acetone/hexane (25:25:50, v/v/v), which contained 0.1% butylhydroxytoluene (BHT). To obtain all the xanthophylls in de-esterified form, the extracts so obtained were saponified at room temperature for 1 h by adding 25 ml of ethanolic KOH (10% w/v). The saponified extracts were eventually taken to 1 ml of a mixture acetone:methanol (1:2, v/v, containing 0.1% BHT) and filtered through Millipore PVDF Millex© filters (13 mm × 0.45 μm) (Bedford, MA, USA) prior to their injection in the HPLC system.

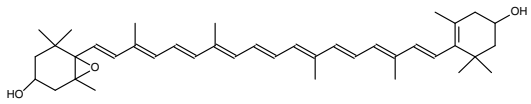
2.2.2. High-performance liquid chromatography

The HPLC method used for this study has long been used in our laboratory and is explained in detail elsewhere (Meléndez-Martínez et al., 2005c). In short, the analyses were performed on an Agilent 1100 system (Agilent, Palo Alto, CA, USA) fitted with a photodiode array detector, a quaternary pump, a column temperature control module set at 17 °C and housing an YMC C_{30} column (5 μm, 250 × 4.6 mm) (YMC, Wilmington, NC, USA), and an autosampler set to draw 20 μl aliquots from the concentrated extracts.

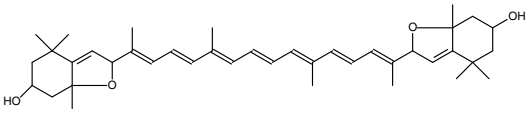
Methanol (MeOH), methyl-ter-butyl ether (MTBE), both of them containing small proportions of BHT and triethylamine (0.1% and 0.05%, respectively), and water, were used in the mobile phase, according to the linear gradient described elsewhere (Meléndez-Martínez, Britton, Vicario, & Heredia, 2006; Mouly et al., 1999): 0 min: 90% MeOH + 5% MTBE + 5% water; 12 min: 95% MeOH + 5% MTBE; 25 min: 89% MeOH + 11% MTBE; 40 min: 75% MeOH + 25% MTBE; 60 min: 50% MeOH + 50% MTBE; 62 min: 90% MeOH + 5% MTBE + 5% water. The mobile phase was pumped at 1 ml/min and the chromatograms were monitored at 430 nm.

2.2.3. Identification of carotenoids

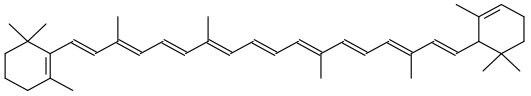
The identification of the orange juice carotenoids detected (chemical structures in Fig. 2) was carried out by the comparison of their spectroscopic and chromatographic characteristics with those of standards isolated by standard procedures (Britton, Liaaen-Jensen, & Pfander, 1995) or obtained by semisynthesis. Thus, α- and β-carotene (β,ε-carotene and β,β-carotene, respectively) were isolated from palm oil (*Elaeis guineensis* Jacq.), (9′Z)-neoxanthin ((9′*Z*)-5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β- carotene-3,5,3′-triol), violaxanthin (5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol) and lutein (β,ε-carotene-3,3′-diol) from spinach leaves (*Spinacia oleracea* L.) and β-cryptoxanthin (β,β-caroten-3-ol) and zeaxanthin (β,β-carotene-3,3′-diol) from red peppers



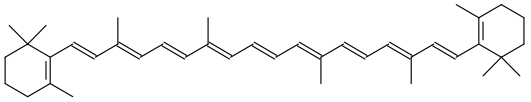
antheraxanthin (5,6-epoxy-5,6-dihydro-β,β-carotene-3,3 -diol)



auroxanthin (5,8:5',8'-diepoxy-5,8,5',8'-tetrahydro-β,-βcarotene-3,3'-diol)



α-carotene (β,ε-carotene)



