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Review

Analytical tools for the analysis of carotenoids in diverse materials

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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

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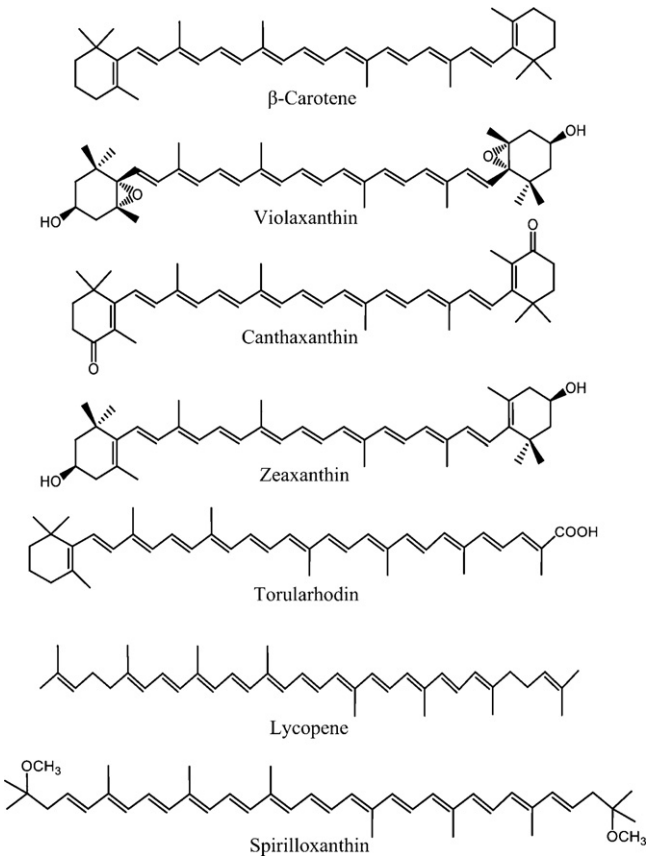

Fig. 1. Lycopene and β-carotene are examples of carotenes while violaxanthin, canthaxanthin, zeaxanthin, torularhodin and spirilloxanthin are examples of xanthophylls.

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.