

SCIENTIFIC, N°2, 2017

NEWSLETTER



**EUROCAROTEN**

EUROPEAN NETWORK TO ADVANCE CAROTENOID RESEARCH AND APPLICATIONS IN AGRO-FOOD AND HEALTH

## CAROTENOIDS: CONTENT IN FOODS, IN DIET AND BIOAVAILABILITY

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Olmedilla-Alonso, B. (2017). Carotenoids: content in foods, in diet and bioavailability. COST Action EUROCAROTEN (CA15136) Scientific Newsletter 2, 1-9.

Carotenoids are considered the most widely distributed pigments in nature<sup>1</sup>. They are mainly present in vegetables and fruits and are also used as food additives. More than 700 different carotenoids have been identified so far<sup>2</sup> and although about 40 carotenoids are provided by the diet<sup>3</sup>, only six are commonly analysed in foods and blood: Three carotenes ( $\beta$ -carotene,  $\alpha$ -carotene, lycopene, which are hydrocarbon compounds) and three xanthophylls ( $\beta$ -cryptoxanthin, lutein, zeaxanthin, which are oxygenated forms).

In addition to their properties as yellowish-red colorants, carotenoids possess important physiological actions, the most widely known is the provitamin A activity, which some of them have. Vitamin A is essential for normal vision, gene expression, embryonic development, immunological functions and the control of metabolic processes. Vitamin A deficiency is one of the major micronutrient deficiencies worldwide. Since vitamin A is not synthesized by the human body, it needs to be obtained through the diet, either preformed as retinol (only obtained from animal food sources) or via conversion of provitamin A carotenoids (mainly present in plants, and used as food additives). These include  $\beta$ -carotene,  $\alpha$ -carotene and  $\beta$ -cryptoxanthin<sup>4,5</sup>.

Apart from the provitamin A activity exerted by some carotenoids, there is extensive evidence that this group of compounds influences diverse molecular and cellular processes, which can provide the basis for their role in the risk reduction of several chronic diseases. A diet rich in carotenoids is frequently associated with a lower risk of developing of certain types of cancer, cardiovascular

disease, type 2 diabetes, age-related macular degeneration (AMD) and cataracts, among others, and even mortality caused by some of these serious diseases<sup>6-9</sup>. Thus, in the context of the relationship between diet / nutrition and health or disease in humans (**Figure 1**)<sup>10</sup>, it is important to know about the carotenoid food sources and individual carotenoid intake, but also about the bioavailability i.e. how much of that intake is absorbed.

### Carotenoid food sources

The most important sources of carotenoids in the human diet are vegetables and fruits. Carotenoids are especially abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables. Also animal-derived food products contribute to some extent and e.g. dairy products, eggs and some fish and seafood can have a significant carotenoid content. Synthetic and natural carotenoids and carotenoid-rich extracts are also widely used as natural colorants in manufactured food<sup>11</sup>.

Food carotenoid content is compiled in food composition tables (FCT) and databases (FCDB), and generally includes carotenoids such as  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lycopene and lutein (i.e. references 12- 18

