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Review

Carotenoids bioavailability from foods: From plant pigments to efficient biological activities

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ABSTRACT

Carotenoid pigments are a group of bioactive compounds that are of interest to the food scientists, nutritionists and food industries due to their positive impact on human health and their economic benefits. Carotenoids are responsible for the attractive colour of many plant food (mainly fruit and vegetables), which is perhaps the first attribute that consumers assess when determining the quality and appearance of a product, and therefore conditions its acceptability. In addition, carotenoids have diverse biological functions and activities, such as the well known provitamin A activity, antioxidant capacity and enhancement of the immune system. There are an extensive number of factors affecting the efficient incorporation of these phytochemicals from the diet, although in many cases no biological activity will be put in action within the consumer body (animal or human) without a first visual attraction. The term bioaccessibility is used to evaluate the amount of a nutrient that is released from a food during the digestion process. The bioaccessibility of lipophilic compounds, such as carotenoids, in natural foods (mainly fruits and vegetables) is usually fairly low and is constrained by various factors, particularly the degree of food processing and matrix composition. There are evidences that homogenisation and thermal treatment have positive effects on the bioaccessibility of these compounds, whereas the presence of dietary fibre has a negative effect. The presence and co-ingestion of fat in the diet are a key factor, with a minimum quantity needed to facilitate carotenoid absorption, and this seems to be one of the advantages of the Mediterranean diet. Most of the relevant data on the bioaccessibility of carotenoids from natural or processed foods has been obtained in postprandial absorption studies and supplementation studies. This approach, although highly valuable, is insufficient for a detailed analysis of the food matrix composition effects, and also it does not take into consideration other factors that may be involved in carotenoid absorption in each stage of the bioaccessibility process (digestibility and absorption). *In vitro* experimental processes that reproduce the physiological conditions and events that take place in the human gastrointestinal tract during digestion have been developed and fine-tuned in recent years. These digestion models become an excellent analytical resource to establish both the significance and scope of diverse factors in the efficiency of digestibility of carotenoids allowing a detailed analysis of the influence of the food matrix composition on the digestive process.

Contents

Abbreviations: BBMV, brush-border membrane vesicles; HDL, high-density lipoproteins; LDL, low-density lipoproteins; O/W, oil in water emulsions; VLDL, very low-density lipoproteins.

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1. Introduction

The colour of food is perhaps the first attribute that consumers assess when determining the quality and appearance of a product, and therefore conditions its acceptability. Colour becomes a measure of quality and also an indication of deterioration. In fruits and vegetables, colour is due mainly to three pigment families: chlorophylls, carotenoids and anthocyanins, which are responsible for green, red-yellow, and blue-violet colourations, respectively. The first function of these pigments that can be observed in plants is the attraction of animals to act as vectors for the dissemination of seeds and fruits, thus ensuring reproductive success and perpetuation of the species (Bartley & Scolnik, 1995). Certainly the acquisition of these striking colours has been selected for throughout evolution and has helped to establish other characteristics among which are the bioactive properties. Humans are not indifferent to this phenomenon of attraction (Mínguez-Mosquera, Pérez-Gálvez, & Hornero-Méndez, 2005).

A large body of epidemiological evidences has demonstrated that eating fruits and vegetables may reduce the risk of several degenerative processes (Fung, Willett, Stampfer, Manson, & Hu, 2001; Gramenzi et al., 1990; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hirvonen et al., 2001; Joshipura et al., 2001; Key, Thorogood, Appleby, & Burr, 1996; Knekt et al., 1994; Law & Morris, 1998; Sasazuki, 2001). Increased consumption of fruits and vegetables leads to an increased intake of water- and fat-soluble vitamins, dietary fibre and other phytochemicals (such as carotenoids, glucosinolates, phenols and chlorophylls), which have beneficial effects on health (Liu, 2004; Lock, Pomerleau, Causer, Altman, & McKee, 2005). This claim is supported by studies that refer to the beneficial effect of some of those compounds (Aggarwal et al., 2004; de Jong, Plat, & Mensink, 2003; Lambert & Yang, 2003; Verhoeven, Goldbohm, van Poppel, Verhagen, & van den Brandt, 1996; Ziegler, 1989).

Promotional campaigns have raised consumer awareness that healthy eating habits are now recognised to improve or increase life expectancy, then positively impacting the consumption of fruits and vegetables. Encouraging consumption of these foods has steadily increased from a mean of 2 to 3 portions a day in the 1970s to the current mean of 5. The recommendation of increasing our consumption of fruits and vegetables also suggests that our intake of phytochemicals should increase, and one of the driving reasons could be the data from studies concerning their bioavailability. The amount of phytochemicals that are efficiently absorbed from raw fruits and vegetables is low in comparison with processed products and supplements. Thus, only 25% of the vegetables consumed provide enough quantities of phytochemicals to obtain favourable effects, and this proportion increases up to 50% in the case of fruits (Johnston, Taylor, & Hampl, 2000).

In particular, an interest in increasing the consumption of carotenoids has been evident since 1981, when Peto, Doll, Buckely, and Sporn (1981) suggested that β-carotene consumption reduces the incidence of some types of cancer, and further evidences were obtained in subsequent studies (Ziegler, Maine, & Swanson, 1996). However, the percentage of carotenoid ingested *versus* the amount assimilated, reaches up to 10% when a natural food is consumed (Boileau, Moore, & Erdman, 1999). In light of this low percentage, it would be interesting, and potentially useful, to include carotenoids in processed foods or new food matrices in order to increase their bioavailability. Food formulations that provide one alternative to natural foods, in terms of carotenoid contribution, are based on hydrophilic matrices that have been enriched with these compounds through dispersion, emulsion or encapsulation with other compounds to modify fat-solubility if necessary (Pérez-Gálvez & Mínguez-Mosquera, 2004; Richelle, 2002). These types of formulations present several advantages to the consumer, including the ability to select a combination of various functional compounds at adequate concentrations for their activity, and the application of additional design criteria that could further increase assimilation of the said compounds.

When claims are made regarding phytochemicals (their presence in food and the health benefit that they provide by strengthening the pool of antioxidants in our bodies), the assessment of truth of these claims is not only a matter of composition but rather whether those components are effective for the claimed function. Then, it has to be shown how these compounds are ingested, digested, and effectively assimilated, passing the pre-systemic metabolism to reach the target tissue where they carry out their function, stages that can be verified through a bioavailability study. This fact is summarised in the Regulation EC 1924/2006 on nutrition and health claims made on foods. Specifically in section 15 it is established that "in order to ensure that the claims made are truthful, it is necessary that the substance that is the subject of the claim is present in the final product in quantities that are sufficient, or that the substance is absent or present in suitably reduced quantities, to produce the nutritional or physiological effect claimed. The substance should also be available to be used by the body. In addition, and where appropriate, a significant amount of the substance producing the claimed nutritional or physiological effect should be provided by a quantity of the food that can reasonably be expected to be consumed".

These statements apply particularly for carotenoids. Bioavailability of carotenoids from dietary sources depends on multiple factors some of them related to food matrix characteristics and the properties of any co-ingested food, other ones related to nutrient status and genetic profile of the host (van het Hof, West, Weststrate, & Hautvast, 2000). These factors condition the efficiency of carotenoid bioavailability from different foods and consequently the exertion of benefits they could develop in humans.

2. Carotenoid content in food: The Mediterranean diet

Although more than 700 carotenoids have been described in Nature, not all natural sources of them are present in our normal diet. It is estimated that we only have access to about 40 carotenoids that can be absorbed, metabolised, and/or used in our bodies. That number is reduced to 6 if we consider the carotenoid profile that is usually detected in human blood plasma. This group includes α- and β-carotene, lycopene, β-cryptoxanthin, zeaxanthin and lutein, which are regularly present in the foods listed in Table 1 (for structures see Fig. 1) (Britton & Khachik, 2009). The carotenoid content of the foods listed in this and other tables can be found in databases that have been developed for this purpose. The database of Mangels, Holden, Beecher, Forman, and Lanza (1993) only included fruits and vegetables as

Table 1
Carotenoid content in foods representative of the Mediterranean diet, expressed in mg/100 g.

