Journal of Chromatography A, 1224 (2012) 1–10

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Review

Analytical tools for the analysis of carotenoids in diverse materials

S.M. Rivera, R. Canela-Garayoa*

Departament de Química, Universitat de Lleida, 25198 Lleida, Spain

ARTICLE INFO

Article history:
Received 19 October 2011
Received in revised form
30 November 2011
Accepted 4 December 2011
Available online 13 December 2011

Keywords:
Atmospheric pressure ionization (API)
Carotenoids
High-performance liquid chromatography (HPLC)
Mass spectrometry (MS)
Raman spectroscopy
Ultra high-performance liquid chromatography (UHPLC)

ABSTRACT

High-performance liquid chromatography (HPLC) has become the method of choice for carotenoid analysis. Although a number of normal-phase columns have been reported, reverse-phase columns are the most widely used stationary phases for the analysis of these molecules. C18 and C30 stationary phases have provided good resolution for the separation of geometrical isomers and carotenoids with similar polarity. More recently ultra high-performance liquid chromatography (UHPLC) has been used. UHPLC has a number of distinct advantages over conventional HPLC. These include: faster analyses (due to shorter retention times), narrower peaks (giving increased signal-to-noise ratio) and higher sensitivity. High strength silica (HSS) T3 and C18 and ethylene bridged hybrid (BEH) C18 stationary phases, with sub-2 μm particles have been used successfully for UHPLC analysis and separation of carotenoids. A number of spectroscopic and mass spectrometric techniques have also been used for carotenoid qualitative and quantitative analysis. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI/TOF-MS), atmospheric-pressure solids-analysis probe (ASAP) and Raman spectroscopy are used to profile rapidly and qualitative carotenoids present in different crude extracts. Such detection methods can be used directly for the analysis of samples without the need for sample preparation or chromatographic separation. Consequently, they allow for a fast screen for the detection of multiple analytes. Quantitative carotenoid analysis can be carried out using absorbance or mass detectors. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is efficient for carotenoid identification through the use of transitions for the detection of analytes through precursor and daughter ions. This approach is suitable for the identification of carotenoids with the same molecular mass but different fragmentation patterns. Here we review critically the latest improvements for carotenoid resolution and detection and we discuss a number of analytical techniques for qualitative and quantitative analysis of carotenoids.

Contents

* Corresponding author. Tel.: +34 973 702843; fax: +34 973 238264.
E-mail address: canela@quimica.udl.cat (R. Canela-Garayoa).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.12.025

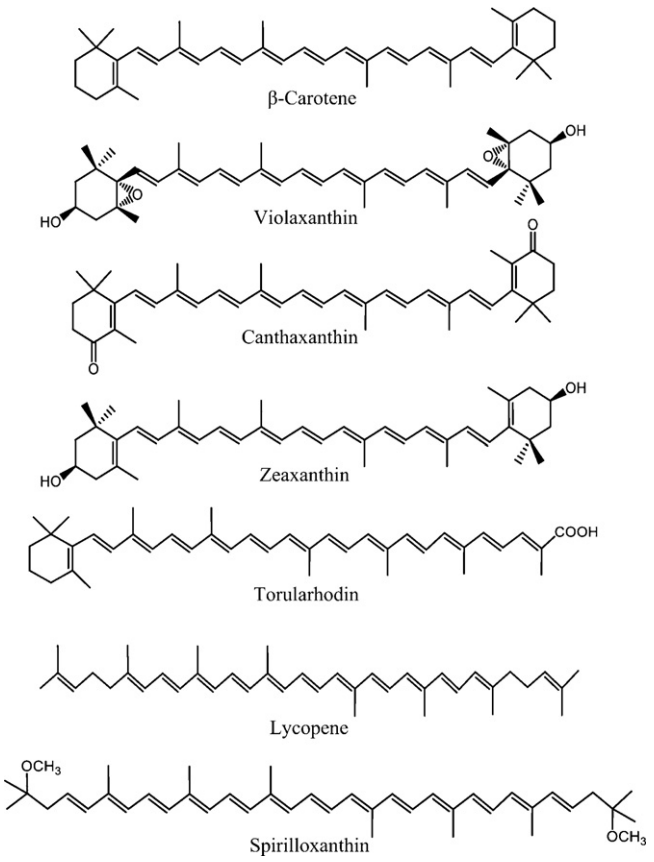

Fig. 1. Lycopene and β-carotene are examples of carotenes while violaxanthin, canthaxanthin, zeaxanthin, torularhodin and spirilloxanthin are examples of xanthophylls.

1. Introduction

Carotenoids are natural pigments synthesized by plants and some microorganisms. Humans and animals are not able to synthesize carotenoids de novo and they need to acquire them through their diet. Carotenoids exhibit yellow, orange and red colors but when they are bound to proteins acquire green, purple or blue colors [1]. They are found in a large number of fruits and vegetables [2], some animal products (eggs, butter, milk) and seafoods (salmon, shrimp, trout, mollusc, etc.) [3].

Carotenoids are classified as (1) carotenes or carotenoid hydrocarbons, composed of only carbon and hydrogen, e.g., lycopene and β-carotene; and (2) xanthophyls or oxygenated carotenoids, which are oxygenated and may contain epoxy, carbonyl, hydroxy, methoxy or carboxylic acid functional groups (Fig. 1). Examples of xanthophylls are violaxanthin (epoxy), canthaxanthin (oxo), zeaxanthin (hydroxy), spirilloxanthin (methoxy) and torularhodin (carboxylic acid)[1] [4].

Carotenoids have received much attention because of their various functions. In animals and humans, these compounds are precursors of vitamin A and retinoid compounds required for morphogenesis [5,6]. In humans, carotenoids contribute to preventing and protecting against serious health disorders such as cancer, heart disease, macular degeneration [7–13]. In plants, they serve as regulators of plant growth and development, as accessory pigments in photosynthesis, as photoprotectors, as precursors for the hormones abscisic acid (ABA) [14] and strigolactones, and as attractants for other organisms, such as pollinating insects and seed-distributing herbivores [15,16]. Furthermore in industry, carotenoids are used in nutrient supplementation, for pharmaceutical purposes, as food colorants and fragrances, and in animal feed[2] [17,18]. Consequently, these pigments have been extensively studied by organic chemists, food chemists, biologists, physiologists, medical doctors and recently also by environmentalists. The widespread interest in carotenoids has led to an increased demand for reliable analytical methodologies for their identification and determination.

The most striking and characteristic feature of the carotenoid structure is the long system of alternating double and single bonds that forms the central part of the molecule. This structure constitutes a conjugated system in which the π-electrons are delocalized along the entire polyene chain. It is this feature that confers carotenoids their unique molecular shape, chemical reactivity, and light-absorbing properties. Based on chemical and physical properties of carotenoids, high-performance liquid chromatography (HPLC) using various absorbance detectors [19,20] has become the most common analytical method for determining carotenoid profiles both qualitatively and quantitatively. However, a number of structurally related molecules coelute. Consequently, their analysis by absorbance is not possible because the ultraviolet–visible (UV–vis) spectra of many carotenoids are similar. Increasing interest in identifying carotenoids directly in the biological matrix (without preliminary sample preparation) has led to the development of other determination techniques for this purpose (e.g., near infrared reflectance spectroscopy (NIRS), Raman spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) [21–24]. These approaches allow a rapid overview of carotenoids while saving on time and cost.

2. High-performance chromatographic analysis

2.1. Separation

Among the high-performance chromatographic methods available, gas chromatography (GC) is unsuitable for the analysis of carotenoids because of the inherent instability and low volatility of these molecules. Therefore, HPLC using absorption and mass detection techniques is currently the most common chromatographic method used for their analysis. Improvements in chromatographic performance using ultra high-performance liquid chromatography (UHPLC) have recently been reported [25–29]. This technique uses narrow-bore columns packed with very small particles (below 2 μm) and mobile phase delivery systems operating at high back-pressures. While in conventional HPLC the maximum back-pressure is in the region of 35–40 MPa depending on the instrument, back-pressures in UHPLC can reach up to 103.5 MPa [30]. Thus, UHPLC offers several advantages over conventional HPLC, such as faster analyses (shorter retention times), narrower peaks (giving increased signal-to-noise ratio) and greater sensitivity [31].

