Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 169–175
Food Chemistry
www.elsevier.com/locate/foodchem

Geometrical isomers of violaxanthin in orange juice

Antonio J. Meléndez-Martínez, Isabel M. Vicario, Francisco J. Heredia *

Laboratory of Food Colour and Quality, Department of Nutrition and Food Science, Faculty of Pharmacy, University of Seville, 41012 Seville, Spain

Received 13 June 2006; received in revised form 19 September 2006; accepted 8 November 2006

Abstract

The geometrical isomers of carotenoids are now attracting considerable interest, due in part to the fact that they are known to behave differently in terms of susceptibility to oxidation, vitamin A activity and bioavailability. The study of such isomers has been facilitated by the development of the C_{30} stationary phase, which has made it possible to evaluate their occurrence in any source in a rapid fashion. In this study, the violaxanthin ((3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol) fraction of orange juice (*Citrus sinensis* L. Osbeck) was isolated by thin-layer chromatography (TLC) to identify the different geometrical isomers occurring in it. Under the HPLC conditions described, several violaxanthin isomers were detected, of which (all-*E*)-violaxanthin and, above all, (9*Z*)-violaxanthin, happened to be the most important in quantitative terms.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: C_{30}; Carotenoids; Citrus; Electron-impact mass spectra (EI-MS); Geometrical isomers; Orange juice; Violaxanthin

1. Introduction

Carotenoid pigments are a ubiquitous group of isoprenoid compounds synthesized by plants, algae, fungi and bacteria. Animals cannot synthesize carotenoids *de novo*, so they rely on the diet as a source of these compounds (Fraser & Bramley, 2004; Isler, 1971; Rodriguez-Amaya, 2001). Carotenoids do not just provide natural yellow, orange or red colours, but also play important roles in photosynthesis. Thus, they contribute to the harvesting of light, prevent the photochemical formation of singlet oxygen ($^1O_2$) from chlorophyll and are able to quench it if this is finally formed, thus protecting the plant against photooxidation (Britton, 1995a; Frank & Cogdell, 1993). In addition to these important functions, carotenoids are beneficial to humans: apart from the well-known vitamin A activity of those showing an unsubstituted β-ring with an 11-carbon polyene chain (Simpson, 1983; Zechmeister, 1962), there is evidence that these pigments may be scavengers of reactive species, thus showing antioxidant activity (Beutner et al., 2001; Edge, McGarvey, & Truscott, 1997; Krinsky, 2001), and that they may protect humans from serious disorders such as skin disorders, cardiovascular disease, several forms of cancer, age-related diseases of the eye, such as macular degeneration, or cataracts (Bramley, 2000; Meléndez-Martínez, Vicario, & Heredia, 2004; Nishino et al., 1999; Olson, 1999; Simpson & Chichester, 1981; Snodderly, 1995). At this point, it is important to note that, although the beneficial effects of carotenoids are mainly linked to their antioxidant activity, there are mechanisms other than this one that may also be implicated in such effects (Stahl, Ale-Agha, & Polidori, 2002).

Due to all the above comments, it is not surprising that carotenoids have been attracting great interest. Many studies have been undertaken in recent years to provide data in relation to the carotenoid content of a number of foodstuffs specially important in human nutrition, such as carrots (Chen, Peng, & Chen, 1995; Skrede et al., 1997), peppers (Collera-Zúñiga, García-Jiménez, & Meléndez-Gordillo, 2004; Pérez-Gálvez, Hornero-Méndez, & Mínguez-Mosquera, 2004), tomatoes (Abushita, Daood, & Biacs, 2000; Fraser, Bramley, & Seymour, 2001), orange juice (Gama & Sylos, 2005; Gross, Gabai, & Lifshitz, 1972; Gross, Carmon, Lifshitz, & Sklarz, 1975; Meléndez-Martínez, Britton,

* Corresponding author. Tel.: +34 95455 6761; fax: +34 95455 7017.
E-mail address: heredia@us.es (F.J. Heredia).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.11.017

Vicario, & Heredia, 2005a; Mouly, Gaydou, Lapierre, & Corsetti, 1999), tropical fruits (Gamage Chandrika, Jansz, Nalinie Wickramasinghe, & Warnasuriya, 2003; Mercadante & Rodriguez-Amaya, 1998), green vegetables (Burns, Fraser, & Bramley, 2003; Kimura & Rodriguez-Amaya, 2002; Tenorio, Villanueva, & Sagardoy, 2004) and many others. In relation to the analysis of these pigments, there is no doubt that the development of C_{30} columns (Sander, Epler-Sharpless, Craft, & Wise, 1994) has widened our knowledge of the carotenoids occurring in nature, owing to the fact that such a stationary phase allows different isomers of the same carotenoid to be separated (Breitenbach, Braun, Steiger, & Sandmann, 2001; Emenhiser, Simunovic, Sander, & Schwartz, 1996). The study of geometrical isomers of carotenoids, whose existence has been long known (Zechmeister, 1962), is becoming of great importance (Godoy & Rodriguez-Amaya, 1998; Humphries & Khachik, 2003; Kull & Pfander, 1997; Marx, Schieber, & Carle, 2000; Marx, Stuparic, Schieber, & Carle, 2003; Meléndez-Martínez, Britton, Vicario, & Heredia, 2006; Nguyen, Francis, & Schwartz, 2001), since it has been observed that their properties (vitamin A activity, susceptibility to oxidation, bioavailability) differ from those of their (all-*E*)-counterparts (Bohm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Henry et al., 2000; Schieber & Carle, 2005; Zechmeister, 1962). Moreover, the study of certain geometrical isomers of violaxanthin and neoxanthin is of great importance in relation to their roles in the photosynthetic apparatus and as precursors of the plant hormone abscisic acid (ABA) (Grudzinski et al., 2001; Phillip, Molnár, Tóth, & Young, 1999; Qin & Zeevaart, 1999; Rodrigo, Alquezar, & Zacarías, 2006). Therefore, the goal of this study was to provide new data on the geometrical isomers of violaxanthin ((3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol) (Fig. 1) occurring in orange juice (*Citrus sinensis* L. Osbeck), some of which have already been reported to be present in the juice and the peel of this citrus (Gross, 1987; Gross et al., 1972; Molnár & Szabolcs, 1980; Stewart, 1977).