	α-Carotene	β-Carotene	β-Cryptoxanthin	Lutein or Zeaxanthin	Lycopene
Green vegetables					
Lettuce	–	1272	–	2635	–
Spinach	–	5597	–	11938	–
Brussels sprouts	6	450	–	1590	–
Vegetables/tubers					
Beans	147	408	–	–	–
Broccoli	1	779	–	2	–
Pepper	59	2379	2205	–	–
Pumpkin	4795	6940	–	–	–
Potato	–	6	–	–	–
Tomato	112	393	–	130	3025
Carrot	4649	8836	–	–	–
Onion	6	–	–	–	–
Fruits					
Pineapple	30	–	–	–	–
Banana	5	21	–	–	–
Grape	5	603	12	13	
Mango	17	445	11	–	–
Melon	27	1595	–	40	–
Orange	16	51	122	187	–
Watermelon	–	295	103	17	4868
Pear	6	27	–	–	–
Cereals					
Corn	33	30	–	884	–
Wheat	–	100	–	35	–
Vegetable oils					
Olive	–	219	30	5990	–
Palm	24	38	–	–	–

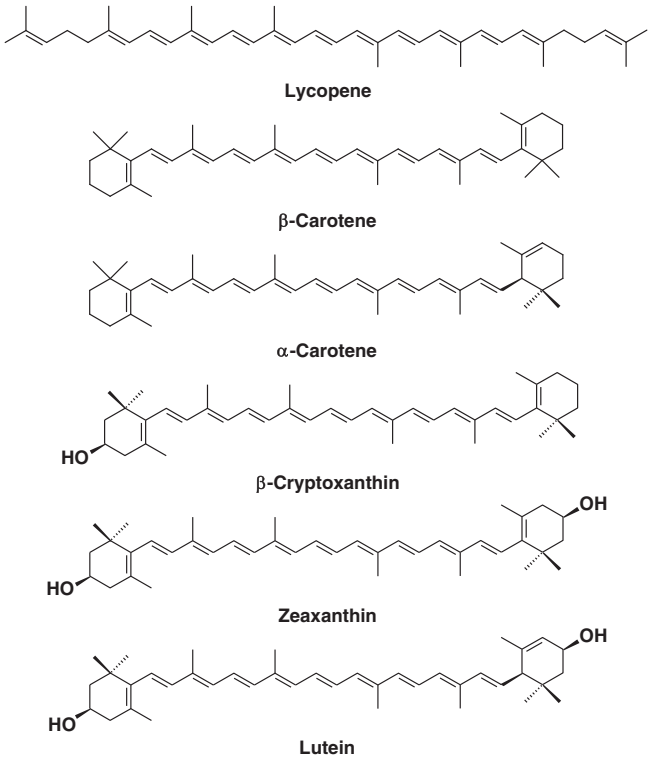

Fig. 1. Chemical structures of the carotenoids most frequently found in fruits, vegetables and human blood plasma.

carotenoid-containing foods. Later on, a new database was developed, published by Holden et al. (1999), that also included other foods such as vegetable oils, butter, eggs, cheese, and other products made of vegetables (pizzas, salads, *etc.*). This database evaluated up to 200 references on the carotenoid content of 215 foods, tabulating the average content and standard deviation, as well as the number of studies conducted for each food. It is available at the following web site: www.nal.usda.gov/fnic/foodcomp (last accessed 20/01/2011). One of the main uses of this database is the estimation of the provitamin A contribution of a given dietary intake. Another database on the average content of carotenoids in foods is offered by the U.S. Department of Agriculture, Agricultural Research Service(2005). In the list of nutrients included in this database (www.nal.usda.gov/fnic/foodcomp/Data/SR18/nutrlist/sr18list.html, last accessed 20/01/2011) there is information on α- and β-carotene, lycopene, β-cryptoxanthin, zeaxanthin and lutein.

Despite the correlation between high carotenoid content in plasma, which comes exclusively from the intake of foods rich in carotenoids, and lower risk of developing severe degenerative processes, adequate intake levels of these components have not been established since the positive health effects may be due to other constituents that are co-ingested with carotenoids. Neither the health-promoting biological actions that these compounds may have in our bodies (antioxidant capacity, immune enhancement, increased intercellular communication) nor the fact that some of them exhibit provitamin A activity has been, at the moment, reasons to establish a recommendation on the appropriate amount of carotenoid intake. However, by using data from epidemiological studies on the consumption of fruits and vegetables and their effect on health, normal values may be set for carotenoid intake, which may be associated with a lower risk of developing degenerative diseases (cancer, cardiovascular disease, *etc.*). Even so, there are discrepancies on mean intake values in the consulted references. Thus, individuals who eat a diet rich in these foods ingest about 6 mg/day according to studies by Lachance (1997) and published guidelines from Health Canada (1997). However, the study published by the WCRF/AICR (1997) raises the average intake value to 9–18 mg/day. For intervention studies conducted with controlled dietary carotenoid content, it is suggested that an intake of 3 to 6 mg/day of carotenoids is sufficient to maintain plasma levels of these components (Micozzi et al., 1992; Yong et al., 1994; Zino, Skeaff, Williams, & Mann, 1997).

In particular, the Mediterranean diet offers perhaps the most diversity and amount of carotenoid intake due to its high content of fruits and vegetables (fresh and/or processed) and vegetable oils (especially olive oil) (Saura-Calixto & Goñi, 2009). Bearing in mind the 6 most representative carotenoids mentioned above, Table 1 shows the amounts found in foods of the Mediterranean diet. All green vegetables contain a considerable amount of lutein, β-carotene, and β-cryptoxanthin, with the concentration varying greatly from one source to another. The best sources of α-carotene are carrots and pumpkins, while β-carotene is found more widely in fruits and vegetables such as carrots, red bell peppers, oranges, potatoes, broccoli, and green vegetables. β-Cryptoxanthin is frequently found at low concentration, although in ripe red peppers and some tropical fruits like papaya is one of the major pigments. Tomato and its derived products (pasta and sauces), together with watermelon and pink grapefruit, are the main sources of lycopene. Rich sources of lutein include green vegetables such as spinach, Brussels sprouts, broccoli, and peas, while zeaxanthin is found in high concentrations in egg yolks and corn.

3. Carotenoids and provitamin A activity

In animals, carotenoid pigments have several important biological activities from nutritional and physiological standpoints. Animals and humans cannot synthesise carotenoids *de novo* although they can metabolise some of them into vitamin A (retinol). Approximately 10% of carotenoids meet the main structural requirement for acting as vitamin A precursors, *i.e.*, contain a β-type non-substituted ring, being β-carotene and β-cryptoxanthin the most representatives. Table 2

shows the provitamin A activity (relative to β-carotene) of the main carotenoids present in the diet. The only source of these retinol precursors is the diet and, in most cases, fruits and vegetables are the food sources in our diet that primarily provide carotenoids with provitamin A activity. The extensive presence and distribution of carotenoids in Nature, mainly in fruits and vegetables (foods that occupy or should occupy an important place in our diet), make carotenoids with provitamin A activity the most important source of retinol. Some groups of people, the vegetarians, even depend almost exclusively on fruits and vegetables as a source of retinol in the form of its precursors. In mammals, therefore, the unique and important biological function of carotenoids with retinol equivalence is their role as vitamin A precursors, which is necessary for vision, growth, cell differentiation, and other physiological processes (Olson, 1996).