2.2. Analysis of carotenoids by HPLC

Normal- and reversed-phase systems, in isocratic or gradient elution modes, have been used to analyze carotenoids. However,

[1] Spirilloxanthin has been isolated as the major carotenoid from purple phototrophic bacteria such as *Rhodospirillum rubrum*, *Rhodomicrobium vannielii* and *Rhodopseudomonas acidophila*, while torularhodin has been isolated from *Rhodotorula* red yeasts, e.g. *R. mucilaginosa*.

[2] Apocarotenoids are well known as food colourings, such as bixin and crocetin, found in annatto seeds and saffron, respectively. Other compounds derived from the degradation of carotenoids, such as ionones, damascones, and damascenones, are used as fragrances.

Table 1
Comparison of the performance of stationary phases for carotenoid separation and chromatographic systems used for separating *cis*- and *trans*-isomers.

Column type	Particle size	Chromatographic conditions	Matrix	Carotenoids determined	Observations	Ref.
Monomeric C18 Polymeric C18 Polymeric C30	5 μm 5 μm 5 μm	Solvent A: methanol, solvent B: MTBE and solvent C: water. Gradient elution: 81% A, 15% B and 4% C to 6% A, 90% B and 4% C in 90 min; column temperature: 20 °C; flow rate: 1.0 mL/min	Standards	Capsanthin, astaxanthin, lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, echinenone, α-carotene, δ-carotene, lycopene and *trans* and 9-*cis*, 13-*cis*-, and 15-*cis*-β-carotene	A better separation of carotenes and xanthophylls was achieved using the polymeric C30 column	[41]
Monomeric C18 Polymeric C18 Monomeric C30 Polymeric C30 PEAA[a] phase	5 μm 5 μm 5 μm 5 μm 3 μm	Solvent A: methanol/water (92:8, v/v) containing 0.05% ammonium acetate and 0.05% TEA and solvent B: MTBE. Gradient elution: 83% A to 59% A in 29 min, 59% A to 30% A in 5 min; NR[b,c]	Standards	Lutein, zeaxanthin and α-and β-carotene	The PEAA phase showed better separation of carotenoids	[33]
Monomeric C30, non-endcapped; Monomeric C30, endcapped	6 μm 6 μm	Solvent A: acetone and solvent B: water. Isocratic elution: 85% A and 15% B for 30 min; NR[c]; flow rate, 1.0 mL/min	Standards	*trans*-Lutein and 13-*cis*-, 13′-*cis*-, 9-*cis*-, 9′-*cis*- and bi-*cis*-lutein	Non-endcapped phase showed slightly better separation, especially for the bi-*cis* isomers	[42]
Polymeric C18, non-endcapped; Polymeric C18, endcapped[d]; Polymeric C18, endcapped[e]	5 μm 5 μm 5 μm	For xanthophylls: solvent A: methanol. Isocratic elution: 10 min; NR[b]; flow rate: 1.5 mL/min. For carotenes: solvent A: methanol and solvent B: ethyl acetate. Isocratic elution: 90% A and 10% B for 20 min; NR[b]; flow rate: 1.5 mL/min	Standards	Lutein, zeaxanthin, α-and β-carotene and lycopene	Carotenes were affected little by endcapping. In contrast, the separation of lutein and zeaxanthin was influenced by silanol activity	[41]
Monomeric C18 Polymeric C30	4 μm 3 μm	For monomeric C18: solvent A: methanol containing 0,1% TEA, solvent B: ethyl acetate and solvent C: water. Gradient elutiom: 80% A, 10% B and 10% C to 70% A and 30% B in 20 min, column temperature: 29 °C; flow rate: 1 mL/min. For polymeric C30: solvent A: methanol containing 0,1% TEA and solvent B: TBME. Isocratic elution: 50% A and 50% B for 35 min; column temperature: 33 °C; flow rate: 1 mL/min	Standards	All-*trans*-Lycopene and 5-*cis*-, 9-*cis*-, 13-*cis*-lycopene	Polymeric C30 showed better separation for the lycopene isomers	[43]
Silica-based nitrile-bonded phase	5 μm	Solvent A: hexane, solvent B: dichloromethane, solvent C: methanol and solvent D: N, N-diisopropylethylamine. Isocratic elution: 75% A, 25% B, 0.3% C and 0.1% D for 60 min; column temperature: 20 °C; flow rate: 0.7 mL/min	Quail plasma	All-*trans*-Zeaxanthin and 9-*cis*-, 13-*cis*-zeaxanthin, 3′-epilutein, all-*trans*-lutein and 9-*cis*-, 9′-*cis*-, 13-*cis*-, 13′-cis-lutein	This chromatographic system allowed the separation of geometric and positional isomers	[44]
YMC C30	5 μm	Solvent A: methanol/MTBE/water (92:4:4, v/v) and solvent B: MTBE/methanol/water (90:6:4, v/v). Gradient elution: 100% A to 94% A in 80 min; column temperature: 20 °C; flow rate: 1.0 mL/min	Standards, sweet corn and spinach	*trans*-Lutein and 13-*cis*-, 13′-*cis*-, 9-*cis*- and 9′-*cis*-lutein, *trans*-zeaxanthin and 13-*cis* and 9-*cis*-zeaxanthin	This chromatographic system allowed the separation of geometric and positional isomers	[45]
YMC C30	NR[f]	Solvent A: acetone and solvent B: D_2O. Isocratic elution: 95% A y 5% B in 25 min; NR[b]; flow rate: 1 mL/min	Standards	All-*trans*-β-Carotene and 9-*cis*-, 13-*cis*-, 9,13-bi-*cis*-, and 13,15-bi-*cis*-β-carotene	–	[22]

Table 1 (*Continued*)

Column type	Particle size	Chromatographic conditions	Matrix	Carotenoids determined	Observations	Ref.
Vydac C18	5 μm	Solvent A: acetonitrile, solvent B: methanol and solvent C: dichloromethane. Isocratic elution: 80% A, 18% B and 2% C for 12 min; NR[b,c]	Carrot and palm oil	α-Carotene, all-*trans*-β-carotene and 9-*cis*- and 13-*cis*-β-carotene	This chromatographic system allowed the separation of geometric and positional isomers	[46]
YMC C30	3 μm	Solvent A: methanol and solvent B: MTBE. Gradient elution: 95% A to 70% A in 30 min, 70% A to 50% A in 20 min; column temperature: 22 °C; flow rate: 0.9 mL/min	Standards and cashew apple juice	All-*trans*-α-Cryptoxanthin, trans-β-cryptoxanthin and 9-*cis*-, 9′-*cis*-, 13-*cis*-, 13′-*cis*-and 15-*cis*-β-cryptoxanthin	This chromatographic system allowed the separation of geometric and positional isomers	[82]
Beckman Ultrasphere C18 phase	5 μm	Solvent A: dichloromethane/methanol/acetonitrile/water, (5.0:85.0:5.5:4.5, v/v). Isocratic elution; column temperature: 25 °C; flow rate: 1.0 mL/min	*Haematococcus pluvialis*	(3S, 3′S)- and (3R, 3′R)-*trans*-astaxanthin and (3S, 3′S)-9-*cis*-, (3S, 3′S)-13-*cis*- and (3S, 3′S)-15-*cis*-astaxanthin	–	[47]
ProntoSil C30 phase	3 μm	Solvent A: methanol/TBME/water (83:15:2, v/v). Isocratic elution for 20 min; column temperature: 30 °C; flow rate: 1 mL/min	Standards and *Haematococcus pluvialis*	All-*trans*-Astaxanthin and 9-*cis*-, and 13-*cis*-astaxanthin	–	[48]
Polymeric C30	3 μm	Solvent A: methanol and solvent B: TBME. Isocratic elution: 89% A and 11% B for 65 min; column temperature: 23 °C; flow rate: 1 mL/min	Standards	All-*trans*-α-Carotene and 9-*cis*-, 9′-*cis*-, 13-*cis*-, and 13′-*cis*-α-carotene	–	[49]
Polymeric C30	5 μm	Solvent A: 1-butanol, solvent B: acetonitrile and solvent C: dichloromethane. Isocratic elution: 30% A, 70% B and 10% C for 35 min; NR[b]; flow rate: 2.0 mL/min	Standards and tomato	All-*trans*-Lycopene and 5-*cis*-, 9-*cis*-, 13-*cis*-, 15-*cis*- and 4 bi-*cis*-lycopene	–	[50]