2. Materials and methods

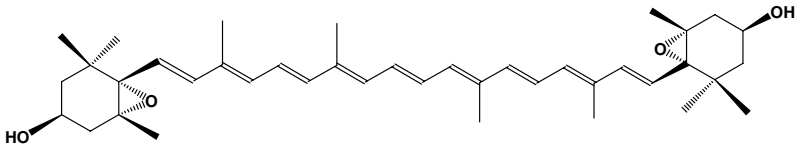
2.1. Isolation and isomerization of the violaxanthin standard

The (all-*E*)-violaxanthin standard was obtained according to standard procedures (Britton, 1995c; Mínguez-Mosquera, 1997) from a saponified extract of spinach leaves (*Spinacia oleracea* L.). An ethanolic solution of this standard, under an atmosphere of nitrogen, was heated in a water bath (80–100 °C) for 30 min and subsequently illuminated overnight by means of an incandescent lamp to obtain a mixture of different geometrical isomers (Meléndez-Martínez et al., 2005a). We opted for using treatments, consecutively, on the basis of the results obtained by other authors (Chen, Chen, & Chien, 1994; Chen & Huang, 1998), which indicated that the profile of geometrical isomers formed differed, depending on the treatment applied.

The isomers so obtained were identified on the basis of their spectroscopic characteristics (Fig. 2, Table 1) (Britton, 1995b; Zechmeister, 1962).

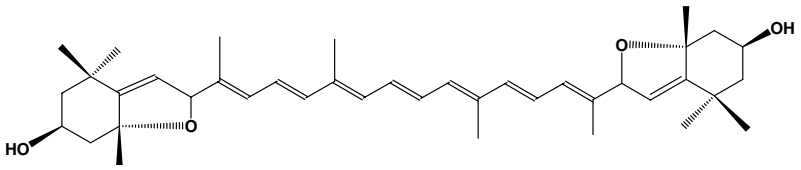
2.2. Carotenoid extraction from orange juice and saponification

The carotenoid extract was obtained from 300 ml of Valencia ultrafrozen orange juice, kindly provided by the company Zumos Vitafresh (Almonte, Spain). After thawing at room temperature, the carotenoid fraction was extracted with 500 ml of a mixture ethanol:hexane (1:1) in a separatory funnel, filtered and saponified (500 ml of 10% ethanolic KOH), overnight, under an atmosphere of nitrogen. Finally, the saponified extract was washed with water (4 × 300 ml) and taken to dryness at a temperature below 35 °C.



violaxanthin

((3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*S*)-5,6:5',6'-diepoxy-5,6,5',6'-tetrahydro-β,β-carotene-3,3'-diol)



auroxanthin

((3*S*,5*R*,8*RS*,3'*S*,5'*R*,8'*RS*)-5,8:5',8'-diepoxy-5,8,5',8'-tetrahydro-β,β-carotene-3,3'-diol)

Fig. 1. Chemical structures of violaxanthin and auroxanthin.

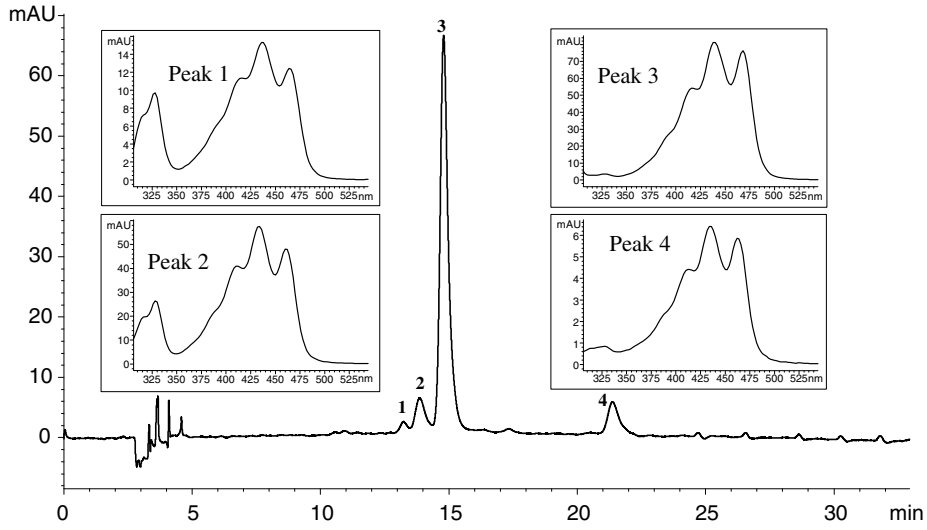


Fig. 2. HPLC chromatogram (detection wavelength: 430 nm) and UV/vis spectra of the geometrical isomers obtained by heat- and light-induced isomerization of an (all-*E*)-violaxanthin standard isolated from spinach leaves (peak identification given in Table 1).