Data published in the study "Global prevalence of vitamin A deficiency in populations at risk 1995–2005" published by the World Health Organisation in 2009, indicate that 190 million preschool-age children and 19.1 million pregnant women had levels of serum retinol less than 0.7 μmol/L, which is the lower limit of normal, and below which is considered a state of vitamin A deficiency. The deficient population is distributed in countries whose gross domestic product (GDP) is less than US$15,000 and in those with 92% of the world's population. *A priori*, this situation may seem paradoxical since the majority of that population bases its diet on plant-derived foods, and therefore represents the main contribution of provitamin A. However, and in light of the data, that contribution is not enough due to the equivalence or efficiency of the conversion of provitamin A into retinol, as it will be discussed later. In order to reach a level higher than that which is considered deficient, it is necessary to consume foods of animal origin that have been fortified. The Institute of Medicine of the National Academy of Sciences (USA) has established recommended daily allowance levels for the population according to age, with an additional distinction for pregnant and lactating women. For adults between 31 and 50 years of age, for example, the RDA value is 900 mg/day for men and 700 mg/day for women.

3.1. Metabolism of carotenoids with provitamin A activity. Bioefficiency

As stated before, not all carotenoids have the structural requirements for conversion into vitamin A. Only those with at least one β-type ring, without oxygenated functional groups, along with one polyene chain containing at least 11 carbon atoms are potential precursors of vitamin A. Among the 700 natural carotenoids described up to now, only 10% show provitamin A activity. The most important ones, as much for their high activity level as for their availability, are α- and β-carotene, and some xanthophylls including β-cryptoxanthin and some apocarotenoids (Mínguez-Mosquera & Hornero-Méndez, 1997). Of these, β-carotene has the greatest provitamin A activity since every molecule of pigment produces two of retinal, which is then reduced to vitamin A (retinol).

Table 2
Carotenoids with provitamin A activity. Provitamin value is relative to β-carotene according to Bauernfeind (1972).

Carotenoid	Percent activity
trans-β-Carotene	100
9-*cis*-β-Carotene	38
13-*cis*-β-Carotene	53
trans-α-Carotene	53
9-*cis*-α-Carotene	13
13-*cis*-α-Carotene	16
trans-β-Cryptoxanthin	57
9-*cis*-β-Cryptoxanthin	27
15-*cis*-β-Cryptoxanthin	42
β-Carotene-5,6-epoxide	21
Mutatochrome	50
γ-Carotene	42–50
β-Zeacarotene	20–40

The metabolic process for conversion of carotenoids with provitamin A activity into retinol occurs *via* an oxidation process with central cleavage of the molecule, which is catalysed by the enzyme β,β-carotene 15,15′-mono-oxygenase (EC 1.13.11.21). This enzyme, originally called β,β-carotene-15,15′-dioxygenase, although discovered 45 years ago, (Goodman & Huang, 1965), had not been purified until recently through the successful cloning of cDNAs in different species, including humans (Yan et al., 2001). That key process is followed by the conversion of retinal into retinyl esters by retinal reductase and lecithin-retinol acyltransferase (Moore, 1930). The proposed mechanism, firstly described by Goodman, Huang, and Shiratori (1966), consists of oxidation of the two central carbon atoms of β-carotene with molecular oxygen and subsequent homolytic cleavage of the dioxyethane intermediate at the C15–C15′ linkage, generating two molecules of retinal (Fig. 2A). However, Leuenberger, Engeloch-Jarret, and Woggon (2001) showed that the enzyme involved in the retinal transformation is really a mono-oxygenase and that the mechanism involves epoxide formation at the 15–15′ bond and the subsequent non-selective ring opening with water and cleavage to generate the corresponding aldehydes. The experimental strategy used in this work is well known and consists of cleaving an asymmetric carotenoid substrate (α-carotene) and its enzymatic incubation in a medium enriched in $^{17}O_2$ and $H_2^{18}O$. The presence of ^{17}O and ^{18}O in the aldehyde obtained at the end of the enzymatic process revealed the previously discussed mechanism (Fig. 2B).

The validity of one cleavage path or another, central or eccentric, has been the subject of profound debate for decades. Although evidence existed for the operation of the eccentric cleavage path, the alternative route of central cleavage was accepted for decades until the publication of the work of Wang, Tang, Fox, Krinsky, and Russell (1991) that demonstrated the production of apocarotenals and retinoic acid as the main products of the breakdown of β-carotene. Both pathways can be considered to occur during the conversion process of β-carotene to retinal with a predominance of one or the other depending on the type of tissue where the process is carried out. All of these evidences support the existence of a family of oxygenase enzymes that produce central or eccentric cleavage of carotenoids with provitamin A activity, giving rise to metabolites with different structures depending on the location of epoxidation and cleavage of the polyene chain (Fig. 3). This group of enzymes is localised in the cytosol of cells and, in mammals its activity is mostly located in the intestine. In fact, in humans it is believed that 60–70% of ingested β-carotene is converted to retinal. Activity has also been detected in this family of enzymes in other tissues such as the liver, lungs, kidneys, and brain. The characteristics of the enzymatic processes involved in the conversion of carotenoids into retinol esters indicate that the processes are regulated within lipid and retinoid metabolism. Factors related to the individual (vitamin A status, malnutrition, intestinal infection/inflammation) and those related to the food matrix (presence of other carotenoids, fat and dietary fibre, processed/cooked) that affect carotenoid bioavailability, all determine the bioefficiency level (Castenmiller & West, 1998).

Bioefficiency is defined as the fraction of ingested carotenoids with provitamin A activity that is absorbed and converted to its active form, that is, retinol. In 1967 the WHO established 6 as the ratio value to calculate β-carotene bioefficacy. In the case of other carotenoids with provitamin A activity, the ratio was doubled. Thus, 1 mg of retinol was obtained from 6 mg of β-carotene or 12 mg of other proactive carotenoids. The establishment of these parameters was based on data showing that the equivalence between pure β-carotene in oil and retinol is 2:1. Estimating that the bioavailability of carotenoids from the diet was one third of that of retinol, the ratio of 6 was set for β-carotene (3×2:1), and 12 for other carotenoids with retinol equivalence (2×3×2:1). However, it was recognised that these ratios could

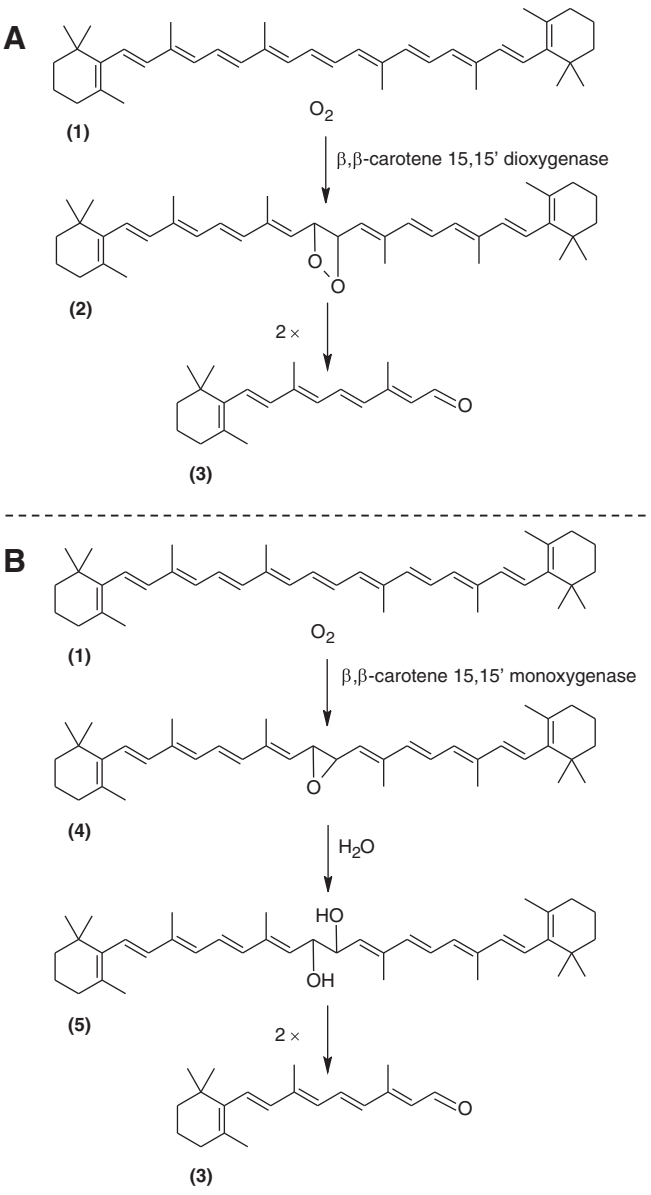

Fig. 2. Reaction of β-carotene (1) to retinal (3) *via* dioxygenase enzyme and dioxyethane intermediate (2) (panel A) or *via* monoxygenase enzyme and monoxyethane (4) and diol (5) intermediates (panel B). Enzymatic activity is performed over central position of β-carotene.