[a] Poly(ethylene-co-acrylic acid).
[b] Column temperature not reported.
[c] Flow rate not reported.
[d] Endcapped with trimethylchlorosilane.
[e] Endcapped with hexamethyldisilazan.
[f] Particle size not reported.

most separations of these compounds reported in the literature involve reversed-phase HPLC using C18 and C30 columns [32]. The performance of the columns is dependent on several parameters (alkyl phase length, silanol activity, bonding density, substrate pore diameter, etc.). The combination of these properties must be considered when separating analytes. Table 1 shows how chemical properties of the stationary phases influence the resolution of the carotenoids. Various mixtures of solvents have been used with these reversed-phases, including water, methanol, acetonitrile, 2-propanol, acetone, ethyl acetate, tetrahydrofuran, t-butyl methyl ether (MTBE), dichloromethane and chloroform [2,21]. In general, polymeric C30 phases provide better separations of carotenoid geometric isomers than C18 ones. This finding is attributed to be the enhanced shape selectivity of the former [33–35]. However, C18 stationary phases have also been reported to yield relatively good separations of these isomers; for example, polymeric C18 phases have provided acceptable selectivity for the separation of the geometric isomers of β-carotene, lutein and zeaxanthin [36,37]. C30 phases also provide a satisfactory resolution for carotenoids with similar polarity [37–40]. Table 1 shows the chromatographic systems used for separating *cis*- and *trans*-isomers employing a range of stationary phases. The silanol activity of the stationary phase has been shown to affect carotenoid separations (Table 1). Sander et al. [41] studied the influence of silanol activity on the selectivity of the stationary phase for carotenes (α- and β-carotene and lycopene) and xanthophylls (lutein and zeaxanthin). These carotenoids were separated using endcapped and non-endcapped polymeric C18 phases. Separations of the three non-polar carotenoids were affected little by endcapping. In contrast, the separation of lutein and zeaxanthin, the most polar carotenoids, was improved with the non-endcapped stationary phase with respect to endcapped phases [2,41]. Using endcapped and non-endcapped C30 phases to separate a mixture of lutein isomers, Szabó et al. [42] also described the effect of the silanol groups on carotenoid separation from a mixture of all-*trans*-lutein and 13-*cis*-, 13′-*cis*-, 9-*cis*-, 9′-*cis*- and bi-*cis*-lutein isomers. The non-endcapped phase showed slightly better separation, especially for the bi-*cis* isomers. Furthermore, the chromatographic retention time and the elution order for the isomers differed in non-endcapped and endcapped phases. Similarly, these studies show that the use of non-endcapped phases can be particularly useful for the separation of xanthophylls and geometrical isomers.

2.3. Analysis of carotenoids by UHPLC

UHPLC is a promising tool for carotenoid analysis. So far, high strength silica (HSS) C18 and T3 and ethylene bridged hybrid (BEH) C18 stationary phases have been successfully used to separate

Table 2
Analytical conditions for the separation of carotenoids using UHPLC systems.

Column type	Column characteristic[a] and temperature	Mobile phase	Flow rate (mL/min)	Carotenoids determined	Run time (min)	Ref.
HSS C18	(2.1 mm × 100 mm); 1.8 μm; NR[b]	Solvent A: 10% isopropanol and solvent B: 100% acetonitrile. Gradient elution: 75% B in 3 min, 75% B to 95% B in 0.2 min and 95% B to 100% B in 7.8 min	0.75	Capsanthin, capsorubin, antheraxanthin, zeaxanthin, β-cryptoxanthin and β-carotene	11	[51]
BEH C18	(2.1 mm × 100 mm); 1.7 μm; NR[b]	Solvent A: Millipore water containing 0.1% TFA and solvent B: methanol/acetonitrile/isopropyl alcohol (54:44:2, v/v/v). Gradient elution: 85% B to 95% B in 1 min, 95% B to 99% B in 1 min, 99% B to 99% B in 3 min, and 99% B to 95% B in 1 min	0.6	Lutein, lycopene and β-carotene	6	[52]
BEH C18	(2.1 mm × 100 mm); 1.7 μm; 32 °C	Solvent A: acetonitrile:methanol (70:30, v/v) and solvent B: 100% water. Gradient elution: 85% A in 2 min, 85% A to 100% A in 1 min, 100% A in 8.6 min, 100% A to 85% A in 1 min and 85% A in 2.4 min	0.4 and 0.6	Antheraxanthin, violaxanthin, neoxanthin, astaxanthin, adonixanthin, zeaxanthin, lutein, β-apo-8′-carotenal, 3-hydroxyechinenone, α and β-cryptoxanthin, echinenone, lycopene, β-carotene, phytofluene and phytoene	15	[53]
HSS T3	(2.1 mm × 150 mm); 1.8 μm; 35 °C	Solvent A: acetonitrile: dichloromethane:methanol (75:10:15, v/v/v) and solvent B: water containing 0.05 M acetate ammonium. Gradient elution: 75% A in 20 min, 75% A to 100% A in 1 min, 100% A to 98% A in 9 min, 98% A to end	0.4	Neoxanthin, violaxanthin, lutein 5,6 epoxide, antheraxanthin, lutein, zeaxanthin, β-cryptoxanthin, echinenone, all-*trans*-β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene	46	[54]
BEH C18	(2.1 mm × 50 mm); 1.7 μm; 32 °C	Solvent A: acetonitrile containing 0.1% formic acid and solvent B: TBME. Gradient elution: 2.5% B in 0.1 min, 2.5% B to 7.5% B in 0.3 min, 7.5% B to 10% B in 0.1 min, 10% B to 12.5% in 1 min and 2.5% B in 1.5 min	0.45	β-Cryptoxanthin, lycopene, and β-carotene	3	[55]
HSS T3	(2.1 mm × 100 mm); 1.8 μm; 35 °C	Solvent A: acetonitrile: methanol (85:15, v/v) and solvent B: 2-propanol Gradient elution: 95% A in 0.8 min, 95% A to 50% A in 2 min. At min 3.5 the system is returned to initial conditions and maintained for 1 min	0.5	Lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene and β-carotene	4.5	[56]

[a] Diameter, length and particle size.
[b] Column temperature not reported.

several carotenoids [51–56]. Chauveau-Duriot et al. [54] compared the performance of HPLC (using RP C18 Nucleosil and Vydac TP54 columns) and UHPLC (using a HSS T3 column) systems in forage samples. The UHPLC system gave better quality results for the separation of forage-based carotenoids: 23 chromatographic peaks were fully resolved with UHPLC in contrast to the 12 chromatographic peaks observed using HPLC. In addition, the HSS T3 column allowed the separation of usual coeluting compounds, such as zeaxanthin and lutein, and *cis*- and all-*trans*-isomers of β-carotene. As indicated above, one of the main differences between HPLC and UHPLC columns is the particle size of the stationary phase. While in HPLC particles are over 2 μm, they are below this size in UHPLC. Smaller particles tend to reduce the value of H, which means that the column is more efficient—that is, it provides more theoretical plates per unit length. Moreover, small particles tend to allow solutes to transfer into and out of the particle more quickly because their diffusion path lengths are shorter. Thus, the solute is eluted as a narrow peak because it spends less time in the stationary and stagnant mobile phase where band broadening occurs. The increase in efficiency boosts the resolution parameter (R_s). Consequently, a higher resolution between analytes can be expected. Table 2 shows a summary of the analysis of carotenoids performed to date using UHPLC. With UHPLC columns (HSS C18 and T3 and BEH C18) as well as with HPLC C18 columns, xanthophylls are eluted before carotenes. The order of elution of the xanthophylls depends on the number and type of functional groups present. Thus, carotenoids containing hydroxyl groups elute earlier than those with keto groups (comparing xanthophylls with the same backbone structure). The HSS T3 column could be practical for the separation of xanthophylls since it was designed for greater retention of polar compounds [57]. The retention of acyclic carotenes by the BEH C18 column is determined by their polarities[3] and number of double bonds. For instance, under the chromatographic conditions applied by Rivera et al. [53], the retention times of the acyclic carotenes lycopene (with 13 double bonds, of which 11 are conjugated), phytofluene (with 10 double bonds, of which 5 are conjugated), and phytoene (with 9 double bonds, of which 3 are conjugated), were 7.66, 10.18 and 11.25 min, respectively.