Table 1
Chromatographic and spectroscopic characteristics of the geometrical isomers obtained by isomerization of the (all-*E*)-violaxanthin standard

Peak	r_t (min)	Isomer	Absorption maxima (nm)				D_B/D_{II}[a]
1	13.24	(15*Z*)-Violaxanthin	328	416	436	464	0.634
2	13.87	(13*Z*)-Violaxanthin	328	412	432	460	0.460
3	14.80	(All-*E*)-violaxanthin		416	440	468	
4	21.37	(9*Z*)-Violaxanthin	326	412	436	464	0.113

[a] Ratio of the absorbance of the *cis* peak in the UV/vis spectrum to that of the second absorption band in the visible region.

2.3. Isolation of violaxanthin from orange juice

An aliquot of the saponified extract of orange juice carotenoids was separated by TLC on silica gel aluminium sheets (Merck, Darmstadt, Germany), using diethyl ether as mobile phase, to obtain preliminary information about its carotenoid profile. The standard isolated from spinach leaves was co-chromatographed to determine the location of the band corresponding to violaxanthin. After 30 min, the TLC aluminium sheet was left to dry to check that the band whose R_f matched with that of the standard turned bluish, which revealed the formation of the corresponding auroxanthin-epimers (Eugster, 1995; Mínguez-Mosquera, 1997).

The rest of the extract was chromatographed on $60GF_{254}$ (Merck, Darmstadt, Germany) silica gel plates (20 × 20 cm, 1 mm thickness) made in the laboratory, using the same solvent system. A small amount of the violaxanthin standard was co-chromatographed to help locate the band of interest. To diminish the isomerization of 5,6-epoxycarotenoids to their 5,8-epoxyderivatives, the inherent acidity of the silica was reduced by adding a pellet of NaOH to the slurry water–silica and by moving the plates over a flask containing NH_3. After 1 h of development, the broad band located beside that corresponding to the standard was scraped off the plates and re-chromatographed on laboratory-made aluminium G type E (Merck, Darmstadt, Germany) plates (20 × 20 cm, 0.5 mm thick), using a mixture of acetone: petroleum ether (40–60 °C) (3:7) as the solvent system. After 1 h, the two sub-fractions observed in the main band were recovered separately from the plate. The extracts were kept dry at −18 °C under a nitrogen atmosphere prior to analysis.

2.4. High-performance liquid chromatography

HPLC analyses were carried out on an Agilent 1100 system fitted with a quaternary pump, a photodiode array detector (PDA), a column temperature control module and an autosampler set to draw 20 μl of sample (Agilent, Palo Alto, CA, United States). The carotenoids were separated by means of an YMC C_{30} column (5 μm, 250 × 4.6 mm) (YMC, Wilmington, NC, USA) kept at 17 °C. The flow rate was 1 ml/min and the chromatograms were monitored and recorded at 430, 450 and 486 nm. Methanol (MeOH), methyl *tert*-butyl ether (MTBE) and water were used in the mobile phase according to a gradient described elsewhere (Meléndez-Martínez, Britton, Vicario, & Heredia, 2005b; Meléndez-Martínez, Britton, Vicario, & Heredia, 2005c; 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. MeOH and

MTBE contained small proportions of butylated hydroxytoluene (BHT) (0.1%) and triethylamine (0.05%) to protect the pigments during the analysis.

2.5. Mass spectrometry

The electron impact mass spectra (EI-MS) were obtained from a Micromass AutoSpec system (Micromass, Manchester, UK) at an ionizing voltage of 70 eV. The temperature of the ion source chamber was 230–240 °C. Before the analysis, the extracts were purified through alumina minicolumns (Brockmann activity grade III) and concentrated to dryness.

3. Results and discussion

The carotenoid profile of the great majority of green leaves is quite constant, being commonly used as a source for the isolation of the major ones, namely (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), lutein (β,ε-carotene-3,3′-diol) and β-carotene (β,β-carotene), to be used as standards (Britton, 1995c; Mínguez-Mosquera, 1997). The spectrum in acetone of violaxanthin standard isolated from spinach leaves showed sharp well-defined absorption bands with maxima at 420, 442 and 472 nm. Upon the addition of a few drops of ethanolic 0.1 M HCl (Eugster, 1995; Rodriguez-Amaya, 2001), a hypsochromic shift of 40 nm was observed in the absorption maximum, due to the two 5,6-epoxy to 5,8-epoxy re-arrangements to give epimers of auroxanthin ((3*S*,5*R*,8*RS*,3′*S*,5′*R*, 8′*RS*)-5,8:5′, 8′-diepoxy-5,8,5′,8′-tetrahydro-β, β-carotene-3, 3′-diol) (Fig. 1), which supported the assumption that the carotenoid isolated was violaxanthin.