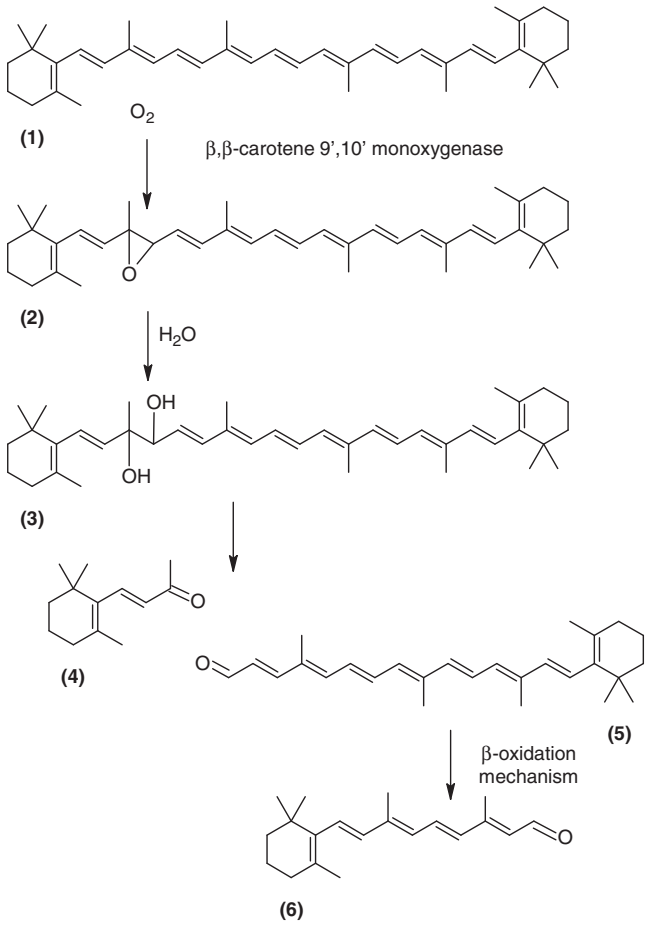

Fig. 3. Reaction of β-carotene (1) to retinal (6) *via* monoxygenase and monoxyethane (2), diol (3), β-ionone (4) and β-apo-10-carotenal (5) intermediates. Enzymatic activity is performed over an eccentric position of β-carotene.

overestimate or underestimate the bioavailability of dietary carotenoids when considering other factors that alter the efficiency of bioavailability, such as carotenoid content in the food, type of food, degree of processing, and so on. Later studies (van het Hof et al., 1999) conducted with stable isotopes have confirmed the fact that they had overestimated the efficiency of bioavailability, which is a more crucial factor than the conversion to retinol, indicating that the efficiency of dietary β-carotene absorption was one seventh of that corresponding to β-carotene administered in oil, thus leading to ratios of 14 (7×2:1) and 28 (2×7×2:1) for β-carotene and other carotenoids with retinol equivalence, respectively. The Institute of Medicine of the National Academy of Sciences (USA) report from 2001 reduced the ratios to 12 for β-carotene and 24 for other carotenoids with retinol equivalence (Scott & Rodriguez-Amaya, 2000).

The abovementioned change in ratios between β-carotene and carotenoids with provitamin A activity obtained from the consumption of fruits and vegetables, prompted by bioavailability studies, indicates that the amount of intake of these foods that is required to meet the daily vitamin A requirement is higher than that which had been established for years, which had overestimated the intake of vitamin A in the diet. Table 3 shows the units and conversion factors for International Units (IU), Retinol Equivalent (RE) and Retinol Activity Equivalent (RAE). This last unit has emerged to replace the RE and correct the overestimation of bioefficacy. All these aspects have recently been reviewed by Thurnham (2007).

4. Carotenoid bioavailability

Since humans cannot synthesise carotenoids and must therefore depend on diet to supply these compounds and their beneficial effects, the bioavailability of carotenoids has always been of interest. The concept of *bioavailability* comes from a fusion of the words *biological* and *availability* (Metzler & Huang, 1983), and its definition has evolved with time. Thus, bioavailability includes accessibility for absorption, absorption, metabolism, transport and tissue distribution, and bioactivity. However the term bioavailability is usually defined as the fraction of an oral dose of a parent compound or active metabolite that reaches the systemic circulation (Schumann et al., 1997), a definition more related with the term *bioaccessibility*. In general, bioaccessibility can be described as the sum of digestibility and assimilation. Although bioavailability and bioaccessibility are often used indistinctly, it is important to note that definition of bioavailability includes metabolism, transport and tissue distribution, and bioactivity. However, bioactivity generally fades into the concept of bioavailability given that, pharmacologically speaking, once the active ingredient has reached its target organ, it is not inert, but rather

Table 3
Units used to express the vitamin A content in foods and the equivalences with retinol, β-carotene and other carotenoids with provitamin A activity.

	μg retinol	μg β-carotene	μg carotenoid[a]
IU retinol[b]	0.3	3.6	7.2
IU β-carotene[b]	–	0.6	1.2
RE	1	6[c]	12
RAE[d] (μg)	1	12	24

[a] Carotenoids with provitamin A activity with a 50% conversion value (see Table 2) with respect to β-carotene (see Table 3).
[b] 1 IU (International Units) retinol = 3 IU β-carotene.
[c] Equivalency with respect to β-carotene from diet. 1 RE (Retinol Equivalent) = 2 μg of pure β-carotene in oil. An alternative equivalency ratio could be 1 RE = 14 μg β-carotene or 28 μg to other provitamin A carotenoids.
[d] 1 RAE (Retinol Activity Equivalent) = 3.33 IU retinol.

carries out some action (Stahl et al., 2002). Digestibility refers specifically to the fraction of food components that are transformed by digestion into potentially accessible matter through all physical–chemical processes that take place in the lumen. Assimilation refers to the uptake of bioaccessible material through the epithelium by some mechanism of transepithelial absorption.

In the field of carotenoids some works that measure digestibility stage refer this term as bioaccessibility while others equate measurement of assimilation with bioavailability (Granado-Lorencio et al., 2007; Hedrén, Diaz, & Svanberg, 2002; Hornero-Méndez & Mínguez-Mosquera, 2007; Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx, 2009; O'Sullivan, Jiwan, Daly, O'Brien, & Aherne, 2010). Finally, digestibility plus assimilation are often referred to as bioavailability (Chitchumroonchokchai, Schwartz, & Failla, 2004; Chitchumroonchokchai & Failla, 2006).