Analysis of carotenoids by UHPLC yields excellent separations for carotenes and xanthophylls. This technique can also greatly reduce the run time, thereby avoiding the risk of degradation, a

[3] Comparison of the polarity among lycopene, phytofluene and phytoene was based on their log *P* values, 14.53, 15.28 and 15.53, respectively. These values were calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994–2011 ACD/Labs).

process caused by the high sensitivity of these compounds to physical and chemical factors.

2.4. Improving the separation of carotenoids in liquid chromatography (LC)

When complex mixtures of carotenoids (regarding the number and diversity of carotenoids which can be found in the samples) need to be separated, the analyst can find problems of coelution in the chromatographic system. For instance, the following compounds can coelute in the same chromatographic peak: neoxanthin and violaxanthin; zeaxanthin and lutein; antheraxanthin and astaxanthin; *cis*- and all-*trans*-isomers of β-carotene and lycopene [53,54]. The separation of these carotenoids could be improved in several ways. One option is to change the column temperature. β-Carotene isomers have been resolved at 11 °C with a non-endcapped polymeric 3-μm C30 column employing a gradient elution and with methanol, TBME and water as mobile phase [35]. Similarly, the separation of lutein and zeaxanthin has been enhanced when a low temperature is applied [58]. A very interesting paper [59] describes the influence of temperature on the retention of several carotenoids using C18, C30 and C34 stationary phases. Another alternative which can be used to improve separation between carotenoids is to decrease the particle size of the stationary phase, as illustrated in Section 2.3. On many occasions, the separation of carotenoid mixtures must be carried out using two separate HPLC columns [13,60].

Chemical reactions can be performed on the carotenoid extract before analysis by LC. These reactions allow confirmation of the presence or absence of a given functional group. Thus, when a carotenoid epoxide coelutes with another that does not hold this functional group, the extract can be treated with acid. This acid can cause the rearrangement of the epoxy group [61]. Therefore, the modification of the carotenoid structure leads to the displacement of the former carotenoid epoxide, which should appear in a new chromatographic region. Thus, it would allow better visualization of the UV–vis spectra of the other carotenoid structure formerly coeluting with it. Table 3 shows the most common chemical reactions carried out for carotenoid identification purposes [61]. Samples obtained after the chemical reaction require treatment before injection into the chromatographic system in order to circumvent incompatibility with the mobile phase or damage to the chromatograph. Thus, for example, acid samples should be neutralized before injection.

3. MS for carotenoid identification

In HPLC, UV–vis instruments are the most common detectors used to identify carotenoids. However, given that the UV–vis spectra of many carotenoids are similar (e.g., α-cryptoxanthin and zeinoxanthin) and a number of structurally related molecules coelute, many researchers have complemented the identification of carotenoids using other detection methods [23,62] (Table 4). Among those, mass detectors have shown great advantages for the analysis of these substances, including the elucidation of their structure on the basis of the molecular mass and their fragmentation pattern. These properties facilitate the quantification of individual carotenoids that coelute. Several ionization methods have been reported for MS analysis of carotenoids, including electron impact (EI), fast atom bombardment (FAB), matrix-assisted laser desorption/ionization (MALDI), electrospray (ESI), atmospheric pressure chemical ionization (APCI) and more recently, atmospheric pressure photoionization (APPI) and atmospheric pressure solids analysis probe (ASAP). Most mass spectra of carotenoids have been acquired using positive ion mode; however, negative ion mode has also been reported [66–68]. An overview of the theory of some of these ionization techniques can be found in the report by van Breemen [68].

3.1. LC–MS

APCI has become the most widely used ionization technique for carotenoids and shows high sensitivity for their analysis [53,69]. APCI has been used to successfully ionize not only xanthophylls and carotenes but also carotenoid esters [70–74], thereby demonstrating the suitability of this approach to ionize carotenoids with different polarities. A highly promising technique to ionize nonpolar compounds, such as carotenoids, is APPI. This method has recently been introduced as a new ionization method for LC–MS and can be considered complementary to the other two atmospheric pressure ionization (API) techniques, namely ESI and APCI. In APPI, the liquid sample is vaporized in a heated nebulizer identical to the one in an APCI source, after which the gaseous analytes are ionized through photo-ionization and gas-phase reactions. The ionization in APPI is initiated by 10 eV photons emitted by a krypton discharge lamp. The photons can ionize molecules that have ionization energies (IEs) below 10 eV. This value includes most analytes, but excludes the solvents generally used in LC, such as methanol, acetonitrile, and water, as well as the gases used in the nebulization or that are otherwise present in the atmospheric pressure ion source [75]. Future research is required to test the effectiveness of this technique to ionize diverse carotenoids.

Although the fragment pattern observed in the carotenoid mass spectra depends on the ionization technique and composition of the mobile phase used, characteristic carotenoid fragments have been observed with various ionization techniques.[4] For example, the ions $[M-92]^{+\bullet}$, $[M-92]^{+}$ and $[M+H-92]^{+}$ correspond to loss of a neutral molecule of toluene and indicate the presence of extensive conjugation within the molecule. These ions have been obtained by using EI [4], ESI [53,68] and APCI [67,70,76]. The ions $[M-106]^{+\bullet}$, $[M-106]^{+}$ and $[M+H-106]^{+}$ are also very characteristic in carotenoids and it is explained by the removal of the xylene molecule from the polyene chain. The ions have been obtained using EI [4,77], ESI [78] and APCI [76]. Losses of 56 and 69 units indicate carotenoids containing a ε-ring and Ψ end group, respectively. The ions $[M-56]^{+\bullet}$, $[M-56]^{+}$ and $[M+H-56]^{+}$ have been achieved by means of EI [4], ESI [68] and APCI [76]. In addition, the ions $[M-69]^{+\bullet}$, $[M-69]^{-}$ and $[M-69]^{+}$ have been described using EI [4,77] and APCI [76,79]. The removal of a hydroxyl group or a molecule of water $[M-17]^{+\bullet}$ or $[M+H-18]^{+}$, respectively, as occurs in carotenoids such as β-cryptoxanthin or lutein, is characteristic of the presence of a hydroxyl group in the compound. These ions have been obtained through EI [4,80], ESI [78] and APCI [67,76]. For carotenoid epoxides, the fragments at m/z 221 and 181 indicate that the epoxy group is in a ring bearing a hydroxy group. Ren and Zhang [81] proposed that the fragment at m/z 221 is produced by cleavage between carbon atoms 10 and 11 of the polyene chain. We hypothesize that the fragment at m/z 181 is produced by cleavage between carbons 8 and 9 of this chain, since this cleavage leads to a monocharged fragment ion of 181 uma. These fragments have been obtained by applying EI [4,77], ESI [78,81] and APCI [76,82]. In addition, carotenoids with a very similar structure can be differentiated through comparison of the intensities of their fragments. Zeinoxanthin and α-cryptoxanthin have the same chemical formula ($C_{40}H_{56}O$) but are distinguishable by the position of the hydroxyl group. In the α-cryptoxanthin, this functional

[4] Polar solvents such as alcohols lead to an increased abundance of protonated carotenoids, and non-polar solvents such as MTBE facilitate the formation of molecular ions.

Table 3
Chemical reactions used for carotenoid identification.

