Chapter 14
Carotenoids as a Source of Antioxidants in the Diet

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Abstract Carotenoids, widely distributed fat-soluble pigments, are responsible for the attractive colorations of several fruits and vegetables commonly present in our daily diet. They are particularly abundant in yellow-orange fruits (carrots, tomatoes, pumpkins, peppers, among others) and, although masked by chlorophylls, in dark green leafy vegetables. Several health benefits have been attributed to carotenoids or to foods rich in these pigments, by means of different mechanisms-of-action, including the role as provitamin A of almost 50 different carotenoids and the antioxidant activity that protects cells and tissues from damage of free radicals and singlet oxygen, providing enhancement of the immune function, protection from sunburn reactions and delaying the onset of certain types of cancer. Common food sources and the efficiency of the absorption of carotenoids, analytical approaches used for measurement of their antioxidant effect and an overview of some epidemiological studies that have been performed to assess the beneficial impact of carotenoids in human health are outlined in this chapter.

Keywords Health benefits • Antioxidant capacity • Antioxidant activity • Carotenoid dietary source • Cancer markers

14.1 Introduction

The definition of an *antioxidant* is commonly subject to the intended aim or use of the antioxidant substance, the nature of the radical species, how and where they are generated and what target of damage is measured. A working definition of antioxidant is any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge 2007). With this definition several natural compounds families could be defined as antioxidants and the carotenoids are one of them. Carotenoids are fat-soluble natural pigments widely distributed in nature with

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a polyenic skeleton of 40 carbon atoms that presents different structural changes, such as cyclization of one or both ends, hydroxylation, and / or the introduction of oxygenated functions. Carotenoids can be classified in two groups: carotenes which are strictly hydrocarbons, and xanthophylls, which are derived from the former and contain oxygenated functions. The presence of the long, extensive system of conjugated double bonds is responsible for one of the most distinctive characteristics of the carotenoids: light absorption. The main biological function of carotenoids in photosynthetic organisms is energy transfer in photosynthesis and photoprotection (Krinsky 1994). In mammals, the only biological function of some carotenoids is their role as precursors of vitamin A, necessary for vision, growth, cell differentiation, and other physiological processes (Olson 1996). Not all carotenoids present the structural requirements for conversion to vitamin A. Only those with at least one type β-ring without any oxygen function and with a polyenic chain containing at least 11 carbon atoms are potential precursors of vitamin A. With these structural requirements only the 10 % of the 700 carotenoids described so far shows activity of provitamin A. The most relevant provitamin A carotenoids, either for their high activity and wide distribution in food, are α- and β-carotene, some xanthophylls such as β-cryptoxanthin and some *apo*-carotenoids (Mínguez-Mosquera and Hornero-Méndez 1997). From all of them, β-carotene presents the highest provitamin A activity since each carotene molecule produces two retinal molecules that are reduced to vitamin A (retinol). Table 14.1 contains the most representative carotenoids with provitamin A activity and their efficiency compared to β-carotene.

One of the first biological actions described for carotenoids was their characterization as very effective *quenchers* of singlet oxygen (Foote and Denny 1968). The quenching mechanism is based on a physical process by which the excess energy of the oxygen molecule is absorbed by the carotenoid getting

Table 14.1 Provitamin A activity of carotenoids relative to β-carotene

Carotenoid	Activity (%)
all-*trans*-β-carotene	100
9-*cis*-β-carotene	38
13-*cis*-β-carotene	53
all-*trans*-α-carotene	53
9-*cis*-α-carotene	13
13-*cis*-α-carotene	16
all-*trans*-β-cryptoxanthin	57
9-*cis*-β-cryptoxanthin	27
15-*cis*-β-cryptoxanthin	42
β-carotene-5,6-epoxide	21
muthatocrome	50
γ-carotene	42–50
β-zeacarotene	20–40

Data according to Bauernfeind (1972) and Zechmeister (1949)

back the oxygen molecule to its ground energy state. Then, the excess energy of the carotenoid is dispersed through the environment without causing damage to neighboring molecules. The continuous reiteration of this process will finally affect the chemical structure of the carotenoid by reactions involving the addition of singlet oxygen to the polyenic chain yielding carotenoid endoperoxides and carbonyl derivatives. Although a single molecule of β-carotene can quench 1000 singlet oxygen molecules before oxidation, the chemical irreversible reaction takes place, giving an end to the quenching process (Liebler 1993). However, the positive effect of the action is beyond any doubt. Indeed there is a great interest in the development of synthetic chemical derivatives combining the excellent efficiency of carotenoids as quenchers with the ability of flavonoids in stabilizing the radical intermediates formed in the quenching process. Thus, it is possible to synthesize compounds resulting from the fusion of these two groups of phytochemicals like the carotenylflavonoids, with a high quenching capacity and greater stability to the chemical oxidation processes that may have potential applications in sunscreens (Beutner et al. 2007).