In principle, many different geometrical isomers of carotenoids might occur, as each double bond could exist in two configurations, namely *E* (*trans*) or *Z* (*cis*). Nonetheless, only a few of these possible geometrical isomers are in practice found, due to the fact that the introduction of a *Z* double bond results in steric hindrance that renders the *Z* isomer less stable than its *E* counterpart. Hence, only *Z* isomers in which steric hindrance is small, normally (9*Z*)-, (13*Z*)-, (15*Z*)- and some (di-*Z*)-isomers, can be formed with relative ease and are commonly found in nature (Britton, 1995a; Weedon & Moss, 1995). In this particular study, it was observed that a mixture of only four geometrical isomers was obtained, as a result of heating and illumination of the ethanolic solution of the (all-*E*)-violaxanthin standard, as is depicted in Fig. 2. The identification of the *E*/*Z* isomers was carried out on the basis of their spectroscopic features in the mobile phase (Table 1). For that purpose, the hypsochromic shifts of the absorption maxima of the (*Z*)-isomers relative to the (all-*E*)-isomer, around 2–6 nm in the case of (mono-*Z*)-isomers and 10–12 nm in the case of (di-*Z*)-isomers, were considered. In addition, the intensities of the "*cis* peaks" that appear in the ultraviolet region of the spectra, which are higher as the *cis* double bonds are closer to the centre of the molecule, were also taken into account (Britton, 1995b; Rodriguez-Amaya, 2001; Zechmeister, 1962). To evaluate the intensity of the *cis* peak, the ratio of its absorbance to that of the second absorption band in the visible region, known as *Q* ratio, D_B/D_{II} or A_B/A_{II}, and commonly used for that purpose (Chen et al., 1994; Deli, Molnár, Tóth, Szabolcs, & Radics, 1988; Phillip et al., 1999), was calculated. Taking into consideration all these observations, peaks 1 and 2 were readily identified as the central and next-to-central isomers ((15*Z*)-violaxanthin and (13*Z*)-violaxanthin, respectively) on the basis of the intensities of their *cis* peaks (0.634 and 0.460, respectively). The location of the absorption maxima of the 15*Z*-isomer at a slightly higher wavelength relative to the (13*Z*)-isomer (436 and 432 nm, respectively), which was coherent with the identification of other central and next-to-central isomers of carotenoids, carried out by other authors (Bohm et al., 2002; Chen et al., 1994; Molnár, Szabolcs, & Radics, 1986; Strand, Kvernberg, Karlsen, & Liaaen-Jensen, 2000), also helped to identify these isomers. Peak 4 was unequivocally identified as the peripheral (9*Z*)-isomer on the basis of its smooth *cis* peak ($D_B/D_{II} = 0.113$) and the slight hypsochromic shift of its absorption maxima (436 nm) relative to the (all-*E*)-isomer (peak 3, $\lambda_{max} = 440$ nm).

Fig. 3 depicts the chromatogram at 430 nm of the main sub-fraction of the band isolated from orange juice. The HPLC analysis of the other sub-fraction isolated revealed the presence of only one isomer, which was also present in the main sub-fraction (peak 5). The existence of "sub-fractions" within the TLC band of a given carotenoid, generally corresponding to geometrical isomers, is well-known (Schiedt, 1995). The identification of the pigment isolated from orange juice as violaxanthin was done according to recommended procedures. Its chromatographic behaviour on the silica TLC plates, where it was co-chromatographed with the violaxanthin standard, was taken into consideration, as well as the change in colour of the band from yellow to blue, due to the acidity of the absorbent, which is a typical feature of carotenoids with two 5,6-epoxy groups (Eugster, 1995; Mínguez-Mosquera, 1997). The band isolated from the juice was also co-chromatographed with the mixture of geometrical isomers obtained from the violaxanthin standard on a C_{30} column according to the HPLC method, which allowed the straightforward identification of the peaks corresponding to (all-*E*)-violaxanthin and (9*Z*)-violaxanthin. To confirm the presence of two 5,6-epoxy groups in the molecule, the corresponding test for their detection by chemical derivatization (Eugster, 1995; Rodriguez-Amaya, 2001) was accomplished. As a consequence of the acidic treatment of the extract with ethanolic 0.1 M HCl, the 5 peaks present in Fig. 3 disappeared and a new chromatogram, in which 12 peaks were detected, was obtained. The absorption maxima of these new compounds (ranging from 396 to 402 nm) were located at a wavelength approximately 40 nm lower than those corresponding to the

