Food Chemistry 122 (2010) 167–172

Contents lists available at ScienceDirect

Food Chemistry

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

Screening of vegetables and fruits from Panama for rich sources of lutein and zeaxanthin

Enrique Murillo [a], Antonio J. Meléndez-Martínez [b,*], Falcón Portugal [a]

[a] Departamento de Bioquímica, Facultad de Ciencias Naturales Exactas y Tecnología, Universidad de Panamá, Panama
[b] Food Colour and Quality Lab., Dept. Nutrition and Food Science, Universidad de Sevilla, Facultad de Farmacia, 41012 Sevilla, Spain

ARTICLE INFO

Article history:
Received 18 November 2009
Received in revised form 5 January 2010
Accepted 15 February 2010

Keywords:
Age-related macular degeneration
Carotenoids
Eye health
Lutein
Phytochemicals
Pigments
Tropical fruits
Xanthophylls
Zeaxanthin

ABSTRACT

In this study we present the results of a screening for good sources of the carotenoid pigments lutein and zeaxanthin, which are related to the eye health. Seventy-four fruits and vegetables available in Panama, including 20 for which there were not previous reports on their carotenoid content have been analysed. The results obtained have revealed the existence of 7 sources with high content (5–20 μg/g) and 8 with very high content (>20 μg/g) of lutein. More importantly, we found 4 sources with high content and 5 with very high content of zeaxanthin, for which only a few good sources were known so far. These results can be useful for the update of carotenoid composition tables, the domestication of some species, the development of ingredients for functional foods, the study of the molecular basis underlying high-zeaxanthin phenotypes, etc.

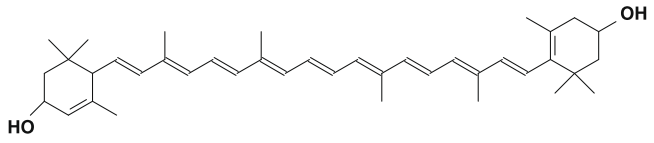
1. Introduction

Carotenoids are isoprenoid compounds that continue to attract the interest of scientists, since their discovery in the 19th century. For many years, the interest in these compounds was mainly due to the fact that they are pigments that account for the colour of many eye-catching structures (fruits, petals, feathers, the exoskeleton of some animals, etc.) and have been consequently attributed importance in relation to, pollination, seed dispersal and signalling in general, food acceptability, etc. However, carotenoids are much more than just pigments. For instance, they play roles of paramount importance in photosynthesis that makes them essential compounds for life as we know it. Moreover, many aroma compounds and the plant hormone, abscisic acid, are formed as a result of the cleavage of carotenoids. Some carotenoids (around 50) are also provitamin A, hence they have been long attributed importance in human nutrition. However, the interest in these compounds from a nutritional standpoint has expanded considerably in the last three decades. This fact has been mainly due to accumulating evidence that these compounds can protect us against the harmful effect of free radicals and have beneficial effects in the prevention and/or alleviation of human disorders, like cardiovascular disease, diverse forms of cancer, eye disorders, light-induced erythemas, etc. (Britton, Liaaen-Jensen, & Pfander, 1995; Krinsky, Mayne, & Sies, 2004; Rodriguez-Amaya, 2001) (Britton, Liaaen-Jensen, & Pfander, 2008).

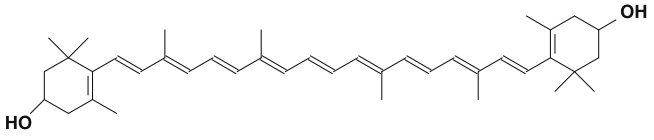
Within the group of carotenoids commonly found in diet of humans, the interest in the possible health benefits associated to lutein (β,ε-carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol) (Fig. 1) has grown considerably during the last years (Granado, Olmedilla, & Blanco, 2003). Although it has been reported that they can act as antioxidant and be beneficial for the prevention of certain cancers and cardiovascular disorders (reviewed in Calvo (2005)), their role in eye health has been better established. Thus, it has been demonstrated that both lutein and zeaxanthin are selectively deposited in the retina and that they prevent against age-related macular degeneration, the main cause of blindness in humans over 60 years old. Likewise, it has been reported that the ratio zeaxanthin/lutein increases towards the centre of the macula, which may indicate that zeaxanthin serves special roles in vision (Krinsky, Landrum, & Bone, 2003). Consequently, the content of both carotenoids have been determined in several common foods and dietary supplements in the current decade (Breithaupt & Schlatterer, 2005; Humphries & Khachik, 2003; Liu, Perera, & Suresh, 2007) and studies on their bioaccessibility and bioavailability have been conducted (Granado-Lorencio et al., 2009; Lakshminarayana, Raju, Krishnakantha, & Baskaran, 2007). It is

* Corresponding author. Tel./fax: +34 954557017.
E-mail address: ajmelendez@us.es (A.J. Meléndez-Martínez).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.02.034



Lutein (β,ε-carotene-3,3'-diol)



Zeaxanthin (β,β-carotene-3,3'-diol)

Fig. 1. Chemical structures of lutein and zeaxanthin.

therefore well-known that green vegetables, pumpkins, other vegetables and egg yolk are rich in lutein, although apart from some genotypes of pepper, the fruits of the Chinese wolfberry *(Lycium barbarum)* and buriti (*Mauritia vinifera*), there are very few known good sources of zeaxanthin (Britton & Khachik, 2009; Inbaraj et al., 2008; Sommerburg, Keunen, Bird, & van Kuijk, 1998).