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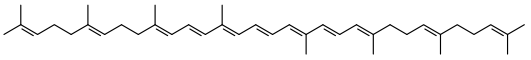
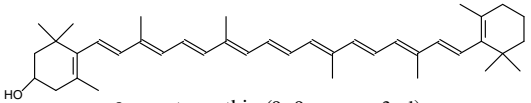
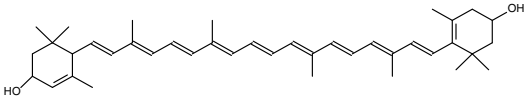


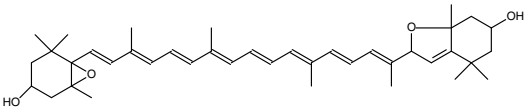
ζ -carotene (7,8,7',8'-tetrahydro-ψ,ψ-carotene)



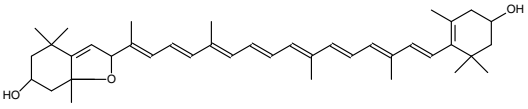
β -cryptoxanthin (β, β -caroten-3-ol)



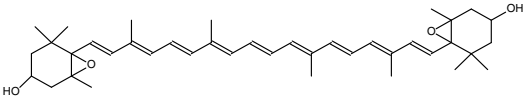
lutein (β,ε-carotene-3,3'-diol)



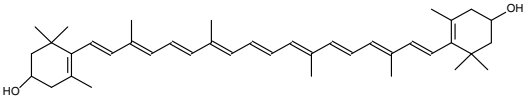
luteoxanthin (5,6:5',8'-diepoxy-5,6,5',8'-tetrahydro-β,β- carotene-3,3'-diol)



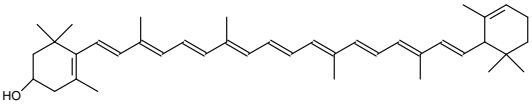
mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3 -diol)



violaxanthin (5,6:5',6'-diepoxy-5,6,5',6'-tetrahydro-β,-βcaroten-3,3'-diol)



zeaxanthin (β, β-carotene-3,3'-diol)



zeinoxanthin (β,ε-caroten-3-ol)

Fig. 2. Chemical structures of the major carotenoids detected in UFOJ.

(*Capsicum annuum* L.). The antheraxanthin (5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol) standard was semisynthesized by treating zeaxanthin with 3-chloroperoxybenzoic according to the procedure explained elsewhere (Barua,

1999), the antheraxanthin fraction being separated from the rest of the extract by thin-layer chromatography (TLC) on silica gel 60 F_{254} plates (20 cm × 20 cm, 0.7 mm thick) (Merck, Darmstadt, Germany), using the mixture petroleum ether (65–95 °C)-acetone-diethylamine (10:4:1) (Mínguez-Mosquera, 1997) as mobile phase. For the identification of some other carotenoids, namely zeinoxanthin (β,ε-caroten-3-ol) and different isomers of violaxanthin, antheraxanthin and ζ-carotene (7,8,7′,8′-tetrahydro-ψ,ψ-carotene), appropriate fractions were isolated from the UFOJ itself and studied as reported in other studies (Meléndez-Martínez, Britton, Vicario, & Heredia, 2005b, 2005c, 2007a, 2007c). As for 5,8-epoxycarotenoids, appropriate standards were obtained by treating their corresponding 5,6-epoxy isomers with a few drops of methanolic 0.1 M HCl. Thus, mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol) was obtained from antheraxanthin, luteoxanthin (5,6:5′,8′-diepoxy-5,6,5′,8′-tetrahydro-β,β-carotene-3, 3′-diol) and auroxanthin (5,8:5′,8′-diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene-3, 3′-diol) from violaxanthin and neochrome (5′,8′-epoxy-6,7-didehydro-5,6,5′,8′-tetrahydro-β,β- carotene-3,5,3′-triol) from (9′Z)-neoxanthin.

To identify geometrical isomers, ethanolic solutions of the standards were heated (80–100 °C) for 30 min and then illuminated overnight by means of a powerful lamp as explained elsewhere (Meléndez-Martínez, Vicario, & Heredia, 2007a). For the tentative assignment of geometrical configurations, the absorption maxima shifts, as well as the intensity of the absorption maxima appearing in the UV region of the spectra (Zechmeister, 1962), were considered, as explained elsewhere (Meléndez-Martínez et al., 2006).