In many cases, the information published on the bioavailability of carotenoids is obtained by determining the quantity of carotenoids in plasma after the intake of food or supplement either in a single dose (postprandial study) or over a certain period of time (supplementation study). Thus, bioavailability is estimated once the total dose has been administered, and the quantity of carotenoids accumulated in plasma is known. Furthermore, experiments may be designed such that relevant factors that modify either digestibility or assimilation may be determined. For example, the carotenoid composition of the ingested food or the type of food matrix may be changed or other components that may interfere or complement absorption may be determined. Considerable amount of data has been obtained with this strategy (Castenmiller & West, 1998; O'Neill & Thurnham, 1998; Furr & Clark, 1997; Stahl & Sies, 1992; van Vliet, Schreurs, & van den Berg, 1995). However, digestion and assimilation involve several steps, and each one could cause an effect on the nutrient or bioactive compound so that a detailed picture is not obtained with an *in vivo* approach. Therefore, application of *in vitro* digestion and assimilation procedures is a suitable process to gain information about factors that modulate different steps of carotenoid bioaccessibility and bioavailability.

4.1. Digestibility: Solubilisation and micellisation

Carotenoids are lipophilic compounds and must first undergo solubilisation from the food matrix followed by micellisation. This refers to their incorporation into micelles, which are molecular aggregates that transport fat-soluble material, making it potentially accessible by the intestinal epithelium (Fig. 4). The degree of food processing is significant for micellisation efficiency as a high processing degree can maximise the amount of compound that is made soluble from the matrix. Mechanical homogenisation, the application of a thermal treatment and the addition of fat during food processing are all techniques that increase the efficiency of this phase (van het Hof, Gärtner, West, & Tijburg, 1998). The above-mentioned techniques modify the subcellular structures where the carotenoids are located, thus improving their release and solubilisation (Gärtner, Stahl, & Sies, 1997; Paetau et al., 1998; Stahl & Sies, 1992).

The micellisation phase involves reduction of the size of lipid particles produced in the previous phase to form molecular aggregates of 3–10 nm (micelles) in which the lipid material will be stored (Johnson & Gerwin, 2001). Micelles may not only contain carotenoids, but also acylglycerols, cholesterol and phospholipids. Secretions from the gallbladder and pancreas are critical in this phase, as the first secretions consist mainly of biliary salts that contribute to reduce micelle size and stabilise these aggregates. These secretions provide digestive enzymes such as the pancreatic lipases that hydrolyse triacylglycerides, cholesterol esters and other esterified compounds, such as carotenoids from fruits. The key factor in this phase is the ingested fatty matter. Its presence stimulates biliary secretions and pancreatic lipase levels, which in turn increases micellisation capacity. It is estimated that the consumption of 3 to 5 g of fat notably increases the bioavailability of carotenoids (Jayarajan, Reddy, & Mohanran, 1980; Roels, Trout, & Dujacquier, 1958). However, some comparative studies indicate that certain types of fats may disfavour this process. The bioaccessibility of carotenoids decreases when short/medium-chain triacylglycerides are ingested, while bioaccessibility appears to be increased by the consumption of long chain-triacylglycerides (Borel et al., 1998; Hollander & Ruble, 1978). Due to the estimation method applied (assessment in plasma of the quantity of carotenoids accumulated during intake) it is not possible to differentiate whether the triacylglyceride chain length has an effect on micellisation or the stage(s) following assimilation.

The fat solubility of individual carotenoids also affects micellisation. Fat solubility depends on the structural particularities of each carotenoid. The general distinction of carotenoids as carotenes or xanthophylls implies also differentiation with respect to fat and solvent solubility. Comparative studies of bioaccessibility indicate that xanthophylls, such as lutein, are absorbed more readily than carotenes (van het Hof et al., 1999). Therefore, the joint intake of carotenoids from both groups somehow leads to an absorption competition in which xanthophylls achieve better bioaccessibility (Fotouhi et al., 1996; Gärtner, Stahl, & Sies, 1996; Henderson et al., 1989). However, these studies did not establish the step of the carotenoid absorption process at which this competition arises. It is possible that these two types of carotenoids are incorporated differently into micelles, due to their different fat solubility, but the absorption difference could also occur during the subsequent assimilation stage (van den Berg, 1999).

Another important factor in the micellisation phase is the presence of soluble fibre. Dietary fibre consumption reduces the efficacy of this process because the biliary content becomes soluble in the gels formed during gastric digestion, affecting the efficacy of carotenoid bioaccessibility (Erdman, Fahey, & White, 1986; Jones & Ntanios, 1998; Rock & Swendseid, 1992; Westrate & Meijer, 1998). It has been demonstrated that the presence of dietary fat substitutes, such as sucrose polyester, notably reduces the bioaccessibility of carotenoids (Cooper, Webb, & Peters, 1997). Pancreatic lipase inhibitors, which are used to treat obesity, also reduce the efficacy of micellisation, decreasing the plasma concentration of carotenoids (James, Avenell, Broom, & Whitehead, 1997; Zhi, Melia, Koss-Twardy, Arora, & Patel, 1996).

Certain structural differences may alter fat solubility and, therefore, modify the efficiency of the micellisation. One of them is the esterification of xanthophyll with fatty acids. This modification has not been widely considered in bioaccessibility studies, although the xanthophylls we consume are usually esterified. Esterified xanthophylls exhibit increased fat solubility relative to their corresponding free xanthophylls and even against carotenes (Fernández-García, Mínguez-Mosquera, & Pérez-Gálvez, 2007). In addition, studies of carotenoid bioaccessibility after intake of food rich in esters show that only the corresponding free forms are detected in the

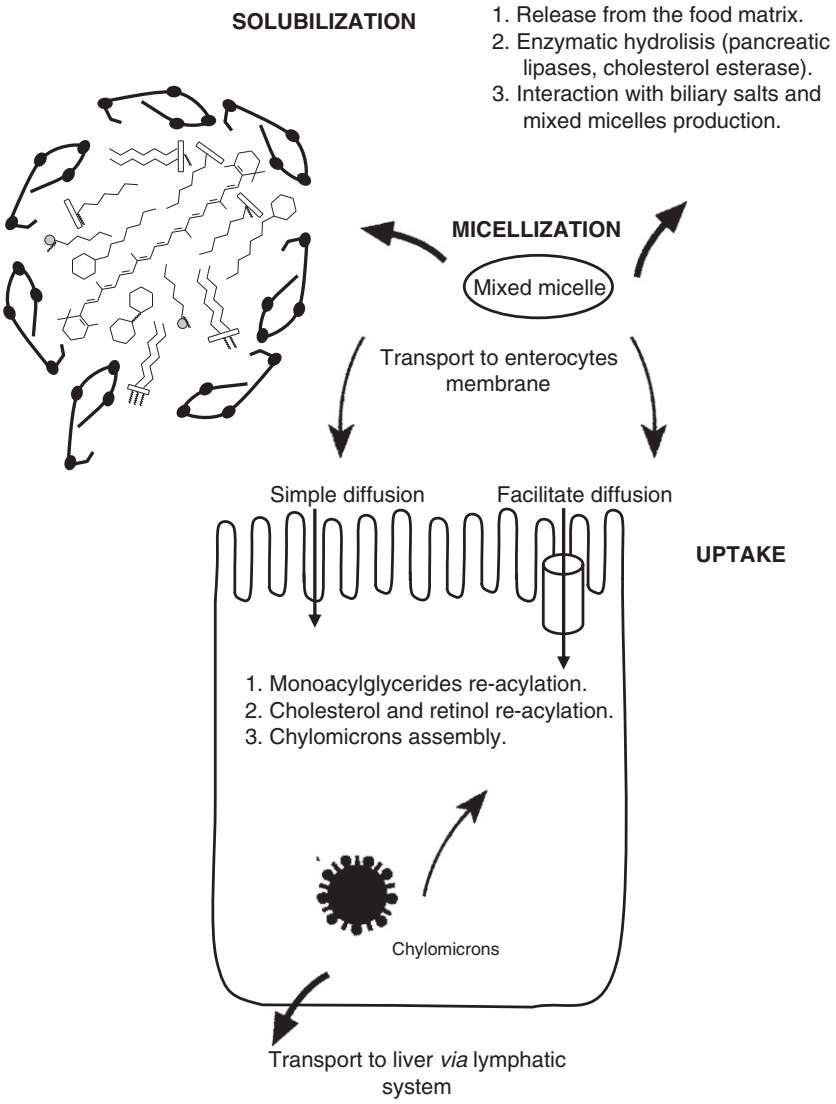

Fig. 4. Representation of different bioaccessibility and uptake stages of lipidic compounds in intestinal epithelium.