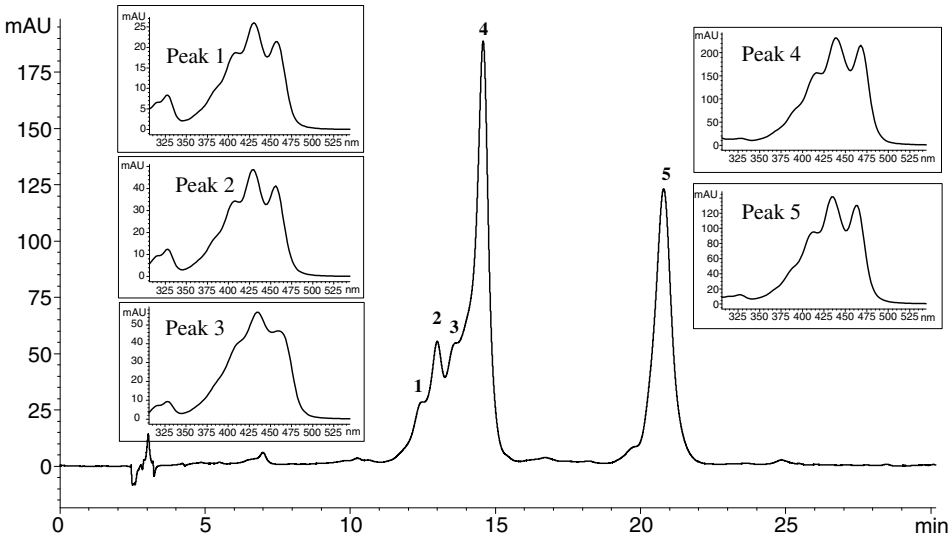


Fig. 3. HPLC chromatogram at 430 nm and UV/vis spectra of the geometrical isomers of violaxanthin detected in orange juice (peak identification given in Table 2).

compounds occurring in the original extract (ranging from 430 to 440 nm, Table 2), which supported their identification as geometrical isomers of violaxanthin. The fact that, as a result of the acidic treatment of violaxanthin, 3 stereoisomers of auroxanthin (namely (8*R*, 8′*R*)-, (8*R*, 8′*S*)- and (8*S*, 8′*S*)-auroxanthin) are obtained (Britton, Liaaen-Jensen, & Pfander, 2004), explains the noteworthy increase in the number of compounds present in the extract after the 5,6-epoxy to 5,8-epoxy re-arrangement. The electron impact mass spectrum (EI-MS) of the mixture of geometrical isomers of violaxanthin isolated from orange juice showed a molecular ion at 600 *m*/*z*, which was consistent with the formula $C_{40}H_{56}O_4$. The fragments at *m*/*z* 582 $[M-18]^+$ and 564 $[M-18-18]^+$, corresponding to the losses of molecules of water, revealed the existence of two hydroxy groups in the molecule, whilst those at *m*/*z* 520 $[M-80]^+$ and 440 $[M-80-80]^+$ accorded with the presence of two epoxy groups. In addition, the fragments at *m*/*z* 352, 221 and 181, typical of epoxycarotenoids, indicated that the epoxy groups were in rings with a hydroxy group. The features of the mass spectrum detailed above were in agreement with the data reported in an extensive compilation of MS data of carotenoids (Enzell & Back, 1995) and with those corresponding to the violaxanthin isolated from mango reported by Mercadante, Rodriguez-Amaya, and Britton (1997).

The chromatographic and spectroscopic characteristics of the different *E*/*Z* isomers of violaxanthin present in the band isolated from orange juice are summarized in Table 2. Peaks 4 and 5 were readily identified as (all-*E*)-violaxanthin and (9*Z*)-violaxanthin, on the basis of those features, which, in addition, were in agreement with those of the isomers thus identified in the mixture of geometrical isomers obtained by isomerization of the violaxanthin standard isolated from spinach leaves (Table 1, peaks 3 and 4, respectively). Peaks 1 and 2 showed virtually identical spectra, with the same absorption maxima (328, 408, 430 and 456 nm) and *cis* peaks of very similar intensity (Table 2), being tentatively identified as (di-*Z*)-violaxanthin isomers on the basis of the large hypsochromic shifts of their absorption maxima (10 nm) relative to the (all-*E*)-isomer. Furthermore, the intensities of the *cis*-peaks in their spectra ($D_B/D_{II} = 0.354$ and 0.289, for peaks 1 and 2, respectively, Table 2) were lower than those of the (13*Z*)- and (15*Z*)-violaxanthin isomers ($D_B/D_{II} = 0.460$ and 0.634, respectively, Table 1), which was in agreement with the identification of (mono-*Z*)- and (di-*Z*)-isomers of violaxanthin carried out by Molnár et al. (1986). Unlike peaks 1, 2, 4 and 5, the identification of peak 3 was more complicated, since both its shape and spectrum in the mobile phase seemingly indicated that different isomers coeluted. The retention time and the location of the absorption maxima of the

Table 2
Spectroscopic and chromatographic features of the geometrical isomers of violaxanthin obtained from orange juice

Peak	r_t (min)	Isomer	Absorption maxima (nm)				D_B/D_{II}[a]
1	12.44	(Di-*Z*)-violaxanthin	328	408	430	456	0.354
2	13.00	(Di-*Z*)-violaxanthin	328	408	430	456	0.289
3	13.64	(13*Z*)- + (Di-*Z*)-violaxanthin	328		434	460	0.204
4	14.58	(All-*E*)-violaxanthin		416	440	468	
5	20.80	(9*Z*)-Violaxanthin	326	412	436	464	0.125

[a] Ratio of the absorbance of the *cis* peak in the UV/vis spectrum to that of the second absorption band in the visible region.