2.2.4. Quantitative analysis

The levels of orange juice carotenoids were worked out from calibration curves normally constructed with the corresponding (all-*E*)-standards in compliance with recommended guidelines (Kimura & Rodriguez-Amaya, 1999). The zeinoxanthin content was inferred from the calibration curve of lutein, due to both carotenoids possessing the same chromophore and hence virtually identical spectra. Likewise, the levels of the two early-eluting unidentified peaks were worked out from the calibration curve for neochrome due to great similarity in their spectra. When coelution took place, quantification was done by taking into consideration the calibration curve of the major carotenoid in the mixture, which was determined by carefully studying the shape of the average spectrum in the mobile phase as compared to those of the coeluting pigments. The total content of carotenoids was estimated as the sum of the levels of the individual pigments.

3. Results and discussion

3.1. Qualitative analysis

Typical chromatograms of model OJFC, encompassing all the carotenoids detected in the survey, are depicted in Fig. 3. The diverse features of the peaks found are summa-

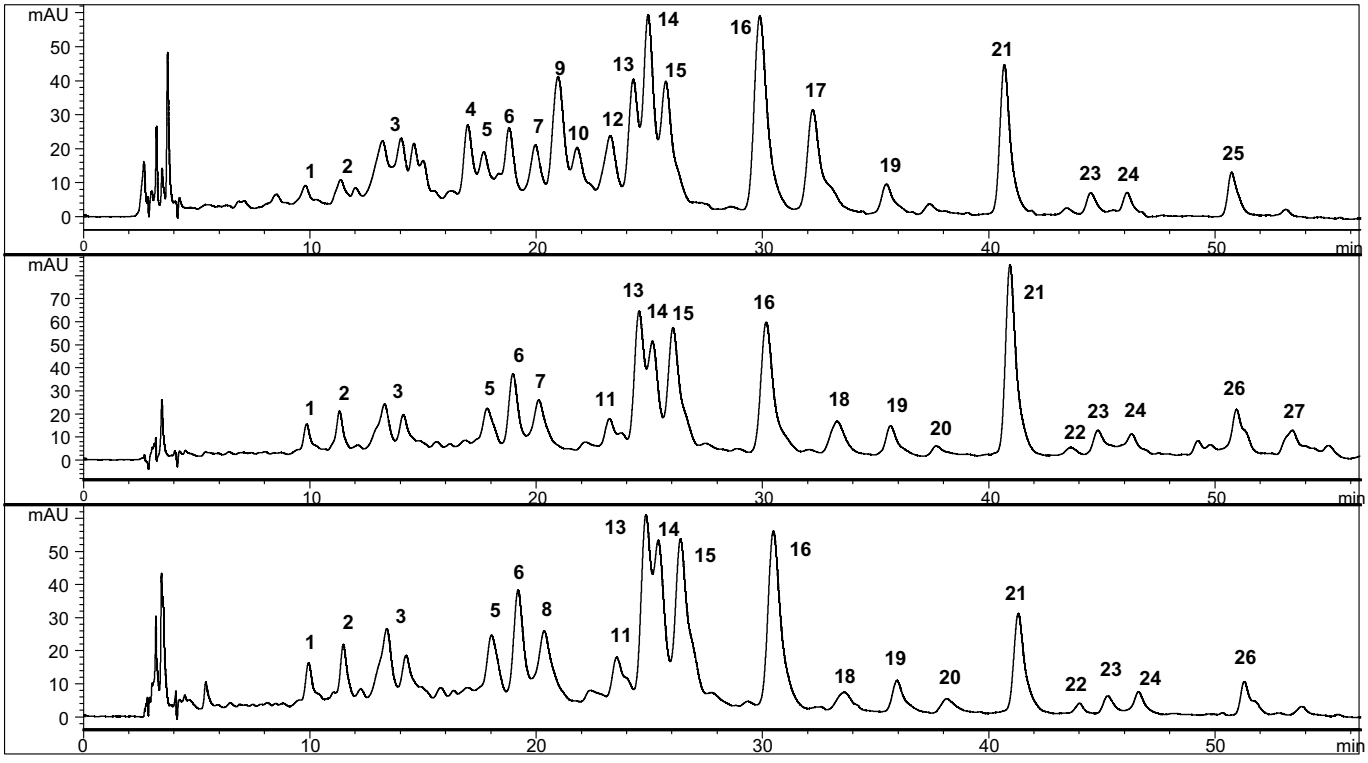


Fig. 3. Chromatograms at 430 nm of representative samples of OJFC (peak identification in Table 1).

rized in Table 1. Due to the efficiency of C_{30} columns in the separation of stereoisomers of carotenoids (Aman et al., 2005; Emenhiser, Simunovic, Sander, & Schwartz, 1996; Marx, Schieber, & Carle, 2000; Mercadante, 1999; Pott, Marx, Neidhart, Mühlbauer, & Carle, 2003), coelution phenomena are bound to take place when such a stationary phase is employed for the analysis of orange juice carotenoids, since these products constitute one of the most intricate food sources of these compounds (Meléndez-Martínez et al., 2007a, 2007b).