However, any other biological action of carotenoids causes greater interest and controversy than the antioxidant action. A helpful notation to understand the relevant features of the antioxidant action is to distinguish *antioxidant action* and *antioxidant activity* concepts, which are different although they are commonly used without distinction. While the antioxidant activity is associated with oxidative processes in vitro, antioxidant capacity is associated with oxidative processes in vivo and really involves a biological action.

14.2 Antioxidant Activity

The term antioxidant activity is defined as the constant rate of the reaction between an antioxidant and radical species. This concept correlates with the chemical reaction process of the antioxidant/radical pair, structure of both substances and the reaction mechanism(s) that take place. Antioxidants may react with radical species mainly by different mechanisms: electron transfer to produce radical cations, reduction, radical adducts formation, and hydrogen atom abstraction. These mechanisms may happen at once but the dominant process depends on the structure of the antioxidant and the characteristics of both the reaction environment and the radical species. Some methods for determining the antioxidant activity may be developed to measure the progress of these mechanisms, while other methodologies measure the progress of one of them. Carotenoids are lipophilic antioxidants and display activity towards reactive oxygen species produced in biological systems. Some of them are the superoxide anions produced within mitochondria, hydroxyl radicals with a very short lifetime, acting close to their site of formation, perhydroxyl radical responsible for initiation of lipid autoxidation. Additionally nitrogen derived radicals may be the target molecules for carotenoid antioxidant activity.

Electron transfer mechanism that yields a carotenoid radical cation takes place when the radical species possess high redox potential (Jomova et al. 2009) while reduction of the carotenoid molecule is a consequence of reduction reactions yielding the corresponding radical carotenoid anion (Bobrowski and Das 1985). These radical intermediates may react with biological substrates and produce oxidative damage (Everett et al. 1996; Miller et al. 1996). The mechanisms reviewed by Britton (1995) and Edge et al. (1997) produce neutral carotenoid radicals. The hydrogen abstraction mechanism only affects those hydrogens in allylic positions to a double bond, which are prone to react with peroxyl radicals. This process generates a resonance-stabilized radical that may continue radical propagation chain. The addition process to the polyenic chain involves the oxidation of the carotenoid via radical addition and the generation of carotenoid-peroxyl adducts that can undergo heterolytic or homolytic breakdown (Liebler 1993). The former process does not continue the radical propagation chain while the latter produces two new reactive species. If the addition reaction takes place at the double bond position of the ring, the product arising is a radical species and the autoxidation process is continued. Figure 14.1 reproduces the reaction mechanisms discussed above and denotes that the production of new radical species during development of the antioxidant process will increase the oxidative potential of the environment

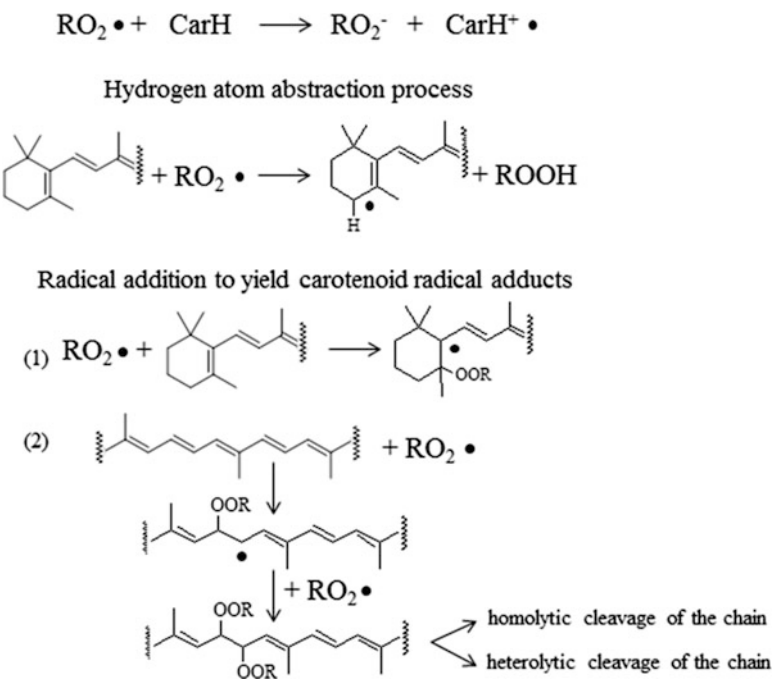

Fig. 14.1 Reaction mechanisms between carotenoids and radical species. (1) Addition to the ring. (2) Addition ot the polyenic chain

where the reaction takes place. When the generation of radical species exceeds the rate at which the antioxidant removes them, the autoxidation process is observed.