Functional group	Reaction	Procedure	Product	Observations
Primary and secondary alcohol	Acetylation	Dissolve the carotenoid (about 0.1 mg) in 2 mL pyridine and add 0.2 mL acetic anhydride. Leave the reaction mixture in the dark at room temperature for 21 h. Then transfer carotenoid to petroleum ether in a separatory funnel with the addition of water. Wash with water, collect, dry with sodium sulfate and concentrate	Acetylated carotenoid	Less polar carotenoid than the original and unchanged UV–vis spectra
Allylic alcohol	Methylation	Dissolve the carotenoid (about 0.1 mg) in 5 mL methanol. Add a few drops of 0.2 M hydrochloric acid. Allow the reaction to proceed at room temperature in the dark for 3 h. Transfer the carotenoid to petroleum ether	Methylated carotenoid	Less polar carotenoid than the original and unchanged UV–vis spectra
5,6-Epoxide	Epoxide-furanoid rearrangement	Dissolve the carotenoid in ethanol and add a few drops of 0.1 M hydrochloric acid	Formation of 5,8-oxo (3-oxolene)	A hypsochromic shift of 20–25 nm indicates the transformation of a 5,6-epoxide to a 5,8-oxo (3-oxolene)
Carbonyl	Reduction	Dissolve the carotenoid in 95% ethanol and add a few crystals of sodium borohydride. Let the reaction mixture stand for at least 3 h in the refrigerator	Hydroxylated carotenoid	The single broad band of a oxocarotenoid is transformed into the three-peak spectrum of the resulting hydroxycarotenoid
Alkene	Iodine-catalyzed *cis*/*trans* isomerization	Dissolve a few crystals of iodine in petroleum ether. Add a drop of iodine solution to the carotenoid solubilized in petroleum ether. Expose the extract to the light for about 1–5 min	*cis*/*trans*-Isomers	The λ_{max} values of *trans* carotenoids will shift 3-5 nm to a lower wavelength whereas those of *cis* carotenoids will shift by the same amount to longer wavelength

Table 4
Techniques used for analyzing carotenoids.

Analytical technique	Matrix	Carotenoid determined	Ref.
NMR	*Haematococcus pluvialis*	All-*trans*-Astaxanthin and 9-*cis* and 13-*cis*-astaxanthin	[48]
NMR	Guava	Phytofluene, all-β-cryptoxanthin, all-rubixanthin, all-lutein, all-neochrome, all-cryptoflavin, all-*trans*-γ-carotene, 9-*cis*-, 13-*cis*-, 15-*cis*- and all-*trans*-isomers of β-carotene and lycopene	[63]
HPLC-NMR	Sweet corn and spinach	*trans*-Lutein and 13-*cis*-, 13′-*cis*-, 9-*cis*-, 9′-cis- and bi-*cis*-lutein, *trans*-zeaxanthin and 13-*cis* and 9-*cis*-zeaxanthin isomers	[45]
HPLC-NMR	Standards	All-*trans*-β-carotene and 9-*cis*-, 13-cis-, 9,13-bi-*cis*- and 13,15-bi-*cis*-β-carotene	[22]
HPLC-NMR	Spinach and chicken and bovine retina	*trans*-Zeaxanthin and 9-*cis*- and 13-*cis*-zeaxanthin, *trans*-lutein and 9-*cis*-, 9′-*cis*-, 13-*cis*- and 13′-*cis*-lutein	[83]
NIRS	Maize grain	Lutein, zeaxanthin, isolutein, α-cryptoxanthin and β-carotene	[24]
Resonance Raman excitation spectroscopy	Antenna and intact thylakoid membranes of higher plants	Neoxanthin, violaxanthin, lutein, zeaxanthin and β-carotene	[64]
Resonance Raman spectroscopy	Spinach, carrot and tomato	Lutein, β-carotene, and lycopene	[65]
Raman Spectroscopic	Human skin	Lutein, zeaxanthin, *trans* and *cis*-lycopene isomers, α, β, γ and ζ-carotene	[62]
FT–Raman spectroscopy	Red pepper, nectarine, yellow carrot root, pumpkin and corn seed	Capsanthin, β-cryptoxanthin, lutein, β-carotene, zeaxanthin, crocetin and *trans* and *cis*-bixin	[97]
FT–Raman spectroscopy	Carrots	β-Carotene, α-carotene, lutein and lycopene	[99]
APCI-MS/MS	Buriti, mamey, marimari, peach palm, physalis and tucuma	Acyclic, cyclic, hydroxylated, epoxides and oxo carotenoids	[76]
APCI-MS/MS ESI-MS/MS APPI-MS/MS	Standards and transgenic maize seed	Antheraxanthin, violaxanthin, neoxanthin, astaxanthin, adonixanthin, zeaxanthin, lutein, β-apo-8′-carotenal, 3-hydroxyechinenone, α-cryptoxanthin, β-cryptoxanthin, echinenone, lycopene, β-carotene, phytofluene and phytoene	[53]
APCI-MS/MS	Human plasma	*trans* and *cis*-Lycopene isomers	[79]
ASAP-MS	Spinach	Canthaxanthin, β-apo-8′-carotenal and β-carotene	[98]
MALDI-TOF-MS	Genetically modified (GM) tomato varieties	Acyclic, cyclic, hydroxylated and ketolated carotenoids	[93]

group is located in the allylic position of the ε-ring while in zeinoxanthin it is located in the β-ring and thus not in an allylic position. Consequently, zeinoxanthin has been reported to show a more intense protonated molecular ion compared to the fragment with m/z 535, while the opposite is observed for α-cryptoxanthin [76]. Zeinoxanthin and α-cryptoxanthin can be differentiated through comparison of the intensity of the protonated molecule ion (m/z 553) with that of the fragment of 535 $[M+H-18]^+$. The same mass spectrometric behavior has been reported for lutein and zeaxanthin. The structural difference between these molecules is the position of a double bond in one of the ionone rings, which causes lutein to have an allylic hydroxyl group. In lutein, the fragment at m/z 551 $[M+H-18]^+$ is a much more abundant ion than the protonated molecular ion (m/z 569), while zeaxanthin exhibits the opposite behavior [71,76,83]. Similarly, EI-MS has identified some differences between lutein epoxide and *cis*-antheraxanthin. Both spectra showed strong molecular ions at m/z 584, consistent with the molecular formula $C_{40}H_{56}O_3$, as well as fragments at m/z 352, 221, and 181. However, fragments at m/z 566 $[M-18]^+$ and 548 $[M-18-18]^+$ were found in the spectrum of lutein epoxide and not in that corresponding to *cis*-antheraxanthin [84]. Antheraxanthin (with two hydroxyl groups located in β-ring) and lutein epoxide (with one hydroxyl group located in β-ring and another in ε-ring) theoretically may give the ions at m/z 566 $[M-18]^+$ and 548 $[M-18-18]^+$. However, the formation of these ions depends on the type of ionization used, conditions of ionization and stability of the ions formed. The loss of water due to the presence of the hydroxyl group in an allylic position (a hydroxyl group located in ε-ring) produces the $[M-18]^+$ ion, which is stabilized by mesomeric effects. Consequently, this ion is more stable than the ion formed by the loss of water due to the presence of the hydroxyl group, which is not in an allylic position (a hydroxyl group located in β-ring). In the antheraxanthin MS spectra it was observed that *cis*-antheraxanthin did not give the ions at m/z 566 $[M-18]^+$ and 548 $[M-18-18]^+$ [84], which could occur as a result of the low stability of these ions under the specific ionization conditions used in this test. In contrast, because lutein epoxide contains one hydroxyl group in an allylic position, we could observe the ion at m/z 566 $[M-18]^+$ in its MS spectra [84]. In addition, the ion at m/z 548 $[M-18-18]^+$ was observed. Nevertheless, the ion at m/z 566 $[M-18]^+$ was much more abundant than the ion at m/z 548 $[M-18-18]^+$, suggesting that the first loss of water occurs mainly as a result of the presence of the hydroxyl group, located in the ε-ring, which provides a much more stable ion. However, the second loss of water occurs mainly as a result of the presence of the hydroxyl group, located in the β-ring. Thus, although some carotenoids show the same or a very similar fragmentation pattern (meaning that their structures are similar and therefore they might coelute), differences between the intensities of their fragments have been reported. These differences can be used to distinguish the molecules. Moreover, these differences can provide an insight into the predominant carotenoid when coelution occurs. In addition, LC–MS has been used not only to characterize carotenoids but also to quantify them. The latter is possible because of the low detection limits and wide linear dynamic range values exhibited by the mass detectors [67,69,85–88].