compound identified as (13*Z*)-violaxanthin, in the mixture of geometrical isomers obtained from the standard, were very similar to those of peak 3. However, the intensities of their corresponding *cis* peaks ($D_B/D_{II} = 0.460$ and 0.204, for (13*Z*)-violaxanthin and peak 3, respectively) differed clearly, as did the shape of their spectra (Figs. 2 and 3). Taking into consideration all of these observations, peak 3 could be tentatively identified as a mixture of (13*Z*)-violaxanthin and a further di-*Z*-isomer. As a result of the coeluetion of these isomers, a merged spectrum would be obtained, which would account for the absence of the typical high degree of fine structure of epoxycarotenoids, and for the smooth *cis* peak. The occurrence of both (13*Z*)- and (di-*Z*)-violaxanthin isomers in orange peel, reported by Molnár and Szabolcs (1980), supports this tentative identification. The existence of various (di-*Z*)-violaxanthin isomers in orange juice is noteworthy, although their occurrence in natural sources has already been reported, concretely in blossoms of the wild pansy (*Viola tricolor* L.), where up to 4 of them (namely the (9*Z*,9′*Z*)-, (9*Z*,13′*Z*)-, (9*Z*,15*Z*)- and (9*Z*,13′*Z*)-isomers) were identified (Molnár et al., 1986).

In quantitative terms, (9*Z*)-violaxanthin was by far the main isomer, followed by the (all-*E*)-isomer. Indeed the (9*Z*)-isomer has been reported to be the major carotenoid, not only in orange juices, but also in orange peels (Gross, 1987; Meléndez-Martínez, 2005; Molnár & Szabolcs, 1980; Oberholster, Cowan, Molnár, & Tóth, 2001). Both (9*Z*)-violaxanthin and (9′*Z*)-neoxanthin are now attracting large interest, due to their involvement in the biosynthesis of the ubiquitous plant hormone abscisic acid (ABA), which is indispensable for the adaptation of plants to different environmental stresses (e.g. salinity and drought) and plays an important role in several physiological processes, such as embryo development or seed dormancy (Parry, 1993; Qin & Zeevaart, 1999; Rodrigo et al., 2006).

References

Abushita, A. A., Daood, H. G., & Biacs, P. A. (2000). Changes in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *Journal of Agricultural and Food Chemistry, 48*, 2075–2081.

Beutner, S., Bloedorn, B., Frixel, S., Hernández Blanco, I., Hoffman, T., Martin, H.-D., et al. (2001). Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β-carotene in antioxidant functions. *Journal of the Science of Food and Agriculture, 81*, 559–568.

Bohm, V., Puspitasari-Nienaber, N. L., 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*, 221–226.

Bramley, P. M. (2000). Is lycopene beneficial to human health? *Phytochemistry, 54*, 233–236.

Breitenbach, J., Braun, G., Steiger, S., & Sandmann, G. (2001). Chromatographic performance on a C_{30}-bonded stationary phase of monohydroxycarotenoids with variable chain length or degree of desaturation and of lycopene isomers synthesized by various carotene desaturases. *Journal of Chromatography A, 936*, 59–69.

Britton, G. (1995a). Structure and properties of carotenoids in relation to function. *The FASEB Journal, 9*, 1551–1558.

Britton, G. (1995b). UV/visible spectroscopy. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Volume 1B: Spectroscopy* (pp. 13–62). Basel, Switzerland: Birkhäuser.

Britton, G. (1995c). Worked examples of isolation and analysis. Example 1: Higher plants. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Volume 1A: Isolation and Analysis* (pp. 201–214). Basel, Switzerland: Birkhäuser.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2004). Carotenoids. Handbook. Basel, Switzerland: Birkhäuser.

Burns, J., Fraser, P. D., & Bramley, P. M. (2003). Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry, 62*, 939–947.

Chen, B. H., & Huang, J. H. (1998). Degradation and isomerization of chlorophyll and β-carotene as affected by various heating and illumination treatments. *Food Chemistry, 62*, 299–307.

Chen, B. H., Chen, T. M., & Chien, J. T. (1994). Kinetic model for studying the isomerization of α- and β-carotene during heating and illumination. *Journal of Agricultural and Food Chemistry, 42*, 2391–2397.

Chen, B. H., Peng, H. Y., & Chen, H. E. (1995). Changes of carotenoids, color and vitamin A content during processing of carrot juice. *Journal of Agricultural and Food Chemistry, 43*, 1912–1918.

Collera-Zúñiga, O., García-Jiménez, F., & Meléndez-Gordillo, R. (2004). Comparative study of carotenoid composition in three Mexican varieties of *Capsicum annuum* L. *Food Chemistry, 90*(1-2), 109–114.

Deli, J., Molnár, P., Tóth, G., Szabolcs, J., & Radics, L. (1988). Determination of the geometrical configuration of naturally occurring mono-*cis*-lutein epoxides. *Phytochemistry, 27*(2), 547–549.

Edge, R., McGarvey, D. J., & Truscott, T. G. (1997). The carotenoids as anti-oxidants. A review. *Journal of Photochemistry and Photobiology B: Biology, 41*, 189–200.

Emenhiser, C., Simunovic, N., Sander, L. C., & Schwartz, S. J. (1996). Separation of geometrical carotenoid isomers in biological extracts using a polymeric C_{30} column in reversed-phase liquid chromatography. *Journal of Agricultural and Food Chemistry, 44*, 3887–3893.

Enzell, C.R., & Back, S. (1995). Mass spectrometry. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Volume 1B: Spectroscopy* (pp. 261–320). Basel, Switzerland: Birkhäuser.