Peaks "unidentified 1" and "unidentified 2" were found to have spectra resembling those of the neochrome stereoisomers obtained upon the acidic treatment of the (9′*Z*)-neoxanthin standard, although they differed noticeably in their retention times. They have been tentatively identified as latochrome (5′,8′-epoxy-5,6,5′,8′-tetrahydro-β,β-carotene-3,5,6,3′- tetrol) isomers in a recent paper, throwing serious doubts about the occurrence of neoxanthin in orange juices (Meléndez-Martínez, Britton, Vicario, & Heredia, 2008). As for the rest of the major carotenoids found in the samples, they can be classified into four distinct groups, specifically epoxycarotenoids, monohydroxycarotenoids, dihydroxycarotenoids and carotenes. Concerning the first group, only some of the violaxanthin isomers known to occur in slightly processed orange juices (Meléndez-Martínez, Vicario, & Heredia, 2007c) were found at detectable level in the OJFC studied; in addition, they coeluted with other compounds, mainly *Z*-auroxanthin isomers. At this point, it is important to stress that several isomers of auroxanthin were found, which were identified by comparing their features with those of the standards obtained by acidifying a violaxanthin fraction from UFOJ. Specifically, when this fraction was so treated and next analyzed according to the same HPLC method used in this work, up to 12 stereoisomers of its 5,8:5′,8′-diepoxy derivative, auroxanthin, were detected. Such a large number of isomers stem from the fact that 3 different stereoisomers of auroxanthin (namely (8*R*, 8′*R*)-, (8*R*, 8′*S*)- and (8*S*, 8′*S*)-auroxanthin, (chemical structures in Fig. 4) are obtained when (all-*E*)-violaxanthin is acidified (Britton, Liaaen-Jensen, & Pfander, 2004). Apart, from these diepoxy derivatives of violaxanthin, some isomers of its monoepoxy derivative, luteoxanthin, were also detected, in some cases coeluting with other carotenoids. Similarly, some geometrical isomers of both the 5,6-epoxycarotenoid antheraxanthin and its 5,8-epoxy counterpart mutatoxanthin were also found in the samples, the former only in some of them. Thus far it is important to note that the occurrence of 5,8-epoxycarotenoids in orange juices, is very likely to be due to the loss of compartmentation caused by the squeezing, which brings together organic acids and 5,6-epoxycarotenoids and therefore promotes the isomerization

Table 1
Chromatographic and spectroscopic features of the orange juice carotenoids detected and quantitative data