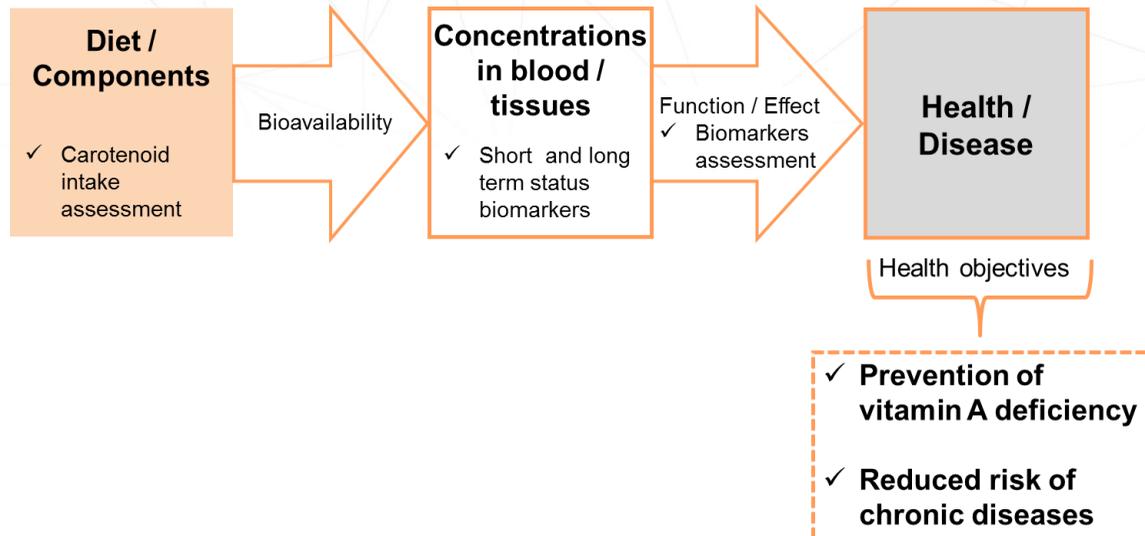


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**Figure 1.** Relationship between diet / nutrition and health / disease (adapted from Olmedilla Alonso (2007)<sup>10</sup>).

from US, Austria, at a European level, Brazil, Spain, Switzerland). In the majority of the FCTs, lutein may include also zeaxanthin content since these two carotenoids were not necessarily separated by all the analytical methods employed until recently. In some cases, FCT and FCDB also include others less extensively studied carotenoids like neoxanthin, violaxanthin, phytoene or phytofluene<sup>16,19</sup>.

The composition and content of carotenoids in foods are affected by several factors, e.g. variety, genotype, season, geographic location/climate, stage of maturity and growing conditions of the particular plant species. Moreover, cultivation practices and methods of cooking and processing food vary widely around the world, and can have a profound effect on the stability and therefore the content of carotenoids. These aspects are well reviewed in Maiani *et al.* (2009)<sup>6</sup>.

In general, the source of the variability observed in the data on the content of carotenoids in foods includes aspects related to the representativeness of the sample, the identification of the food (scientific name, variety, part of the plant consumed, etc.), form in which the food is consumed and the extraction method (e.g., with or

without saponification) and analysis tools (HPLC, column chromatography, spectrophotometry). These aspects can result in databases that provide an over- or underestimation of the carotenoid content of certain foods<sup>20, 21</sup>. Thus, the carotenoid intake and the relative contribution of provitamin A carotenoids to the vitamin A intake, based on consumption among the general population, can vary widely depending on the food composition table used. Thus, to easily identify good food sources of the dietary carotenoids  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, lycopene and zeaxanthin, by providing an indication of the likely carotenoid content, Britton and Khachik (2009)<sup>11</sup> proposed a classification of the carotenoid content in fruits and vegetables according to the following levels:

- Low: 0 – 0.1 mg/100 g
- Moderate: 0.1 – 0.5 mg/100 g
- High: 0.5 - 2 mg/100 g
- Very high: > 2 mg/100 g

Hence, as an example, foods identified with a high, high-very high content of a given carotenoid are shown in **Table 1**.

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**Table 1.** Examples of foods with a high, high-very high content of a given carotenoid (adapted from Britton *et al.*<sup>11</sup>).

