Trends in Analytical Chemistry 56 (2014) 49–73

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

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

Review

Review of methods for analysis of carotenoids ☆

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ARTICLE INFO

Keywords:
Analytical method
Bioavailability
Carotenoid
Extraction
Food analysis
Food composition
Foodomics
Human sample
Metabolomics
Profiling

ABSTRACT

This review covers current analytical techniques, instruments and methodologies used in the analysis of carotenoids in foods and human samples. We also cover the importance of carotenoids for human health, carotenoid content in foods, bioavailability of carotenoids and evaluation of human intake of carotenoids. There are a wide variety of extraction methods and analytical techniques for determination of carotenoids. Recent advances in analytical instruments and the discovery of unknown metabolites of carotenoids widened the scope of carotenoid studies, especially through the application of metabolomics tools. Omics instruments and statistical methods perform untargeted and targeted profiling of carotenoids in foods and human samples, thus advancing knowledge of the composition of food containing carotenoids and their role in human health. Aimed at collating valuable information about recent analytical methodologies for carotenoids, this review mainly focuses on studies released in the past five years (2009–13).

Contents

1. Introduction

Carotenoids are lipid-soluble pigments responsible for the color of a wide variety of foods. They may be divided into two groups:

- xantophylls, molecules containing oxygen, such as lutein and zeaxanthin; and,

☆ This paper was originally submitted to the recently published Special Issue: 'Modern Food Analysis and Foodomics' (http://www.sciencedirect.com/science/journal/01659936/52).

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http://dx.doi.org/10.1016/j.trac.2013.12.011
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- carotenes, non-oxygenated molecules such as α-carotene and lycopene [1].

Some of them are pro-vitamin A carotenoids, subsequently transformed into vitamin A, which can prevent serious eye diseases, such as night blindness, susceptibility to infection, rough, scaly skin, and retarded tooth and bone development. There are about 700 carotenoids in nature, but only about 50 have pro-vitamin A activity. Of those 50 compounds, we found the three most important precursors of vitamin A in humans to be α-carotene, β-cryptoxanthin and β-carotene, which are the major pro-vitamin A components of most carotenoid-containing foods [2,3].

The consumption of carotenoids, provitamin A or not, has been associated with a number of health benefits, such as cancer chemoprotection [4], prevention of heart and vascular disease [5], and prevention of other chronic diseases (e.g., cataracts and age-related macular degeneration) and degenerative diseases (e.g., Alzheimer's Disease) [6–8].

Almost all carotenoids, to a greater or lesser degree, show scavenging properties against excessive numbers of free radicals that may be produced over the course of a cell's life cycle [9]. This antioxidant capacity has been the most investigated and it has been suggested as the main mechanism of action of the carotenoids. However, other mechanisms have been reported and some of the most exciting progress in understanding the actions of carotenoids have come out of recent investigations on the effect of carotenoids on cellular processes [10] (e.g., gene modulation, cell cycle, cell-cell communication, cytotoxicity and apoptosis) [11–17]. As an example, Teodoro et al. [18] treated human cell lines with lycopene, showing its capacity to inhibit cell proliferation, to arrest cell cycles in different phases and to increase apoptosis, mainly in breast, colon and prostate lines after 96 h of treatment. The authors indicated that the anti-proliferative effect of lycopene depended on cellular type, time and dose.

Some studies have shown that carotenoids may mediate their effects via gap-junction communication (GJC), which allows the direct transmission of ions, small hydrophilic metabolites, and messengers <1–2 kDa in size among neighboring cells. GJC plays an important role in normal development and physiology, and failure of GJC has been related to various human diseases and pathologies [5].

Several studies have highlighted the ability of carotenoids to modulate gene expression [13,18–24]. Genetic variations may be involved in inter-individual variability in carotenoid metabolism and carotenoid status [25], and responsiveness to specific carotenoid diets were associated with individuals with genetic variants of the carotenoid-metabolizing enzyme β-carotene 15, 15′-monooxygenase [26].