Peak	r_t[a] (min)	Identification	Absorption maxima (nm)	Quantitative data[b]
1	9.8	Unidentified 1 (latochrome isomer)	400, 422, 448	0.07 ± 0.03 (0.02–0.16)
2	11.2	Unidentified 2 (latochrome isomer)	400, 422, 448	0.09 ± 0.04 (n.d.[c]–0.23)
3	11.9–14.2	Mixture of both violaxanthin and auroxanthin isomers		0.25 ± 0.15 (n.d.–0.60)
4	17.0	Luteoxanthin	400, 422, 448	(n.d.–0.20)
5	17.8	*Z*-Isomer of auroxanthin	376, 396, 420	(n.d.–0.39)
6	18.9	Auroxanthin A	382, 402, 426	0.29 ± 0.11 (0.09–0.54)
7	20.1	Auroxanthin B	382, 402, 426	0.27 ± 0.09 (0.05–0.44)
8	20.3	Mixture of luteoxanthin and a *Z*-auroxanthin isomer		(n.d.–0.30)
9	20.6	Antheraxanthin	444, 472	(n.d.–0.29)
10	22.8	*Z*-Luteoxanthin isomer	396, 416, 442	(n.d.–0.20)
11	23.3	Auroxanthin C	382, 402, 426	(n.d.–0.29)
12	23.4	*Z*-Luteoxanthin isomer		(n.d.–0.24)
13	24.6	Mutatoxanthin epimer A	426, 452	0.38 ± 0.13 (0.11–0.73)
14	25.2	Lutein	424, 444, 472	0.23 ± 0.09 (0.06–0.42)
15	26.1	Mutatoxanthin epimer B	426, 452	0.46 ± 0.17 (0.16–0.97)
16	30.3	Zeaxanthin	450, 474	0.35 ± 0.11 (0.08–0.53)
17	31.1	(9*Z*)- or (9′*Z*)-Antheraxanthin	332, 440, 468	(n.d.–0.27)
18	33.3	(13*Z*)- or (13′*Z*)-β-Cryptoxanthin	338, 444, 466	(n.d.–0.17)
19	35.8	Zeinoxanthin	424, 444, 472	0.07 ± 0.02 (0.04–0.13)
20	38.0	(9*Z*)-Zeaxanthin	340, 446, 470	(n.d.–0.06)
21	41.1	β-Cryptoxanthin	452, 476	0.41 ± 0.22 (0.14–0.87)
22	43.6	(13*Z*)-β-Carotene	338, 444, 470	(n.d.–0.17)
23	45.0	*Z*-Isomer of ζ-carotene	380, 400, 424	0.08 ± 0.05 (n.d.–0.21)
24	46.4	α-Carotene	424, 446, 472	0.03 ± 0.04 (n.d.–0.25)
25	51.1	β-Carotene	452, 476	(n.d.–0.96)
26	51.1	Mixture of β-carotene and a *Z*-isomer of ζ-carotene		(n.d.–0.23)
27	53.0	ζ-Carotene	382, 402, 426	(n.d.–0.17)

[a] Retention time in minutes.
[b] In mg/l. Mean content ± standard deviation (for carotenoids found in all the samples surveyed) and range, the latter between parentheses.
[c] n.d.: not detected.

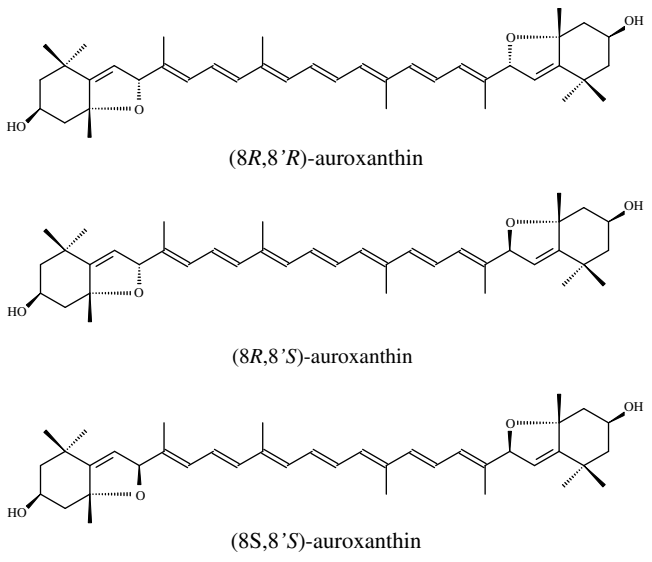


Fig. 4. Chemical structures of (8*R*, 8′*R*)-auroxanthin, (8*R*, 8′*S*)-auroxanthin and (8*S*, 8′*S*)-auroxanthin.

of the latter into their 5,8-epoxy counterparts. This reaction has been long known to be triggered even by traces of acids.