plasma, which implies that de-esterification has occurred at some point along the absorption (Pérez-Gálvez, Martin, Sies, & Stahl, 2003; Roodenburg, Leenan, van het Hof, Westrate, & Tijburg, 2000). Therefore, it was logical to assume that this reaction could be carried out by pancreatic lipases during the micellisation phase (Khachik, Beecher, Goli, Lusby, & Smith, 1992; Olson, 1984). These two aspects of xanthophyll ester metabolism (higher fat solubility and subjection to the de-esterification process) also increase the requirements for biliary salts and digestive enzymes to guarantee xanthophyll ester assimilation. In fact, the bioaccessibility studies that have been performed indicate that a greater fat intake is required to achieve an efficient absorption of xanthophyll esters at levels similar to those of their corresponding free forms (Aman, Bayha, Carle, & Schieber, 2004; Bowen, Herbst-Espinosa, Hussain, & Stacewicz-Sapuntzakis, 2002; Roodenburg et al., 2000).

Special mention should be given to the question of what enzyme/s is/are responsible for performing the de-esterification process. Some have suggested that pancreatic lipases could efficiently perform ester hydrolysis and convert xanthophyll esters to their corresponding free forms. However, Breithaupt, Bamedi, and Wirt (2002) demonstrated that human pancreatic lipase (EC 3.1.1.3) does not efficiently hydrolyse esterified forms of xanthophylls such as β-cryptoxanthin, zeaxanthin, lutein and capsanthin, whereas porcine pancreatic lipase and *Candida rugosa* lipase did posses this activity. Although it is unexpected that human pancreatic lipase does not act on xanthophyll esters, it has also been shown to lack activity towards other esterified compounds (Rigtrup, McEwen, Said, & Ong, 1994). This study also investigates the activity of carboxyl-ester lipase (EC 3.1.1.13), which showed the xanthophyll esterase efficiency. Carboxyl-ester lipase is a non-specific lipase that is active in the presence of biliary salts and hydrolyses cholesterol esters, tri-, di- and monoacylglycerides, phospholipids, lysophospholipids and ceramides (Hui & Howles, 2002). This enzyme role complements the hydrolytic activity of other lipases that act upon the previously mentioned substrates (Harrison, 1998) and it has even been suggested that its activity is directly involved in vitamin A ester hydrolysis (Rigtrup et al., 1994; Rudd & Brockman, 1984).

If carboxyl-ester lipase is responsible for the hydrolysis of xanthophyll esters then significant efforts should be directed at determining where this enzyme activity is located. There are evidences that its activity takes place in the membrane of the intestinal epithelial cells (Gallo, Newbill, Hyun, Vahouney, & Treadwell, 1977; Lechene De la Porte, Aboukail, Lafont, & Lombardo, 1987). As a result, only lipid material that is finally emulsified and micellised and reaches the membrane of these epithelial cells is susceptible to hydrolysis by this enzyme. It has been proven that xanthophyll esters are incorporated into micelles, which it is necessary to reach the enterocyte membrane where they can be hydrolysed (Chitchumroonchokchai & Failla, 2006).

4.2. Absorption

Carotenoid uptake by enterocytes involves contact between the assimilable material of the micelles and the intestinal epithelium, which acts as a "selective barrier" for nutrients. Our understanding of the events that take place at this stage has been significantly enhanced over the last years, demonstrating the existence of different absorption mechanisms from those initially considered. It was supposed that the assimilation of lipid micelle contents, including carotenoids, took place in a passive diffusion process where the micelles collided with the cellular membrane and released their contents into the cytosol (El-Gorab, Underwood, & Loerch, 1975; Hollander & Ruble, 1978). Parker (1996) suggested that the concentration gradient between the micelle and the cytosol determined the diffusion speed and even explained how assimilation could be saturated at certain carotenoid micelle concentrations by reducing the concentration gradient. However, several studies have significantly updated the model of cholesterol, carotenoid and other lipid compounds absorption mechanisms (von Bergmann, Sudhop, & Lütjohann, 2005; Werder et al., 2001). The biokinetic profile of absorption, obtained in model systems such as brush-border membrane vesicles (BBMV) and intestinal epithelium cell cultures (Caco-2), shows some characteristics of a facilitated diffusion (Compassi et al., 1997; Thurnhofer & Hauser, 1990). Based on this observation, the type B residual receptors (SR-BI), cluster of differentiation 36 (CD36) and Niemann–Pick C1-Like 1 protein (NPC1L1) were eventually identified as facilitators of the absorption of cholesterol and carotenoids, although their precise role in this process is still under debate and a matter of further investigations (van Bennekum et al., 2005).

The model of facilitated diffusion has been supported by the finding of macromolecules such as α- and β-peptides or ezetimibe (a drug that lowers cholesterol absorption) that inhibits lipid compound absorption (Altmann et al., 2002; Boffelli et al., 1997). In addition, this mechanism is consistent with the significant variability of lipid absorption efficacy that is observed (*e.g.* cholesterol) among individuals within the same population, which is attributed to genetic differences expressed in the intestinal epithelium (Wang, Paigen, & Carey, 2001). A similar variability has also been observed in studies of postprandial carotenoid absorption (Paetau, Chen, Goh, & White, 1997; Pérez-Gálvez et al., 2005). These achievements are largely due to the development of *in vitro* absorption models, based on obtaining BBMV and cellular cultures. These models have been used as analytical tools in experiments aimed at determining the biokinetics of lipid absorption in general and of the carotenoids in particular (During, Hussain, Morel, & Harrison, 2002; During, Dawson, & Harrison, 2005; Moussa et al., 2008; Reboul et al., 2005; van Bennekum et al., 2005; Wilson, 1990).

Once the lipid material is internalised by the cells, it is then packed into lipoproteins, called *chylomicrons*. These molecular aggregates are assembled in the Golgi apparatus, reach a size of 75 to 1200 nm and are characterised by their apoprotein B-48 content, which is essential to the assembly of these particles. Chylomicrons are excreted into the lymphatic system and are eventually taken up by the liver, where they are stored or re-excreted into circulation within very low-density lipoproteins (VLDLs). Some chylomicrons degrade before reaching the liver due to the activity of lipoprotein lipase therefore part of the lipid content of these particles is released and absorbed into the endothelial tissue. However, most chylomicrons reach the liver and are incorporated into VLDLs; once released into the circulatory system, they are transformed into low-density lipoproteins (LDLs) and finally into high-density lipoproteins (HDLs). Apolar carotenoids such as β-carotene and lycopene are concentrated at the centres of lipoproteic particles and are not frequently exchanged with other lipoproteins; thus, LDLs experience an increase in carotene content, whereas HDLs are comprised of xanthophylls (Paetau et al., 1998). This point is relevant as it indicates that carotenes or xanthophylls will accumulate differentially in certain tissues depending on their receptors density for LDLs or HDLs (Handelman, Snodderly, Adler, Russett, & Oratz, 1992; Schmitz, Poor, Wellman, & Erdman, 1991).