High content	High-very high content
<b>β-carotene</b>	
Brussels sprouts ( <i>Brassica oleracea</i> (Gemmera)) "Karat" banana ( <i>Musa troglodytarum</i> ) Peach ( <i>Prunus persica</i> ) Pepper (red, orange, green) ( <i>Capsicum annuum</i> ) West Indian cherry ( <i>Malpighia glabra</i> )	Apricot ( <i>Prunus armeniaca</i> ) Broccoli ( <i>Brassica oleracea</i> (Italica)) "Buriti" ( <i>Mauritia vinifera</i> ) Carrot ( <i>Daucus carota</i> ) "Gac" oil ( <i>Momordica cochinchinnensis</i> ) Kale ( <i>Brassica oleracea</i> (Acephala)) Mango ( <i>Mangifera indica</i> ) Red palm oil ( <i>Elaeis guineensis</i> ) Spinach ( <i>Spinacia oleracea</i> ) Sweet potato ( <i>Ipomoea batatas</i> ) Tomato ( <i>Lycopersicon esculentum</i> ), "high-beta"
<b>β-cryptoxanthin</b>	
Persimmon ( <i>Diospyros kaki</i> ) Pitanga ( <i>Eugenia uniflora</i> )	
<b>Lutein</b>	
Broccoli ( <i>Brassica oleracea</i> (Italica)) Green leafy vegetables Pepper (yellow, green) ( <i>Capsicum annuum</i> )	
<b>Zeaxanthin</b>	
"Buriti" ( <i>Mauritia vinifera</i> ) Chinese wolfberry ( <i>Lycium chinensis</i> ) Pepper (orange, red) ( <i>Capsicum annuum</i> )	
<b>Lycopene</b>	
Carrot (red) ( <i>Daucus carota</i> ) Guava ( <i>Psidium guajava</i> ) Tomato ( <i>Lycopersicon esculentum</i> ) Water melon ( <i>Citrullus lanatus</i> )	

## Carotenoid intake

The contribution of each of the food sources of dietary carotenoids will depend not only on the carotenoid concentration in the food, but on the frequency with which it is consumed. The estimated intakes of individual

dietary carotenoids vary widely on an individual, regional and national level, and significant seasonal variations have also been reported in some countries (e.g. Spain)<sup>22</sup>. Carotenoid intake assessment, at both the individual and group level, has been shown to be complicated mainly due to the high variability within-subject and between-subject intakes, inaccuracies associated with methods of dietary assessments, and

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inconsistencies in FCT and FCDB<sup>6</sup>. The different intake levels found in the literature are largely attributable to the differences among databases for carotenoid composition in foods and the types of dietary questionnaires employed<sup>22</sup>. Food frequency questionnaires (FFQ) have been reported to overestimate carotenoid intake<sup>23</sup>, especially that of lutein and zeaxanthin when comparing with 3-day food records<sup>22</sup>.

Few studies have been carried out to ascertain the total intakes of carotenoids in the European diet. This information, along with the major foods contributing to carotenoid intake in adults in five European countries, is included in the review from Maiani *et al* (2009)<sup>6</sup>. The relative contribution of each carotenoid intake to total carotenoid intake according to food balance sheet data from several European countries is shown in **Table 2**. Lutein, zeaxanthin and  $\beta$ -carotene are those most frequently found in European diets. However, when the relative contribution of each carotenoid intake to total carotenoid intake in five European countries is assessed

using the same dietary method and database,  $\alpha$ -carotene and  $\beta$ -carotene show a consistent contribution in the European countries (3- 9 % and 31- 39 %, respectively), regardless of the dietary habits and geographical origin of the groups assessed. On the contrary, for lutein and lycopene, a different contribution pattern is observed between Spain (37 and 17 %, respectively) and the rest of the European countries (11- 16 % and 30-35 %, respectively). Regarding  $\beta$ -cryptoxanthin, a clearly distinct relevance is observed with Spain showing two to three-fold greater contribution than others, especially northern European countries<sup>6</sup>. Lutein intake is higher than that of zeaxanthin in all populations<sup>7, 24</sup>; in a typical western diet, a ratio of 7:1 (lutein:zeaxanthin) has been reported<sup>25</sup>.