As far as the dihydroxycarotenoid fractions was concerned, both lutein and zeaxanthin were found at detectable levels in all the orange juices studied, as well as low amounts of the (9*Z*)-isomer of the latter in some samples. Similarly, the monohydroxycarotenoids, zeinoxanthin and the provitamin A β-cryptoxanthin, were also detected in all the samples surveyed, whereas small amounts of the (13*Z*)- or (13′*Z*)-isomers of the latter were occasionally found.

In relation to the visible carotenes fraction (neither phytoene nor phytofluene were determined), those commonly found in slightly processed orange juices (i.e., the *Z*-isomer of ζ-carotene eluting before α-carotene, and α- and β-carotene), were also found in the samples analyzed. However, detectable levels of another *Z*-isomer of ζ-carotene, which coeluted with β-carotene, as well as of (all-*E*)-ζ-carotene were also detected in some samples. In addition, another compound tentatively identified as (13*Z*)-β-carotene was also occasionally found.

To shed some light on the impact of the processing and long-term storage of OJFC on their carotenoid profile, it is useful to compare the carotenoid pattern of OJFC with that of UFOJ. The latter type of juice, which has been comprehensively studied by our research group (Meléndez-Martínez et al., 2007a), does not undergo thermal treatments, so after thawing it is virtually identical to fresh squeezed juice. As a result of the comparison, it was observed that the 5,8:5′,8′-diepoxycarotenoid auroxanthin, which was not detectable in UFOJ, was present in all the OJFC samples, as were some geometrical isomers of its isomer the 5,6:5′,6′-diepoxycarotenoid, violaxanthin. However, (9*Z*)-violaxanthin, which was the major carotenoid in UFOJ, was not found at detectable levels in OJFC. Analogous results have been also reported in fresh and processed products of mango (Mercadante & Rodriguez-Amaya, 1998). As regards the 5,6:5′,8′-diepoxycarotenoid luteoxanthin, which is an intermediate compound in the conversion of violaxanthin into auroxanthin that can be detected in all the samples of UFOJ, it was only detected in the OJFC samples with a shorter shelf-life, that is, in those that were to be kept in the refrigerator. Likewise, the 5,6-epoxycarotenoid antheraxanthin was not detected in many samples of OJFC. However, both (all-*E*)- and (9*Z*)- or (9′*Z*)-antheraxanthin, the latter being a major carotenoid in UFOJ (Meléndez-Martínez et al., 2005b, 2007a), were found at low levels in the OJFC samples to be kept in the refrigerator, which are subjected to less severe thermal treatments and therefore exhibit a shorter self-life. From these findings it can be inferred that a rapid assessment of the age of the juices could be achieved by simply considering their epoxycarotenoids content, the absence of antheraxanthin and (9*Z*)-violaxanthin denoting that the juice has been stored for a relatively long period.

Concerning the mono- and dihydroxycarotenoid fractions, the detection of (13Z)- or (13′Z)-β-cryptoxanthin and (9Z)-zeaxanthin in OJFC samples to be stored at room temperature (as well as the carotene tentatively identified as (13*Z*)- β-carotene) but not in those to be kept in the refrigerator nor in UFOJ (Meléndez-Martínez et al., 2007a), was worthy of note. Possibly such geometrical isomers are formed as a consequence of the thermal treatments that these types of OJFC are subjected to over the concentration process. Indeed, the formation of diverse isomers of carotenoids as a consequence of thermal treatments of the matrix that contain them, is well-known (Meléndez-Martínez, Vicario, & Heredia, 2004a; Marx, Stuparic, Schieber, & Carle, 2003; Rodriguez-Amaya, 1999).