5. Carotenoids bioaccessibility from hydrophilic matrices

Carotenoid bioaccessibility is, as discussed previously, limited and conditioned by different factors, mainly the degree of food processing and matrix composition (Deming & Erdman, 1999). Fat (type and quantity) is a relevant factor that is required in minimum quantities to ensure bioaccessibility (Jalal, Nesheim, Agus, Sanjur, & Habicht, 1998; van het Hof et al., 2000). However, it has been demonstrated that carotenoid bioaccessibility from fatty foods (*e.g.* oily concentrates such as tomato and paprika oleoresins) is not as high as expected, particularly when compared with the bioaccessibility provided by hydrophilic matrices elaborated from these vegetables (Oshima, Ojima, Sakamoto, Ishiguro, & Terao, 1997; Pérez-Gálvez et al., 2003; Richelle, 2002). Could hydrophilic matrices be considered as an alternative to oily formulations to increase carotenoid bioaccessibility? Lipophilic compound formulation in a hydrophilic matrix requires an adequate solubilisation technique (emulsion, encapsulation) but in this case it would be possible to diversify the traditional sources of these bioactive compounds. In this sense, applying any of the appropriate techniques could be an adequate strategy to achieve stable, digestible and well-assimilated bioactive compounds. To this end, liquid formulations in which carotenoids have been incorporated as oil in water emulsions have been developed (Amar, Aserin, & Garti, 2003; Spernath, Yaghmur, Aserin, Hoffman, & Garti, 2002). In theory, the adequate incorporation of a carotenoid into a hydrophilic environment emulsion should increase bioaccessibility, although this fact has scarcely been determined. Then, quality design of the emulsion formulation will be completed when the estimation of digestibility/assimilation from that matrix is incorporated as a criterion during the optimisation process of the formulation composition (Fernández-García, Rincón, & Pérez-Gálvez, 2008).

6. *In vitro* digestion models

Over the last decades, the available information on carotenoid bioavailability was obtained with postprandial absorption and supplementation studies. This approach, although highly valuable, is not sufficient for a detailed analysis of the effects of food matrix composition or other factors on each stage in the process of absorption. There were *in vitro* digestion models ready for the estimation of digestibility of minerals such as iron and phosphorus (Liu, Ledoux, & Veum, 1998; Miller, Schricker, Rasmussen, & van Campen, 1981), cholesterol (Fouad, Farrell, Marshall, & van de Voort, 1991) and hydrosoluble vitamins (Ekanayake & Nelson, 1986). These are static gastrointestinal models, simulating the transit through the human digestive tract, and reproducing the physiological conditions of gastric and intestinal digestion. These models are easy to apply to a large number of samples and are thus well suited for studies of the effects of various digestion conditions or other factors linked to the food matrix on the bioaccessibility (digestibility and assimilation) of a nutrient.

The procedure consists of subjecting the food to the digestive process which is divided into two stages, gastric and intestinal, whose standard conditions are generally based on those in Miller et al. (1981). During the gastric phase, a portion of the homogenised food is acidified to pH 2 with 1 M HCl and a porcine pepsin suspension is added. The homogenised portion is transferred to a test tube and incubated at 37 °C in a water bath with circular agitation. Then, to simulate the intestinal phase, the pH is increased to 5.3 with sodium bicarbonate solution. The pH is again increased to 7.5 and the mixture is incubated for 2 h under the temperature and agitation conditions mentioned above. When this digestion procedure is applied to estimate digestibility of a hydrosoluble nutrient, it is not necessary

to isolate the micellised content from the rest of the non-digested or partially emulsified food. However, these models were not wholly applicable for digestibility studies of fat-soluble compounds, since they did not include a micelle fraction separation step, and this separation is essential when the digestibility and/or assimilation of fat-soluble materials are to be analysed. This procedure was not developed until 1990. Hernell, Staggers, and Carey (1990) conducted a detailed study of the physical–chemical properties of the duodenal contents during the digestion of food rich in triglycerides ingested by volunteers. The application of an ultracentrifugation step allowed the separation of non-digested triglycerides, the micelle fraction and a precipitate fraction. The combination of an *in vitro* digestion model with the micelle fraction separation method by ultracentrifugation yields a complete experimental tool for the analysis of fat-soluble component digestibility, because the micelle lipid content, which is susceptible to assimilation by epithelial tissue cells, can be separated.

The experimental conditions of any digestion model should not differ significantly from those just mentioned, as it closely reproduces the physiological stages and conditions of the former model, but these consist of specific changes or modifications to the basic model that depend on the particular problem being targeted or the application to be developed. *In vitro* digestion models have multiple applications and can contribute to knowledge in diverse areas. Some uses have included toxicology analyses of possible food contaminants, the study of dietary factors involved in the bioaccessibility of certain nutrients or food components and determination of the effect that different or unusual hepatobiliary conditions have on the bioaccessibility of lipid compounds (Leo et al., 1995; Oomen, Tolls, Sips, & Groten, 2003). Adaptations to this model have also been used in the design of food matrices, in which bioaccessibility is a matrix component selection criterion along with physical structure, stability or palatability (Manzocco & Nicoli, 2002). Bioaccessibility must be a criterion to be considered in the design of functional foods, given that any claim of health benefits established through the intake of these foods is not valid unless it can be shown that the bioactive component is absorbed efficiently (Aggett et al., 2005). Therefore, *in vitro* digestion models are an effective analytical tool that measures bioactive compound digestibility from different matrices and can be used as a starting point for later analysis of assimilation without resorting to *in vivo* experimentation models.

In the particular case of carotenoid pigments, *in vitro* digestion has been applied to measure the digestibility of these compounds from natural foods. The study by Garrett, Failla, and Sarama (1999) is the first application of an *in vitro* digestion model in combination with an intestinal assimilation model, based on the Caco-2 cell line, to determine carotenoid bioavailability in food. Their experimental procedure follows the stages described above: a gastric phase, an intestinal phase and separation of the micelle fraction. Children's food based on fruits and vegetables and tomato paste was used to analyse various factors that control the digestive process. Therefore, it was determined that omitting the gastric digestion phase does not modify the efficiency of the micellisation process in the intestinal digestion phase. The quantities of biliary salts and pancreatic enzyme suspension that provide the best transfer of carotenoids from food to micelles were then defined and were found to correspond to the physiological concentrations estimated in earlier studies (Charman, Porter, Mithani, & Dressen, 1997; Tso, 1994).

Subsequently, the method developed by Garrett et al. (1999) has been applied to and adapted for other studies of digestibility and/or assimilation of carotenoids, with slight modifications. Hedrén et al. (2002) studied the digestibility of carotenes in fresh and cooked carrots, applying similar conditions in terms of digestive enzyme and biliary salts concentrations as well as incubation times, but modifying the conditions of micelle fraction separation. In works published by Breithaupt et al. (2002) and Zorn, Breithaupt, Takenberg, Schwack, and Berger (2003) the gastric digestion phase is eliminated and the pancreatin enzymatic pool is replaced by pancreatic lipase and carboxyl-ester lipase. The use of the carboxyl-ester lipase enzyme, together with pancreatin lipase, in the intestinal digestion phase is the main modification to the method of Garrett et al. (1999) that has been introduced in other studies, as this enzyme mixture seems to have a positive effect on micellisation and subsequent carotenoid assimilation (Chitchumroonchokchai & Failla, 2006). In the study of Granado-Lorencio et al. (2007), an initial mastication simulation phase incorporating a salivary solution with organic and inorganic components and α-amylase (EC 3.2.1.1) is applied before the two digestion phases, gastric and intestinal. *In vitro* digestion model and experimental design of mixtures were used as analytical tools to measure carotenoid bioaccessibility and to optimise the formulation of an O/W emulsion, including carotenoids as functional ingredients (Fernández-García et al., 2008). Two experimental stages were applied. First, a screening phase was completed to detect the critical factors that exerted a significant effect on the response (bioaccessibility). During this phase, it was observed that the response was modified mainly by secondary effects such as synergies and antagonisms of the emulsifying mixture. Due to the effect on the response, a group of four emulsifiers was selected at this phase to perform the second experimental stage, the optimisation phase. This allowed obtaining the mixture that produced the maximum carotenoid bioaccessibility.