3.2. LC–MS/MS

Tandem mass spectrometry (MS/MS) provides many advantages for the analysis of carotenoids. LC–MS/MS offers added selectivity and specificity to the simple LC–MS systems. This more selective detection method reduces interference by impurities in the extract and allows the following: (a) a minimal sample clean-up (leading to a high sample throughput); (b) distinguishing between carotenoids that coelute; (c) information about structural isomers; and (d) a decrease in overall analysis time. Thus, using LC–MS/MS, it is possible to distinguish between lycopene and its structural isomers α-carotene and β-carotene. Fang et al. [79] observed that lycopene, α-carotene, and β-carotene produced molecular ions of m/z 536 during LC–MS analysis using APCI negative ion mode. However, during collision-induced dissociation (CID) only the molecular ion of lycopene formed an abundant and unique fragment ion of m/z 467. This ion corresponds to the loss of a terminal isoprene group $[M-C_5H_9]^-$. Although isomeric with lycopene, α-carotene and β-carotene contain terminal rings instead of acyclic isoprene groups and consequently do not form fragment ions of m/z 467 using APCI negative ion and CID modes. Therefore, the MS/MS transition 536 > 467 was used to quantify the lycopene and distinguish it from its structural isomers. Rivera et al. [53] have also verified the use of transitions for improving the selectivity of carotenoid analysis. They observed that antheraxanthin and astaxanthin coelute under the chromatographic conditions used in the UHPLC analysis. However, these carotenoids were distinguished using the specific transitions found for each carotenoid using APCI. Antheraxanthin was identified using the MS/MS transitions 585.3 > 93.1 and 585.3 > 105.2, while astaxanthin presented the transitions 597.6 > 147.1 and 597.6 > 579.4. Neither compound showed the corresponding transitions of its counterpart species. Thus, the MS/MS transitions allow the individual quantification of these substances in spite of the fact that they show the same chromatographic retention time. MS/MS spectra have also proven especially valuable for confirming the presence of specific components. The analyses can be carried out in complex mixtures without a prior chromatographic separation step, even at trace levels [89].

3.3. Improving carotenoid ionization

Carotenoid analysis is sometimes difficult with soft ionization techniques such as FAB or ESI [89]. These molecules fail to ionize efficiently with these systems. Consequently, the mass spectra present poor structural information with a lack of molecular ions. Moreover, the multiple fragments observed often do not provide any valuable information about the structural characteristic of the compound. However, carotenoid ionization can be improved by adding chemical compounds that facilitate ionization. Examples of such compounds are as follows: (a) ammonium acetate, which has been used to increase the abundance of deprotonated xanthophyll molecules using ESI in negative ion mode; (b) acetic acid, which has been applied to increase the abundance of protonated xanthophyll molecules using ESI in positive ion mode; and (c) halogen-containing eluents, which have been used to increase the molecular ions of xanthophylls and carotenes using ESI in positive ion mode [90].

van Breemen [90] observed that abundant $[M+Na]^+$ ions for xanthophylls, such as astaxanthin, were detected using ESI when sodium acetate was added to the mobile phase. In this case, the protonated or molecular ions were not produced. Similar behavior was found for canthaxanthin using the same ionization system [85]. Therefore some carotenoids can be detected using this adduct since a higher signal is obtained. Nevertheless, this approach cannot be used for carotenes because of the absence of a heteroatom in their structure—heteroatoms, such as oxygen, must be present in the corresponding compound to form adducts with sodium cations.[5] Another type of adducts formed with carotenoids were reported by Rentel et al. [91]. They verified the ionization of several xanthophylls and carotenes as stable silver adducts $[M+Ag]^+$ when an $AgClO_4$ solution was used as a postcolumn additive in LC–MS

[5] Efficient ionization of other low-polar compounds, such as lipids and steroids, using ESI has been done with prior derivatization as their Na^+ and Li^+ adducts via the addition of sodium or lithium salts.

analysis. In their experiments, they also identified the molecular ions $[M]^{+\bullet}$ for these compounds, but these ions were in lower abundance when compared with the silver adducts. In addition, the use of dopants in APPI has contributed to increasing the signal strength of carotenoids. Rivera et al. [53] performed a study on the effect of four dopants on the ionization of 11 xanthophylls and 4 carotenes by APPI. Acetone, toluene, anisole and chlorobenzene (which were introduced in the eluent before introducing the APPI probe) achieved an increase in the signal strength of most of the carotenoids tested. In particular, anisole enhanced the signal strength of phytofluene and phytoene 16- and 178-fold, respectively. Gao et al. [92] produced an interesting review about the additives used in APCI. Although that review was not specific for carotenoids, the authors describe the sensitivity enhancement of various analytes on the basis of the functional group that they bear.

4. Qualitative analysis of carotenoids

A range of qualitative carotenoid analyses can be conducted depending on the aim of the experiment (which also determines the analytical technique to be used). Qualitative analysis of carotenoids can be used for many purposes, among these to: (a) obtain a rapid overview of the carotenoids present in a sample; (b) study carotenoid compositions in their natural environment; (c) study the conformational changes of carotenoids; and (d) classify samples. MALDI/TOF-MS [93], Raman spectroscopy [94], IR spectroscopy [24] and ASAP-MS [95] have been used for this type of analysis. Fraser et al. [93] used MALDI/TOF-MS to differentiate genotypes of genetically modified tomato varieties displaying altered carotenoid contents. They determined the m/z values of about 30 carotenoid standards in order to acquire characteristic fragments for acyclic, cyclic, hydroxylated and ketolated carotenoids. Thus, on the basis of the m/z profiles of the samples, several genotypes were identified. Using MALDI/TOF-MS as a rapid chemical fingerprinting method, large sample populations can be classified on the basis of the traits associated with the presence of one or diverse target metabolites, such as carotenoids. Once the samples have been classified, the most characteristic ones can be selected for further analysis. This approach allows a reduction in the number of samples to be analyzed, thereby facilitating a more comprehensive analysis. A few samples are then chosen to determine their carotenoid composition and content. NMR is used mainly for the assignment of *cis* and *trans* carotenoid isomers [22]; however; it could also be useful as a rapid chemical fingerprinting method for these compounds as it has already been used successfully in metabolome analysis [96]. Raman spectroscopy and ASAP-MS have been applied for direct analysis of carotenoids [97,98]. Fourier transform (FT)–Raman spectroscopy was used for in situ analysis of naturally occurring carotenoids in red pepper, nectarine, yellow carrot root, pumpkin, and corn seed. Carotenoids show strong bands in the Raman spectrum within the 1500–1550 cm^{-1} and 1150–1170 cm^{-1} ranges (due to in-phase C=C (ν_1) and C—C stretching (ν_2) vibrations of the polyene chain),[6] thus allowing the characterization of several of these compounds. Thus, Schulz et al. [97] described characteristic bands of the main carotenoids present in the red pepper (main carotenoid: capsanthin), nectarine (main carotenoid: β-cryptoxanthin), yellow carrot root (main carotenoid: lutein), pumpkin (main carotenoid: β-carotene), and corn (main carotenoid: zeaxanthin). Raman spectroscopy was also useful for the identification of *cis–trans* isomers. When Schulz et al. [97] determined the spectra of *cis* and *trans* bixin, they found that these isomers could be differentiated by the position of the band that appears within 1500–1550 cm^{-1}. Therefore, FT–Raman spectroscopy can be efficiently applied to study the conformational changes of carotenoids during processing and storage.[7] Similarly, Baranska et al. [99] identified several carotenoids (β-carotene, α-carotene, lutein and lycopene) in carrot roots of different color using FT–Raman spectroscopy. In addition, the Raman mapping provided further information on the carotenoid spatial distribution of carotenoids [97,99]. This information allows a semi-quantitative comparison of the presence of carotenoids in specific sample areas. Baranska et al. [99] analyzed carrots of diverse origins to assess the distribution of the main carotenoids occurring in roots of different colors. Using Raman mapping, these researchers found relationships in carotenoid distribution and linked this distribution with root tissues. This new approach can provide valuable information on the spatial differentiation of carotenogenesis and may serve as a basis for further molecular research on gene expression and regulation. Furthermore, ASAP-MS can also be used for the rapid analysis of carotenoids. McEwen et al. [98] described the qualitative detection of carotenoids in spinach leaves by this technique. In ASAP, samples are introduced directly into the mass spectrometer using a sealed melting-point glass capillary ("probe") approximately 10 cm in length. A sample in liquid form can be loaded onto the tip of the probe. The probe is then inserted into the mass spectrometer and the sample is volatilized by heated gas (usually nitrogen). The sample in the gas phase is subsequently ionized via corona discharge at atmospheric pressure using standard voltages for APCI. ASAP can offer a direct (without any sample pre-treatment or chromatographic separation) analysis for several compounds and provides robust data within minutes [95]. However, ASAP is a very recent ionization technique and little information is available about its potential. Nevertheless, it is anticipated that in the future it will be possible to use these types of techniques with accurate mass measurement and mass-selected fragmentation to determine metabolites in complex matrices.