As for the visible carotenes fraction, the occurrence in some of the OJFC samples of geometrical isomers of ζ-carotene other than the one commonly found in UFOJ, was observed. More specifically, detectable amounts of another *Z*-isomer of such carotene, which coeluted with β-carotene, as well as of (all-*E*)-ζ-carotene, were observed in some samples of OJFC, although neither of them were detected in our recent study on the carotenoid pattern of UFOJ. This observation could indicate that the formation of such ζ-carotene stereoisomers may be related to the industrial processing of OJFC and/or to the impact of the acidity of the product over relatively long storage periods at room temperature. In relation to the latter hypothesis, it is noteworthy that the *Z*-isomer of ζ-carotene, commonly found in OJFC and UFOJ, isomerizes into other geometrical isomers on silica gel TLC plates, probably due to the acidity of the absorbent (Meléndez-Martínez, 2005).

3.2. Quantitative analysis

The quantitative analysis of the carotenoids occurring in OJFC is somewhat impaired by the intricacy of their profile

which, added to the efficiency of C_{30} in the separation of isomers, leads unavoidably to the coelution of some carotenoids, notably epoxycarotenoids. This source of inaccuracy adds up to other factors that make it difficult to obtain reliable quantitative data on carotenoids, such as the inaccuracy of some of the absorption coefficients tabulated and the lack of appropriate coefficients for different isomers of a given carotenoid (Britton, 1992; Britton et al., 2004).

For the assessment of the total carotenoid contents, the sum of the levels of the individual pigments were taken into consideration rather than the traditional spectrophotometric determinations (Britton & Young, 1993), since the latter was found to lead to a noticeable underestimation of the carotenoid concentration (Meléndez-Martínez, 2005). According to this, the total contents of the juices studied fell into the bracket 1.37–5.89 mg/l (with an average content of 3.75 mg/l), being clearly inferior to those we calculated in the same terms and reported recently for UFOJ, which ranged from 17.2 to 29.4 mg/l (Meléndez-Martínez et al., 2007a).

Regarding individual carotenoids, the relative contribution of 5,8-epoxides to the total carotenoid content was higher as compared to UFOJ (Meléndez-Martínez et al., 2007a), to the extent that, when all the isomers were considered together, auroxanthin and mutatoxanthin were the major carotenoids in OJFC, as their respective 5,6-epoxide counterparts, violaxanthin and antheraxanthin, were the major carotenoids in UFOJ. Nevertheless, in absolute terms, the average levels of the two major mutatoxanthin isomers were higher in UFOJ (0.61 and 1.20 mg/l, than the 0.38 and 0.46 mg/l in OJFC), despite high levels of antheraxanthin still being present in the juice (Meléndez-Martínez et al., 2007a), which was due to the fact that the total carotenoid content of the UFOJ samples was much higher, as stated earlier.

As for the monohydroxycarotenoid and dihydroxycarotenoid fractions, it was found that the former was more important in quantitative terms. Within the dihydroxycarotenoid fraction, the average level of zeaxanthin (0.35 mg/l) was higher than that of lutein (0.23 mg/l) as also was the case in UFOJ, although the levels of such carotenoids were much higher in the latter juices (2.00 and 0.93 mg/l for zeaxanthin and lutein, respectively) (Meléndez-Martínez et al., 2007a). Something similar was observed in relation to the monohydroxycarotenoid fraction, where it was clearly seen that the mean level of zeinoxanthin was lower than that of β-cryptoxanthin (0.07 and 0.41 mg/l, respectively). The concentrations of both pigments, especially in the case of zeinoxanthin, were lower than those recently reported by our group in UFOJ (0.50 and 1.19 mg/l for zeinoxanthin and β-cryptoxanthin, respectively) (Meléndez-Martínez et al., 2007a).

The visible carotenes fraction was the less important, quantitatively, of all the fractions detected (Table 1), as it is common in most orange juices, those from lycopene-accumulating varieties being an exception (Dhuique-Mayer, Caris-Veyrat, Ollitrault, Curk, & Amiot, 2005; Fanciullino et al., 2006; Meléndez-Martínez et al., 2007a; Xu, Fraser, Wang, & Bramley, 2006).

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