7. *In vitro* absorption models

Overall, the processes taking place during assimilation may be the most complex to reproduce with *in vitro* models. Apart from the physical–chemical processes that take place during assimilation, biochemical events must also be included to adequately reproduce intestinal absorption. The intestinal mucosa is not only a barrier in the physical sense, but also a biochemical barrier. The cell layer carries out complex metabolic processes that are part of the pre-systemic metabolism, which is separated from the nutrient/bioactive compound transport *via* simple or facilitated diffusion or active transport. However, despite this complexity, there are numerous alternatives to reproducing the intestinal assimilation process. Therefore, it is worth mentioning the existence of *ex vivo* (Ungell, 2002), *in situ* (Schanker, Tocco, Brodie, & Hogben, 1958) and *in vivo* techniques (Poelma & Tukker, 1987) before describing *in vitro* assimilation models.

The various types of *in vitro* models include those based on a physical reproduction of the membrane, those based on cell cultures and those that use isolated or reconstituted cell membranes. Models based on artificial membranes aim to specifically measure the membrane permeability of the nutrient or bioactive compound usually following the methodology of Kansy, Senner, and Gubernator (1998). The membrane is reproduced artificially by integrating phospholipids and other membrane constituents in a solution containing organic solvent. This solution is placed over a porous filter that serves as physical support between the donating buffer solution below and the receiving buffer solution above. The forte of membrane-type models lies in the fact that the lipid composition of the artificial membrane that is placed over the filter and can reproduce, as far as possible, the membrane composition of intestinal epithelium cells. The greatest number of modifications has been introduced in this parameter to optimise the model. From the original composition described by Kansy et al. (1998) to the modifications made by Sugano et al. (2001) or by Avdeef, Nielsen, and Tsinman (2004), improvements have made it possible for membrane permeability models to reproduce the results obtained with *in vivo* models. Another recurring theme is the attempt to reproduce the lumen/intestinal epithelium dynamic in these models. Although the conditions of these models are essentially static, this approach of modelling has great potential. Technical articles from R&D pharmaceutical companies have predicted that they will displace *in vitro* models based on cell cultures, considering their lower costs and a more acceptable

correlation with results obtained *in vivo* (Kansy et al., 1998). This technique is ideal as a high-throughput screening model for drug or phytochemical compound development.

Models based on cell cultures are very popular and have been largely accepted by the scientific community as valid references to estimate nutrient and bioactive compound assimilation. Given that primary cultures of enterocytes from explants of epithelial tissue do not form an epithelial monolayer and therefore do not have apical and basolateral areas it is necessary to resort to cellular lines (usually from tumours) that do have the structural characteristics of a cell monolayer. The cell monolayer is cultured over a supporting filter such that the apical area contacts the donating buffer solution and the basolateral area contacts the receiving buffer solution. Different cell lines have been described, such as Madin–Darby Canine Kidney cells (MDCK), cells from rat foetus intestine (2/4/A1), cells from pig kidney epithelium (LLC-PK1) and the best known cell line, Caco-2. This cell line comes from human adenocarcinoma colonocytes and when cultured under appropriate conditions present the morphological characteristics of enterocytes and also produce a large majority of membrane enzymes and proteins (Jumarie & Malo, 1991). Active transport of glucose, peptides, amino acids, biliary acids, carotenoids and flavonoids has been characterised in this cell line, demonstrating they are a reliable *in vitro* model (Bleasby, Chauhan, & Brown, 2000; Delie & Rubas, 1997; Garrett et al., 1999; Manach & Donovan, 2004). Miniaturisation, the development of physiological buffer solutions and standardisation with reference pattern substances to enable comparison of results between laboratories, are all being introduced to bring these models closer to the *in vivo* reality (Ingels & Augustijns, 2003). As in the case with models based on artificial membranes, models based on cell cultures are excessively static and thus they are subject to successive improvements. Additional effort is being directed towards enabling cells to conveniently express the large variety of protein transporters that is usually found in intestinal epithelial enterocytes. It is important to bear in mind that this line comes from the colon, not from the small intestine, which implies a handicap in expressing the adequate number of transporters, given that more receptors are expressed in the small intestine than in the colon (Seithel, Karlsson, Hilgendorf, Bjorquist, & Ungell, 2006). In order to improve this aspect, Caco-2-derived clonal lines such as TC7 have been developed (Carriere et al., 1994; Reboul et al., 2005; Verma, Hansch, & Selassie, 2007). *In vitro* assimilation models based on cell cultures consist of an experimental scanning technique (high-resolution throughput screening) that is largely used in the pharmaceutical industry to estimate drug assimilation, which uses specialised instruments to perform certain experiments many times in parallel. This model has provided excellent insights of the carotenoid cellular uptake and transport increasing the possibility of data correlation with the results from *in vivo* studies (Chitchumroonchokchai et al., 2004; Chitchumroonchokchai & Failla, 2006; During et al., 2002; During et al., 2005; Moussa et al., 2008; Reboul et al., 2005; van Bennekum et al., 2005).

Preparations of BBMV are a classic model for mechanistic studies of nutrient bioactive compounds or drug transport through the cell membrane. The vesicles, cell membranes in suspension, act as receptors as they preserve the proteinic transporters and membrane enzymes with which they interact together with the nutrients from the donor solution in which they were contacted. The vesicles are obtained by isolating cells from the intestinal mucosa by curettage or vibration and then removing any contaminating tissue or basolateral membrane residues. The purified mucosal cells are fractioned by centrifugation and precipitation into BBMVs separated from the nuclear and mitochondrial fraction. This technique, described by Miller and Crane (1961) has been somewhat modified to improve the purity, stability and yield of the preparation, but overall the technique has remained quite unaltered. The preparation of BBMVs in isotonic buffer is incubated with the donating solution or a suspension that contains the nutrients of interest. After some incubation time, the solution is filtered or centrifuged to separate the BBMV fraction and the buffer. This technique has been used successfully in mechanistic studies of the assimilation of glucose, biliary salts, lipids, xenobiotics, cholesterol and carotenoids (Alcorn, Simpson, Leahy, & Peters, 1991; Burckhardt, Kramer, Kurz, & Wilson, 1983; Fernández-García, Carvajal-Lérida, Rincón, Ríos, & Pérez-Gálvez, 2010; Hopfer, Nelson, Perrotto, & Isselbacher, 1973; Ikeda, Tanaka, Sugano, Vahouny, & Gallo, 1988; Keelan, Burdick, Wirzba, & Thomson, 1992; Kessler et al., 1978; Ling, Lee, & Hollander, 1989; Moore, Gugger, & Erdman, 1996; Proulx, McNeil, Biglez, & Williamson, 1982). Intestinal tissue from pigs, rabbits, chickens, rats, mice, and humans has been used in these studies, although human BBMV studies are less frequent due to the limited availability of tissue in the conditions necessary for these trials. This model also has limitations, mainly because it is only predictive for the step of the absorption process that occurs on the interior of the membrane. Furthermore, the vesicles are in suspension, which is the reason why dynamic conditions are applied to the assay; however, this prevents the differentiation of a basolateral area that represents transport through the membrane.

8. Conclusions

Carotenoid pigments are a group of bioactive compounds that are of interest to the food scientists, nutritionists and food industries due to their positive impact on human health and their economic benefits. Carotenoids are responsible for the attractive colour of most fruit and vegetables, having diverse biological functions and activities. An extensive number of factors determine the efficient incorporation of these phytochemicals from the diet. So, if we want to take advantage of all of these benefits we must consider the bioaccessibility event as a necessary stage in the complex process of converting a visual attraction into biological actions. As it can be appreciated from this review, there is a wide range of experimental models that can be used to obtain information about the events that occurred during each stage of the carotenoid digestive process, as well as the mechanisms involved in every step. Each of these models focuses on a particular aspect (digestibility or assimilation efficiency, structural modifications due to the digestive process or the intestinal metabolism, mechanisms involved in the assimilation or inhibition of assimilation), thus providing information that is relevant to carotenoid bioavailability.

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