Regarding the role of carotenoids in DNA integrity, Azqueta and Collins [27] reviewed the positive effects of vitamin A and pro-vitamin A carotenoids (the carotenes and β-cryptoxanthin) and non-pro-vitamin A carotenoids (lycopene, lutein, astaxanthin and zeaxanthin) on DNA damage. Santocono et al. [9] evaluated the ability of the zeaxanthin, lutein, and astaxanthin to protect SK-N-SH human neuroblastoma cells against DNA damage induced by different RNOSs (reactive nitrogen oxide species) and found that their ability to reduce DNA damage depends on the type of RNOS donors and the carotenoid concentration used. The immune response was reviewed by Hughes [28] and, considering its importance, that needs to be updated.

It is important to highlight that cell-line and animal findings cannot be directly extrapolated to humans and that the definitive scientific evidence will come from human studies. Providing more details about the mechanisms of action of carotenoids and their function on human health was not the aim of this review which can be easily found in the review articles cited above. In the following sections, we review in detail the current analytical methodologies and extraction procedures for determination of carotenoids. Table 1 shows some of the most important carotenoid compounds.

2. Carotenoids in human diet

2.1. Carotenoid content of food

Although carotenoids are present in many common human foods, deeply pigmented fruits, juices and vegetables constitute the major dietary sources with:

- yellow-orange vegetables and fruits providing most of the β-carotene and the α-carotene;
- orange fruits providing α-cryptoxanthin;
- dark green vegetables and egg yolk providing lutein and zeaxanthin and tomatoes and tomato products lycopene [29].

As examples, good sources of β-carotene include collard, turnip, spinach, lettuce, mangos, cantaloupe melons, peppers, pumpkin, carrots and sweet potato. α-Carotene is found in a limited number of orange vegetables, such as carrots, sweet potato, pumpkin, and dark green vegetables, such as broccoli, green beans and spinach. Food sources of α-carotene also tend to be good food sources of β-carotene. Lycopene occurs primarily in tomatoes, as stated before, in watermelon and guava. β-Cryptoxanthin can be found in mandarin, orange, papaya, corn, peas and egg yolks. Good sources of lutein and zeaxanthin are leafy greens, such as spinach, collard and kale, and other foods, such as corn, persimmons, and broccoli.

Carotenoids can also be found in the animal kingdom (bird plumage, fish, crustaceans, and insects), and astaxanthin and cantaxanthin are found in salmon and crustaceans. However, animals, (including humans) cannot synthesize carotenoids, so food is their only source of these compounds [4,30–32].

But, the level of a particular phytochemical, such as carotenoids, varies in different fruits and vegetables, according to the variety cultivated. In addition to dependence on cultivar variation, the content of phytochemical substances is influenced by numerous factors, such as ripening time, genotype, cultivation techniques, and climatic conditions that occur during the pre-harvest period [33,34].

Carotenoid content may vary even according to the part of the plant. For example, the studies performed by Ranveer et al. [35] on tomato revealed that the peel has the highest amount of lycopene, followed by industrial waste, whole tomato and pulp.

Other than pre-harvest factors, various post-harvest steps, including food-processing operations, also have a great influence on the stability of phytochemicals in fruit and vegetables and in their products. Conventional (thermal), modern or non-thermal (e.g., high-pressure processing, pulsed electric field, ultrasound/sonication, ozone and ultraviolet), domestic (e.g., washing, peeling, and cutting) and industrial (e.g., canning, and drying) processing may significantly degrade the level of phytochemicals in food products [36]. Also, the results obtained by some authors suggest that cooking methods may influence the carotenoid content [35].

2.2. Bioaccessibility and bioavailability

Many bioactive compounds (e.g., carotenoids) must be released from the food matrix and reach their site of action to exert their biological effects, so bioaccessibility and bioavailability are critical features in assessing the role of these compounds in human health [37,38]. Bioaccessibility represents the maximum amount of carotenoids released from the food that are available for absorption in the enterocytes [39], while the fraction of the dose entering the