5. Conclusions

Several techniques can be used to improve the separation and detection of carotenoids. HPLC and, more recently, UHPLC are used for their separation. Both chromatographic systems are usually linked to UV–vis detectors and, more recently, to MS and MS/MS detectors. The latter provides more confirmative information, thereby allowing the analysis of coeluting compounds. Qualitative or semi-quantitative analysis can be carried out using a large number of spectroscopic and mass spectrometric methods (MS-TOF, IR, Raman and NMR). This variety of detection techniques has contributed to extending information about carotenoids, such as their distribution in their natural environment and the type of chemical changes they undergo during and after food processing.

Acknowledgements

We thank Dr. P. Christou (PVCF and Institucio Catalana de Recerca i Estudis Avancats, Barcelona, Spain) for critical comments on the manuscript and the Comissionat per a Universitats i Recerca del DIUE de la Generalitat de Catalunya (Barcelona, Spain) and to

[6] These bands are influenced not only by the length of the polyene chain and the molecular structure of the terminal groups of carotenoids but also considerably by their interaction with other plant constituents (proteins, fatty acids, etc.).

[7] Raman spectroscopy can be applied to measure carotenoids *in situ* in skin and in the retina. Macular pigment density of lutein and zeaxanthin can be measured and correlations can be made with carotenoid intake, as a functional indicator of the bioavailability of these two carotenoids. A portable Raman device is available for measuring carotenoids in the stratum corneum layer. It is claimed that this approach allows correlations to be made between tissue carotenoid levels and the risk of degenerative diseases related to oxidation stress, such as cancers and macular degeneration.

the European Social Fund (ESF) for the PhD fellowship of Sol M. Rivera. This work was supported by the University of Lleida, Ministry of Science and Innovation (MICINN), Spain (BFU200761413 and CTQ2009-14699-C02-01) and an ERC Advanced Grant (BIOFORCE) to Paul Christou.