The carotenoid intakes in populations from Spain, the United States and Brazil are shown in **Table 3**, using data on food consumption obtained from Dietary Intake Surveys<sup>26-29</sup>. Dietary intake of provitamin A carotenoids was higher in Spain<sup>26</sup> than in Brazil<sup>28</sup> (1.79 vs 1.24

**Table 2.** Relative contribution (%) of each carotenoid intake to the total carotenoid intake according to food balance sheet data (Maiani *et al.* (2009)<sup>6</sup>, Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).

Country	Total intake* µg/day	Lutein (+ zeaxanthin), %	$\beta$ - cryptoxanthin, %	Lycopene, %	$\alpha$ - carotene, %	$\beta$ - carotene, %
Germany	9.4	52	3	8	3	33
Denmark	10.1	52	4	7	3	34
Italy	15.8	45	4	15	3	33
Sweden	7.5	48	5	11	3	32
UK	8.7	50	4	9	3	33
Greece	21.0	40	3	21	4	32
France	14.0	50	4	9	3	34
The Netherlands	8.8	48	5	10	3	33
Spain	12.8	45	4	14	3	34
Europe	11.8	48	4	12	3	33

\*Sum of lutein (zeaxanthin),  $\beta$ -cryptoxanthin, lycopene,  $\alpha$ -carotene and  $\beta$ -carotene.

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**Table 3.** Carotenoid intake (mg/person/day) from fruit and vegetables (F, V) from national surveys.

	SPAIN (2009-2010) <sup>26,29</sup>	USA (2009-2010) <sup>27</sup>	BRAZIL (2008-2010) <sup>28</sup>
<b>Lutein &amp; zeaxanthin</b>	0.84	1.4	0.83
<b>Lycopene</b>	3.0	5.1	0.66
<b>β-carotene</b>	1.31	1.9	0.92
<b>α-carotene</b>	0.27	0.4	0.16
<b>β-cryptoxanthin</b>	0.22	0.2	0.16
<b>Provitamin A carotenoids</b>	1.79	2.5	1.24
<b>Provitamin A, carotenoids supplied by F + V (% RDA for vitamin A, RE)</b>	32.2 <sup>a</sup>	45.3	22.1 <sup>b</sup>

<sup>a</sup>3.9% from fruits and 28.3% from vegetables.

<sup>b</sup>4.8% from fruits and 17.4% from vegetables

RDA: Recommended Dietary Allowance<sup>5</sup> (using 800 µg RE as mean for men and women).

mg/person/day), due to higher intakes of all the provitamin A carotenoids. The intakes of lutein plus zeaxanthin were similar but that of lycopene was higher in Spain<sup>30</sup> than in Brazil<sup>28</sup>. In the US population<sup>27</sup>, the intake of every carotenoid was around twice that of the consumption reported in Brazil. The United States National Health and Nutrition Examination Survey (NHANES) dietary intake was estimated in adults (over 20 y) by means of two 24-h recall registers<sup>27</sup>, and the differences in the food carotenoid composition cannot justify the wide differences in carotenoid intake in the two populations. However, the difference in lycopene intake reported compared to the findings in Spain<sup>29</sup> and the US<sup>27</sup> was related to the food items considered, i.e. only raw fruits and vegetables (tomato, papaya, watermelon and raw salad and fruit salad) were considered in Brazil, whereas the whole diet, including a large number of processed tomato products and manufactured foods that contain lycopene, in general, in higher amounts than those found in fresh foods was considered for the lycopene intake assessment in Spain and in USA<sup>27</sup>.