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. Volume 1A: Isolation and analysis* (pp. 71–80). Basel, Switzerland: Birkhäuser.

Frank, H., & Cogdell, R.J. (1993). Photochemistry and function of carotenoids in photosynthesis. In A. Young & G. Britton (Eds.), *Carotenoids in photosynthesis* (pp. 253–326). London: Chapman and Hall.

Fraser, P. D., & Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research, 43*, 228–265.

Fraser, P. D., Bramley, P., & Seymour, G. B. (2001). Effect of Cnr mutation on carotenoid formation during tomato fruit ripening. *Phytochemistry, 58*, 75–79.

Gamage Chandrika, U., Jansz, E. R., Nalinie Wickramasinghe, S. M. D., & Warnasuriya, N. D. (2003). Carotenoids in yellow- and red-fleshed papaya (*Carica papaya* L.). *Journal of the Science of Food and Agriculture, 83*, 1279–1282.

Gama, J. J. T., & Sylos, C. M. (2005). Major carotenoid composition of Brazilian Valencia orange juice: identification and quantification by HPLC. *Food Research International, 38*, 899–903.

Godoy, H. T., & Rodriguez-Amaya, D. B. (1998). Occurrence of cisisomers of provitamins A in Brazilian vegetables. *Journal of Agricultural and Food Chemistry, 46*, 3081–3086.

Gross, J. (1987). Pigments in fruits. Orlando, FL: Academic Press, Inc.

Gross, J., Carmon, M., Lifshitz, A., & Sklarz, B. (1975). Structural elucidation of some orange juice carotenoids. *Phytochemistry, 14*, 249–252.

Gross, J., Gabai, M., & Lifshitz, A. (1972). A comparative study of the carotenoid pigments in juice of Shamouti, Valencia and Washington oranges, three varieties of *Citrus Sinensis*. *Phytochemistry, 11*, 303–308.

Grudzinski, W., Matula, M., Sielewiesiuk, J., Kernen, P., Krupa, Z., & Gruszecki, W. I. (2001). Effect of 13-*cis* violaxanthin on organization of light harvesting complex II in monomolecular layers. *Biochimica et Biophysica Acta, 1503*, 291–302.

Henry, L. K., Puspitasari-Nienaber, N. L., Jarén-Galán, M., VanBreemen, R. B., Catignani, G. L., & Schwartz, S. J. (2000). Effects of ozone and oxygen on the degradation of carotenoids in an aqueous model system. *Journal of Agricultural and Food Chemistry, 48*(10), 5008–5013.

Humphries, J. M., & Khachik, F. (2003). Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. *Journal of Agricultural and Food Chemistry, 51*, 1322–1327.

Isler, O. (1971). *Carotenoids*. Basel: Stuttgart: Birkhäuser Verlag.

Kimura, M., & Rodriguez-Amaya, D. B. (2002). A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chemistry, 78*, 389–398.

Krinsky, N. I. (2001). Carotenoids as antioxidants. *Nutrition, 17*(10), 815–817.

Kull, D. R., & Pfander, H. (1997). Isolation and structure elucidation of two (*Z*)-isomers of lutein from the petals of rape (*Brassica napus*). *Journal of Agricultural and Food Chemistry, 45*, 4201–4203.

Marx, M., Schieber, A., & Carle, R. (2000). Quantitative determination of carotene stereoisomers in carrot juices and vitamin supplemented (ATBC) drinks. *Food Chemistry, 70*, 403–408.

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*, 609–617.

Meléndez-Martínez, A. J. (2005). Estudio de los carotenoides y del color de zumos de naranja. Ph. D. Thesis. University of Seville.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2005a). Color and carotenoid profile of Spanish Valencia late ultrafrozen orange juices. *Food Research International, 38*, 931–936.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2005b). Identification of isolutein (lutein epoxide) as *cis*-antheraxanthin in orange juice. *Journal of Agricultural and Food Chemistry, 53*(24), 9369–9373.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2005c). Identification of zeinoxanthin in orange juices. *Journal of Agricultural and Food Chemistry, 53*(16), 6362–6367.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2006). HPLC analysis of geometrical isomers of lutein epoxide isolated from dandelion (*Taraxacum officinale* F. Weber ex Wiggers). *Phytochemistry, 67*, 771–777.

Meléndez-Martínez, A. J., Vicario, I. M., & Heredia, F. J. (2004). Importancia nutricional de los pigmentos carotenoides. *Archivos Latinoamericanos de Nutrición, 54*(2), 149–154.

Mercadante, A. Z., & Rodriguez-Amaya, D. B. (1998). Effects of ripening, cultivar differences, and processing on the carotenoid composition of mango. *Journal of Agricultural and Food Chemistry, 46*, 128–130.

Mercadante, A. Z., Rodriguez-Amaya, D. B., & Britton, G. (1997). HPLC and mass spectrometric analysis of carotenoids from mango. *Journal of Agricultural and Food Chemistry, 45*, 120–123.

Mínguez-Mosquera, M.I., (1997). Clorofilas y carotenoides en Tecnología de los Alimentos. Sevilla: Secretariado de publicaciones de la Universidad de Sevilla.

Molnár, P., & Szabolcs, J. (1980). β-Citraurin epoxide, a new carotenoid from Valencia orange peel. *Phytochemistry, 19*, 633–637.