References

[1] G. Britton, R.J. Weesie, D. Askin, J.D. Warburton, L. Gallardo-Guerrero, F.J. Jansen, H.J.M. de Groot, J. Lugtenburg, J.P. Cornard, J.C. Merlin, Pure Appl. Chem. 69 (1997) 2075.
[2] E. Lesellier, C. Tchapla, J. Chromatogr. A 633 (1993) 9.
[3] G. Britton, S. Liaaen-Jensen, H. Pfander, Carotenoids, in: Nutrition and Health, vol. 5, 2009.
[4] G. Britton, S. Liaaen-Jensen, H. Pfander, Carotenoids Handbook, Birkhäuser, 2004.
[5] A. Bendich, J.A. Olson, FASEB J. 3 (1989) 1927.
[6] D. Yuan, L. Bassie, M. Sabalza, B. Miralpeix, S. Dashevskaya, G. Farre, S.M. Rivera, R. Banakar, C. Bai, G. Sanahuja, G. Arjó, E. Avilla, U. Zorrilla-López, N. Ugidos-Damboriena, A. López, D. Almacellas, C. Zhu, T. Capell, G. Hahne, R.M. Twyman, P. Christou, Plant Cell Rep. 30 (2011) 249.
[7] P.D. Fraser, P.M. Bramley, Prog. Lipid Res. 43 (2004) 228.
[8] E. Kotake-Nara, A. Nagao, Mar. Drugs 9 (2011) 1024.
[9] P. Palozza, R.E. Simone, A. Catalano, M.C. Mele, Cancers 3 (2011) 2333.
[10] A.J. Meléndez-Martínez, I.M. Vicario, F.J. Heredia, Arch. Latinoam. Nutr. 54 (2004) 149.
[11] A. Rao, L. Rao, Pharmacol. Res. 55 (2007) 207.
[12] C.E. Scott, A.L. Eldridge, J. Food Comp. Anal. 18 (2005) 551.
[13] C. Zhu, S. Naqvi, J. Breitenbach, G. Sandmann, P. Christou, T. Capell, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 18232.
[14] P.D. Matthews, R. Luo, E.T. Wurtzel, J. Exp. Bot. 54 (2003) 2215.
[15] C. Zhu, C. Bai, G. Sanahuja, D. Yuan, G. Farré, S. Naqvi, L. Shi, T. Capell, P. Christou, Arch. Biochem. Biophys. 504 (2010) 132.
[16] E. Fernández-García, I. Carvajal-Lérida, M. Jarén-Galán, J. Garrido-Fernández, A. Pérez-Gálvez, D. Hornero-Méndez, Food Res. Int. (2011).
[17] G. Britton, S. Liaaen-Jensen, H. Pfander, Carotenoids, in: Natural Functions, vol. 4, 2008.
[18] C. Vílchez, E. Forján, M. Cuaresma, F. Bédmar, I. Garbayo, J.M. Vega, Mar. Drugs 9 (2011) 319.
[19] S. Gueguen, B. Herbeth, G. Siest, P. Leroy, J. Chromatogr. Sci. 40 (2002) 69.
[20] G.P.M. Barreto, J.P. Fabi, V.V. De Rosso, B.R. Cordenunsi, F.M. Lajolo, J.R.O. do Nascimento, A.Z. Mercadante, J. Food Comp. Anal. 24 (2011) 620.
[21] L. Feltl, V. Pacakova, K. Stulik, K. Volka, Curr. Anal. Chem. 1 (2005) 93.
[22] S. Strohschein, M. Pursch, H. Händel, K. Albert, Fresenius J. Anal. Chem. 357 (1997) 498.
[23] Q. Su, K.G. Rowley, N.D.H. Balazs, J. Chromatogr, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 781 (2002) 393.
[24] N. Berardo, O.V. Brenna, A. Amato, P. Valoti, V. Pisacane, M. Motto, Innov. Food Sci. Emerg. Technol. 5 (2004) 393.
[25] M.E. Swartz, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 1253.
[26] S.A.C. Wren, P. Tchelitcheff, J. Pharm. Biomed. Anal. 40 (2006) 571.
[27] S. Chen, A. Kord, J. Chromatogr. A 1216 (2009) 6204.
[28] L. Nováková, H. Vlčková, Anal. Chim. Acta 656 (2009) 8.
[29] M.E. Swartz, Separation Science Redefined (Special Issues of LC–GC N. Am.), 2005.
[30] L. Nováková, L. Matysová, P. Solich, Talanta 68 (2006) 908.
[31] J. Gruz, O. Novák, M. Strnad, Food Chem. 111 (2008) 789.
[32] H.C. Furr, J. Nutr. 134 (2004) 281S.
[33] C.A. Rimmer, L.C. Sander, S.A. Wise, Anal. Bioanal. Chem. 382 (2005) 698.
[34] L.C. Sander, M. Pursch, S.A. Wise, Anal. Chem. 71 (1999) 4821.
[35] J. Breitenbach, G. Braun, S. Steiger, G. Sandmann, J. Chromatogr. A 936 (2001) 59.
[36] D.B. Rodriguez-Amaya, M. Kimura, HarvestPlus handbook for carotenoid analysis, in: HarvestPlus Technical Monograph 2, 2004.
[37] A.J. Meléndez-Martínez, I.M. Vicario, F.J. Heredia, J. Food Comp. Anal. 20 (2007) 638.
[38] J. Jayaraj, Z.K. Punja, Plant Physiol. Biochem. 46 (2008) 875.
[39] P. Crupi, R.A. Milella, D. Antonacci, J. Mass Spectrom. 45 (2010) 971.
[40] A.C.E. Rhodes, J. Plankton Res. 29 (2007) i73.
[41] L.C. Sander, K.E. Sharpless, N.E. Craft, S.A. Wise, Anal. Chem. 66 (1994) 1667.
[42] Z. Szabó, R. Ohmacht, C.W. Huck, W.M. Stöggl, G.K. Bonn, J. Sep. Sci. 28 (2005) 313.
[43] I.L. Nunes, A.Z. Mercadante, Braz. J. Pharm. Sci. 42 (2006) 539.
[44] F. Khachik, F.F. de Moura, D.Y. Zhao, C.P. Aebischer, P.S. Bernstein, Invest. Ophthalmol. Visual Sci. 43 (2002) 3383.
[45] R. Aman, J. Biehl, R. Carle, J. Conrad, U. Beifuss, A. Schieber, Food Chem. 92 (2005) 753.
[46] M.H. Saleh, B. Tan, J. Agric. Food Chem. 39 (1991) 1438.
[47] J.P. Yuan, F. Chen, J. Agric. Food Chem. 46 (1998) 3371.
[48] K. Holtin, M. Kuehnle, J. Rehbein, P. Schuler, G. Nicholson, K. Albert, Anal. Bioanal. Chem. 395 (2009) 1613.
[49] C. Emenhiser, G. Englert, L.C. Sander, B. Ludwig, S.J. Schwartz, J. Chromatogr. A 719 (1996) 333.
[50] M.T. Lee, B.H. Chen, Chromatographia 54 (2001) 613.
[51] I. Guzman, S. Hamby, J. Romero, P.W. Bosland, M.A. O'Connell, Plant Sci. 179 (2010) 49.
[52] P.V. Hung, D.W. Hatcher, Food Chem. 125 (2011) 1510.
[53] S. Rivera, F. Vilaró, R. Canela, Anal. Bioanal. Chem. 400 (2011) 1339.
[54] B. Chauveau-Duriot, M. Doreau, P. Nozière, B. Graulet, Anal. Bioanal. Chem. 397 (2010) 777.
[55] I. Epriliati, G. Kerven, B. D'Arcy, M.J. Gidley, Anal. Methods 2 (2010) 1606.
[56] F. Granado-Lorencio, C. Herrero-Barbudo, I. Blanco-Navarro, B. Pérez-Sacristán, Anal. Bioanal. Chem. 397 (2010) 1389.
[57] P. D. McDonald, D. McCabe, B. A. Alden, N. Lawrence, D. P. Walsh, P. C. Iraneta, E. Grumbach, F. Xia, P. Hong, Waters Whitepaper, Topics in Liquid Chromatography: Part 1. Designing a Reversed-Phase Column for Polar Compound Retention. [Online], http://www.waters.nl/watersdivision/SiteSearch/AppLibDetails.ASP?LIBNUM=720001889EN.
[58] M.G. Sajilata, R.S. Singhal, M.Y. Kamat, Comp. Rev. Food Sci. Food Saf. 7 (2008) 29.
[59] C.M. Bell, L.C. Sander, S.A. Wise, J. Chromatogr. A 757 (1997) 29.
[60] S. Naqvi, C. Zhu, G. Farre, K. Ramessar, L. Bassie, J. Breitenbach, D.P. Conesa, G. Ros, G. Sandmann, T. Capell, P. Christou, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 7762.
[61] D.B. Rodriguez-Amaya, A Guide to Carotenoid Analysis in Foods, International Life Science Institue, 1999.
[62] T.R. Hata, T.A. Scholz, I.V. Ermakov, R.W. McClane, F. Khachik, W. Gellermann, L.K. Pershing, J. Invest. Dermatol. 115 (2000) 441.
[63] A.Z. Mercadante, A. Steck, H. Pfander, J. Agric. Food Chem. 47 (1999) 145.
[64] A.V. Ruban, A.A. Pascal, B. Robert, P. Horton, J. Biol. Chem. 276 (2001) 24862.
[65] P. Bhosale, I.V. Ermakov, M.R. Ermakova, W. Gellermann, P.S. Bernstein, J. Agric. Food Chem. 52 (2004) 3281.
[66] Q. Tian, C.J.G. Duncan, S.J. Schwartz, J. Mass Spectrom. 38 (2003) 990.
[67] R.B. van Breemen, C.R. Huang, Y. Tan, L.C. Sander, A.B. Schilling, J. Mass Spectrom. 31 (1996) 975.
[68] R.B. van Breeman, Current Protocols in Food Analytical Chemistry, John Wiley & Sons Inc., 2001, p. F2.4.
[69] Z. Hao, B. Parker, M. Knapp, L.L. Yu, J. Chromatogr. A 1094 (2005) 83.
[70] T. Řezanka, J. Olšovská, M. Sobotka, K. Sigler, Curr. Anal. Chem. 5 (2009) 1.
[71] C. Kurz, R. Carle, A. Schieber, Food Chem. 110 (2008) 522.
[72] J.D.J. Ornelas-Paz, E.M. Yahia, A. Gardea-Bejar, J. Agric. Food Chem. 55 (2007) 6628.
[73] P. Weller, D.E. Breithaupt, J. Agric. Food Chem. 51 (2003) 7044.
[74] D. Giuffrida, L. La Torre, S. Manuela, T.M. Pellicanò, G. Dugo, Flavour Fragrance J. 21 (2006) 319.
[75] T. Kauppila, Atmospheric pressure photoionization-mass spectrometry (2004). [Online], http://ethesis.helsinki.fi/julkaisut/far/farma/vk/kauppila/atmosphe.pdf.
[76] V.V. De Rosso, A.Z. Mercadante, J. Agric. Food Chem. 55 (2007) 5062.
[77] C.H. Azevedo-Meleiro, D.B. Rodriguez-Amaya, J. Food Comp. Anal. 17 (2004) 385.
[78] V.V. De Rosso, A.Z. Mercadante, J. Agric. Food Chem. 55 (2007) 9135.
[79] L. Fang, N. Pajkovic, Y. Wang, C. Gu, R.B. Van Breemen, Anal. Chem. 75 (2003) 812.
[80] A.J. Meléndez-Martínez, G. Britton, I.M. Vicario, F.J. Heredia, J. Agric. Food Chem. 53 (2005) 6362.
[81] D. Ren, S. Zhang, Food Chem. 106 (2008) 410.
[82] L.Q. Zepka, A.Z. Mercadante, Food Chem. 117 (2009) 28.
[83] M. Dachtler, T. Glaser, K. Kohler, K. Albert, Anal. Chem. 73 (2001) 667.
[84] A.J. Meléndez-Martínez, G. Britton, I.M. Vicario, F.J. Heredia, J. Agric. Food Chem. 53 (2005) 9369.
[85] H. Li, S.T. Tyndale, D.D. Heath, R.J. Letcher, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 816 (2005) 49.
[86] H. Matsumoto, Y. Ikoma, M. Kato, T. Kuniga, N. Nakajima, T. Yoshida, J. Agric. Food Chem. 55 (2007) 2356.
[87] R. Tonhosolo, F.L. D'Alexandri, V.V. de Rosso, M.L. Gazarin, M.Y. Matsumura, V.J. Peres, E.F. Merino, J.M. Carlton, G. Wunderlich, A.Z. Mercadante, E.A. Kimura, A.M. Katzin, J. Biol. Chem. 284 (2009) 9974.
[88] M. Careri, L. Elviri, A. Mangia, J. Chromatogr. A 854 (1999) 233.
[89] F.L. D'Alexandri, R. Tonhosolo, E.A. Kimura, A.M. Katzin, Methods Mol. Biol. 580 (2009) 109.
[90] R.B. van Breemen, Anal. Chem. 67 (1995) 2004.
[91] C. Rentel, S. Strohschein, K. Albert, E. Bayer, Anal. Chem. 70 (1998) 4394.
[92] S. Gao, Z.P. Zhang, H.T. Karnes, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 825 (2005) 98.
[93] P.D. Fraser, E.M.A. Enfissi, M. Goodfellow, T. Eguchi, P.M. Bramley, Plant J. 49 (2007) 552.
[94] J. Lademann, M.C. Meinke, W. Sterry, M.E. Darvin, Exp. Dermatol. 20 (2011) 377.
[95] R.J. Fussell, D. Chan, M. Sharman, TrAC – Trends Anal. Chem. 29 (2010) 1326.
[96] K. Hollywood, D.R. Brison, R. Goodacre, Proteomics 6 (2006) 4716.
[97] H. Schulz, M. Baranska, R. Baranski, Biopolymers 77 (2005) 212.
[98] C.N. McEwen, R.G. McKay, B.S. Larsen, Anal. Chem. 77 (2005) 7826.
[99] M. Baranska, R. Baranski, H. Schulz, T. Nothnagel, Planta 224 (2006) 1028.