In the Spanish population, the proportion of vitamin A intake contributed by provitamin A carotenoids is 42 % and the most abundant provitamin A carotenoid is β-carotene. Provitamin A carotenoids are provided mainly by vegetables and fruits (74 and 14 %, respectively) and by orange and tomato juices<sup>26</sup>. This dietary carotenoid intake in Spain can be compared with the intakes in the US<sup>27</sup> and Brazil<sup>28</sup>, also obtained from representative samples of the population during the same or similar periods of time, and considering data on the carotenoid contents of foods generated by HPLC. The highest contribution from fruit and vegetable intake was observed in the US, followed by Spain and Brazil. However, these food intakes were not completely comparable, as the estimates for Spain and the US included raw and processed foods (for example, oranges, tomato sources) and the data for Brazil was based only on raw fruits and vegetables. In addition, consumption in Brazil was underestimated, as the survey was limited to commonly consumed foods, and fruits and vegetables from the native biodiversity that are consumed in different regions of Brazil were not

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included. In any case, in all three countries, the amount of provitamin A carotenoids supplied by fruits is lower than that of vegetables. The highest intake was that of  $\beta$ -carotene, which, in the three countries, was about two and a half times higher than the intake of  $\alpha$ -carotene plus  $\beta$ -cryptoxanthin.

The results of dietary carotenoid intake can be interpreted on the basis of the contribution of some of them to the vitamin A intake or regarding the effects of individual carotenoids. In general, the intake of carotenoids have been associated with the incidence of certain chronic diseases (e.g., lutein and zeaxanthin with ocular diseases such as cataracts and AMD; lycopene with prostate cancer;  $\beta$ -cryptoxanthin with an increase in bone mass). The individual assessment of each individual carotenoid makes it easier to perform a proper study of the relationship between carotenoid intake and the different situations of health or disease; something of great importance since, as was observed in the latest survey on nutrition and health in the U.S., each of them has different effects in relation to the causes of mortality and, in addition, are seen to interact <sup>7</sup>.

For a proper assessment of the contribution of carotenoids to vitamin A intake, it is necessary to consider not only the provitamin carotenoid concentration in the foods, but also the bioavailability and the capacity to be converted into retinol<sup>6, 30</sup>.

However, these aspects have not yet been included in the FCT, although they are aspects taken into account in the formulas utilized to assess the contribution of each food to vitamin A intake, as retinol equivalents (RE) or as retinol activity equivalents (RAE).

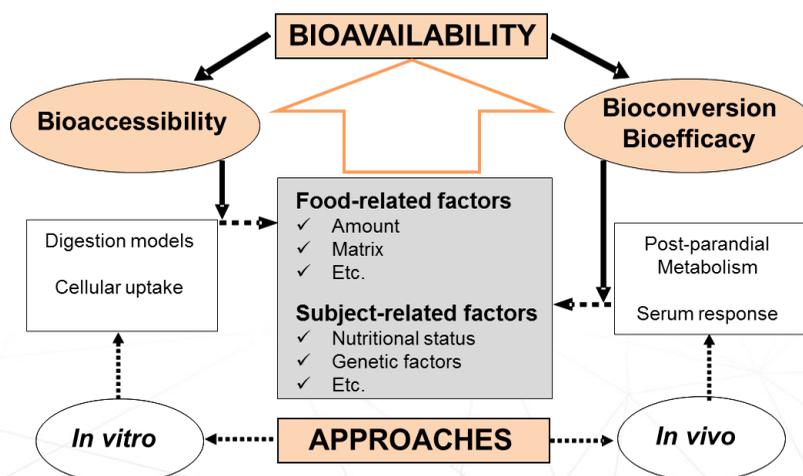
$$RE = \text{retinol} + (\beta\text{-carotene}/6) + (\alpha\text{-carotene}/12) + (\beta\text{-cryptoxanthin}/12)^{31, 32}$$

$$RAE = \text{retinol} + (\beta\text{-carotene}/12) + (\alpha\text{-carotene}/24) + (\beta\text{-cryptoxanthin}/24)^5.$$

However, these equal contributions of  $\alpha$ -carotene and  $\beta$ -cryptoxanthin (which, in turn, are half the bioconversion factor of  $\beta$ -carotene) to the vitamin A intake expressed as RAE are being questioned<sup>30, 33</sup>.