Panama possesses a great plant biodiversity, which has been however little studied with a view to find new interesting sources of carotenoids and other micronutrients. In this study we present the results of a comprehensive screening for good sources of total carotenoids, lutein and zeaxanthin in which 74 fruits and vegetables available in Panama have been analysed. Amongst the foods studied there are 20 for which there are not previous reports on their carotenoid content to the best of our knowledge. Within this group there are 11 wild fruits little known by most of the population but very consumed by farmers and aborigines.

2. Materials and methods

2.1. Samples

Known carotenoid-containing foods and other fruits and vegetables whose edible portion exhibited greenish, yellowish, orange or reddish colours, and that may therefore be potential sources of these pigments, were chosen for the study. Most of the samples were procured from retailers based on Panama City. Other fruits, such as yellow mombin (*Spondias mombin*), red mamey (*Pouteria sapota*), purple mombin (*Spondias purpurea*), "guanabana toreta" (*Annona purpurea*), Chinese passion fruit (*Cionosicyos macranthus*), hill cherry (*Bunchosia nitida*), corozo (*Aiphanes aculeata*), membrillo (*Gustavia superba*), Chinese rose (*Pereskia bleo*), canistel (*Pouteria campechiana*), sastra (*Garcinia intermedia*), black palm (*Astrocaryum standleyaum*) and nance (*Birsominia crassiflora*), were collected directly from the trees. Four samples of every fruit or vegetable were analysed. The samples were collected at different times during the period February 2004 through May 2006.

2.2. Extraction and saponification of carotenoids

The carotenoid pigments were extracted and saponified according to recommended procedures (Rodriguez-Amaya, 2001). Depending on their colour intensity, between 2 and 10 grams of the edible portion of the samples were accurately weighed, and then mixed with 0.2–1.0 g of sodium bicarbonate and extracted to colour exhaustion with acetone. The acetonic extract (25 ml) was concentrated to dryness (in a rotary evaporator at temperature below 40 °C) and then equal volumes of a mixture of ether and hexane (1:1) and water were added to remove traces of acetone, which may promote the formation of artifacts of carotenoids with carbonylic groups in alkaline conditions. The epiphase was concentrated to dryness (in a rotary evaporator at temperature below 40 °C) and the residue re-dissolved in equal volumes of diethyl ether and methanolic KOH (5%). The saponification was carried out under an atmosphere of nitrogen for 2 h. Finally, the mixture was washed several times with saline to remove all the alkali and was split into two aliquots. One of the aliquots was used for the spectrophotometric estimation of the total carotenoids and the other for the HPLC analysis.

2.3. Estimation of the total carotenoid content

Orientative assessments of the total carotenoid content of the samples analysed were carried out by spectrophotometry as recommended anywhere else (Rodriguez-Amaya, 2001). In brief, one of the saponified aliquots was dried out and re-dissolved in an appropriate volume of hexane depending on its colour intensity, after which the absorbance reading at 450 nm was taken. The total carotenoid content was expressed as β-carotene equivalents.

2.4. HPLC

A model 1050 Hewlett Packard HPLC system fitted with a quaternary pump, an automated injector and a diode-array detector was used for the HPLC analyses. The chromatograms were registered using ChemStation software (Agilent Technologies, Palo Alto, CA). A Spherisorb ODS2 column (5 μm, 250 × 4.6 mm) was used as stationary phase. The compounds were elute isocratically using the mixture acetonitrile:dichloromethane:methanol (82:13:5) at 1.5 ml/min. The volume of injection was 30 μl. Lutein and zeaxanthin were identified by comparison of their retention times and online UV–Vis spectra with those of standards.

2.5. Lutein and zeaxanthin standards

The lutein standard was isolated from spinach leaves (*Spinacea oleracea*) and the zeaxanthin standard from orange peppers (*Capsicum annuum*) by column chromatography according to Rodriguez-Amaya (2001). Their identity was confirmed by the study of their UV–Vis spectra in methanol (recorded on a UV-1203 Shimatzu spectrophotometer) and by HPLC–APCI-MS, using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) coupled with a JEOL MS LCmate detector (JEOL Ltd., Peabody, MA).

The quantification of lutein and zeaxanthin in the samples was carried out by external calibration. For this purpose dose–response curves were obtained from the standards according to recommended procedures (Rodriguez-Amaya, 2001).

3. Results and discussion

3.1. Separation, identification and quantification of carotenoids

Lutein and zeaxanthin are dihydroxycarotenoids differing in the position of one of their endocyclic double bonds (Fig. 1). Their polarity is therefore very similar, which makes difficult their baseline separation with certain stationary phases and under certain chromatographic conditions. Although the development of new stationary phases made possible the baseline separation of both pigments, their quantification was impaired for many years to the extent that in some composition tables the levels of both carotenoids are expressed as lutein or lutein + zeaxanthin (E-Siong, Ah-Heng, & Swan-Choo, 1995; Holden et al., 1999; O'Neill et al., 2001), but not individually.

Fig. 2 depicts a chromatogram corresponding to a carotenoid extract from the tree tomato. This chromatogram serves to illus-