Molnár, P., Szabolcs, J., & Radics, L. (1986). Naturally occurring di-*cis*-violaxanthins from Viola tricolor: isolation and identification by ^{1}H NMR spectroscopy of four di-*cis*-isomers. *Phytochemistry, 25*(1), 195–199.

Mouly, P. P., Gaydou, E. M., Lapierre, L., & Corsetti, J. (1999). Differentiation of several geographical origins in single-strength Valencia orange juices using quantitative comparison of carotenoid profiles. *Journal of Agricultural and Food Chemistry, 47*, 4038–4045.

Nguyen, M., Francis, D., & Schwartz, S. (2001). Thermal isomerisation susceptibility of carotenoids in different tomato varieties. *Journal of the Science of Food and Agriculture, 81*, 910–917.

Nishino, H., Tokuda, H., Satomi, Y., Masuda, M., Bu, P., Onozuka, M., et al. (1999). Cancer prevention by carotenoids. *Pure Applied Chemistry, 71*(12), 2273–2278.

Oberholster, R., Cowan, A. K., Molnár, P., & Tóth, G. (2001). Biochemical basis of color as an aesthetic quality in *Citrus sinensis*. *Journal of Agricultural and Food Chemistry, 49*(1), 303–307.

Olson, J. A. (1999). Carotenoids and human health. *Archivos Latinoamericanos de Nutrición, 49*(1-S), 7–11.

Parry, A.D. (1993). Abscisic acid metabolism. In P. J. Lea (Ed.), *Methods in Plant Biochemistry*, Vol. 9. *Enzymes of secondary metabolism* (pp. 381–402). London: Academic Press.

Pérez-Gálvez, A., Hornero-Méndez, D., & Mínguez-Mosquera, M. I. (2004). Changes in the carotenoid metabolism of Capsicum fruits during application of modelized slow drying process for paprika production. *Journal of Agricultural and Food Chemistry, 52*, 518–522.

Phillip, D., Molnár, P., Tóth, G., & Young, A. J. (1999). Light-induced formation of 13-*cis* violaxanthin in leaves of Hordeum vulgare. *Journal of Photochemistry and Photobiology B.Biology, 49*, 89–95.

Qin, X., & Zeevaart, J. A. D. (1999). The 9-*cis*-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. *PNAS, 96*(26), 15354–15361.

Rodrigo, M. J., Alquezar, B., & Zacarías, L. (2006). Cloning and characterization of two 9-*cis*-epoxycarotenoid dioxygenase genes, differentially regulated during fruit maturation and under stress conditions, from orange (*Citrus sinensis* L. Osbeck). *Journal of Experimental Botany, 57*(3), 633–643.

Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in foods*. Washington, DC: ILSI Press.

Sander, L. C., Epler-Sharpless, K., Craft, N. E., & Wise, S. A. (1994). Development of engineered stationary phases for the separation of carotenoid isomers. *Analytical Chemistry, 66*, 1667–1674.

Schieber, A., & Carle, R. (2005). Occurrence of carotenoid *cis*-isomers in food: technological, analytical and nutritional implications. *Trends in Food Science and Technology, 16*, 416–422.

Schiedt, K. (1995). Chromatography: Part III. Thin-layer chromatography. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Volume 1A: Isolation and analysis* (pp. 131–144). Basel, Switzerland: Birkhäuser.

Simpson, K. L. (1983). Relative value of carotenoids as precursors of vitamin A. *Proceedings of the Nutrition Society, 42*, 7–17.

Simpson, K. L., & Chichester, C. O. (1981). Metabolism and nutritional significance of carotenoids. *Annual Review of Nutrition, 1*, 351–371.

Skrede, G., Nilsson, A., Baardseth, P., Rosenfeld, H. J., Enersen, G., & Slinde, E. (1997). Evaluation of carrot varieties for production of deep fried carrot chips. III Carotenoids. *Food Research International, 30*, 73–81.

Snodderly, D. M. (1995). Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *American Journal of Clinical Nutrition, 62*, 1448S–1461S.

Stahl, W., Ale-Agha, N., & Polidori, M. C. (2002). Non-antioxidant properties of carotenoids. *Biological Chemistry, 383*(3-4), 553–558.

Stewart, I. (1977). Provitamin A and carotenoid content of citrus juices. *Journal of Agricultural and Food Chemistry, 25*(5), 1132–1137.

Strand, A., Kvernberg, K., Karlsen, A. M., & Liaaen-Jensen, S. (2000). Geometrical *E*/*Z* isomers of (6*R*)- and (6*S*)-neoxanthin and biological implications. *Biochemical Systematics and Ecology, 28*, 443–455.

Tenorio, M. D., Villanueva, M. J., & Sagardoy, M. (2004). Changes in carotenoids and chlorophylls in fresh green asparagus (*Asparagus officinalis* L.) stored under modified atmosphere packaging. *Journal of the Science of Food and agriculture, 84*(4), 357–365.

Weedon, B.C.L., & Moss, G.P. (1995). Structure and nomenclature. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Volume 1A: Isolation and analysis* (pp. 27–70). Basel, Switzerland: Birkhäuser.

Zechmeister, L. (1962). *Cis–trans* isomeric carotenoids, vitamins A and arylpolyenes. Viena, Berlin: Springer Verlag.