## Bioavailability

Given the importance of carotenoids and their role in a variety of biological activities, it is of great interest to know not only the amounts consumed but to what extent they are absorbed from the different dietary sources and thus, bioavailability is a key factor in the relationship among intake, absorption and utilization<sup>34</sup> (**Figure 2**). Bioavailability is defined as the fraction of carotenoids that is absorbed and available for utilization in normal



**Figure 2.** General approach to study carotenoid bioavailability<sup>34</sup>.

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physiological functions or for storage and it is influenced by a combination of dietary and physiological factors. Thus, bioavailability can be broken down to encompass other terms such as bioaccessibility, which is the amount of carotenoids released from the food matrix during digestion and made available for absorption.

Bioaccessibility is studied by means of *in vitro* methodologies (including *in vitro* digestion, cellular and *in silico* models) and supplies information regarding the effect of dietary factors such as the influence of the food matrix, chemical state of the components, amount ingested, the species of carotenoids, interactions, and absorption modifiers. Although the information provided by bioaccessibility is incomplete, as it does not include data on host-related factors, the *in vitro* models are simple, low cost and reproducible tools for the studies of stability, digestive micellization and intestinal transport, and help to predict, at least to some extent, the bioavailability of food components.

*In vitro* methods attempt to mimic physiological conditions *in vivo*, simulating digestion processes, and typically include the oral, gastric and small intestine phases, and, occasionally, large bowel fermentation. The majority of the methods reported in the literature are static and, although they all take into account e.g. the presence of digestive enzymes, pH, digestion time and salt concentrations, the models vary widely in the use of the parameters making it quite difficult to compare results<sup>33-35</sup>.

The host related factors i.e. nutrient status of the host, genetic factors and interactions are studied *in vivo* (in animals and humans), making possible to evaluate bioconversion i.e. the proportion of absorbed carotenoids that are converted to retinol. Thus, a bioconversion rate of 100% would mean that all the absorbed  $\beta$ -carotene is converted to retinal and then reduced to retinol. Bioefficacy, combines absorption and bioconversion, and at present, a bioefficacy of 100% consider that 1  $\mu$ mol of dietary  $\beta$ -carotene results in 2  $\mu$ mol of retinol. With the provitamin A carotenoids, it is of great interest not only to know the amount absorbed but also to what extent they are converted into retinol, not only in the gut but also in other tissues. In addition, it is important to learn, to what extent each carotenoid and the form in which it is present in the food matrix, is a substrate for different enzymes.

The term bioavailability usually refers to *in vivo* studies, in animals and in humans, the gold standard being the studies in humans. From these, those most frequently used are short-term interventions i.e., single-dose, pharmacokinetic studies and long-term interventions involving multiple doses. Moreover, the use of radioactive and stable isotopes is of great interest for a better understanding of the bioconversion of provitamin A carotenoids.

In human studies, for a reliable and precise information on the host related factors and to allow the comparison among studies, different aspects regarding the subjects, methods, biomarkers and statistical analysis are crucial and need to be properly documented. Information should be included on all the variables known to have a possible influence on the metabolism of the compound being studied (e.g. as inclusion criteria or as variables that could be used in the statistical analysis of the results).

The identification of the major common sources of the dietary carotenoids and their intake in different groups of population can be useful for decisions related to the public health settings regarding both nutritional aspects and prevention of chronic diseases (**Figure 1**). In addition, for an accurate assessment of vitamin A intake as well as of other non-provitamin A carotenoids, there is also a need for information on their bioavailability and capacity of conversion of the carotenoids consumed as it varies depending on the type of carotenoid and food matrix.

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*When referring to this article, please use:*

Olmedilla-Alonso, B. (2017). Carotenoids: content in foods, in diet and bioavailability. COST Action EUROCAROTEN (CA15136) Scientific Newsletter 2, 1-9.

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