Although these reaction mechanisms are the general pattern of the antioxidant activity of carotenoids, the rate of the process depends on the presence of functional groups, so that the structural characteristics of each carotenoid modulate its oxidation and consequently its antioxidant activity. Particularly, the intermediate peroxyl radical is essential in determining the progress of the reaction mechanism because its stabilization by electron delocalization is different depending on the functional groups located at the ends of the polyenic chain. Furthermore, these groups may prevent oxidation progress through one of the reaction mechanisms discussed above and, therefore, reduce the tendency to autoxidation of the carotenoid (Liebler 1993; Pérez-Gálvez and Mínguez-Mosquera 2002).

Two decades ago there was a methodologies span devoted to measurement of antioxidant activity of different natural, synthetic substances, food extracts, and even complete food systems (Cao et al. 1993, 1995; Frankel and Meyer 2000; Karadag et al. 2009; Ou et al. 2001; Prior et al. 2003). The chemistry behind these methods has been detailed in the reviews of Huang et al. 2005 and Prior et al. 2005. Some of the commonly used methods are the oxygen radical absorbance capacity, total radical trapping antioxidant parameter, trolox equivalent antioxidant capacity, total oxyradical scavenging capacity and peroxyl radical scavenging capacity methods (note that the word capacity is used to name these methods). In the case of carotenoids, the methods for determining antioxidant activity generally applied are the one described by Terao (1989) and the TRAP method (Bartosz et al. 1998), methodologies based on the reactions chain of the lipid autoxidation process. Results obtained from the application of these and other chemical assays of the antioxidant activity are worthy for comparison of the activity of different substances and to gain information regarding the influence of the chemical structure for a family of compounds, but they are not good models to determine the behavior of the antioxidant in biological systems.

14.3 Antioxidant Capacity

Antioxidant capacity is defined as the amount of radical species which is removed or neutralized from the reaction environment by the action of a certain amount of antioxidant. This concept is applied to the antioxidant action in tissues and biological samples such as plasma and cell cultures. Measurement methods that are applied to determine the antioxidant capacity of carotenoids are the TEAC method (Re et al. 1999), the DPPH method (Brand-Williams et al. 1995) and the method of ex vivo oxidation of LDLs (Carpenter et al. 1997). These methods are an approximation (more or less realistic) to ascertain the antioxidant capacity of carotenoids. In biological systems, lipids constitute most of the cell membranes and other cellular structures, and lipid oxidation has a negative impact on the functionality of the membrane and its integrity. Since carotenoids are accommodated in such structures,

they are able to delay radical propagation chain generated by lipid oxidation, so that these compounds together with tocopherols are known as membrane antioxidants. Thus, the antioxidant effect of carotenoids in vivo is observed in LDLs and cellular membranes where they exert their ability through the reaction mechanisms described above (Ringer et al. 1991). The fact that carotenoids exercise this capacity in biological systems is one of the main supports to the oxidative stress theory and the promise that a high intake of foods rich in carotenoids will lower the risk of developing degenerative processes. However, the results obtained from analytical approaches designed for estimation of the antioxidant capacity introduce diverse uncertainties about the efficiency of the antioxidant action in such biological systems. Particularly, the ex vivo oxidation of LDLs method evaluates the antioxidant action of carotenoids when they are added to plasma or isolated LDLs, or introduced into them by enriching the diet with supplements or with fruit and vegetables. Most studies show that carotenoids including β-carotene, zeaxanthin, lutein and lycopene are effective antioxidants (Dugas et al. 1998; Panasenko et al. 2000) but some publications report no protection of LDLs to oxidation although they increased their carotenoid content (Carroll et al. 2000; Chopra et al. 1996; Krinsky 1994; Rock et al 1996). This controversy claims to an acute screening of the factors that might affect LDLs proneness to oxidation, not only carotenoid content but also characteristics of the study population, design of the intervention trial, the selected carotenoid source, the efficiency of assimilation of antioxidants present in the diet, i.e. what proportion of the ingested amount is absorbed and therefore bioavailable, possible biotransformation into other compounds with lower antioxidant efficiency, or the generation of new free radicals, as indicated in the description of the antioxidant activity concept, the amount deposited in the tissue where it will develop its action, the nature of the oxidative stress biomarker used to determine the correlation, and the participation of other antioxidants naturally present in the food source (Wright et al. 2002).

14.4 Antioxidant and Non-Antioxidant Actions Related to Modification of Carcinogenesis Markers

One research line regarding the antioxidant action of carotenoids that has generated a huge amount of relevant evidences is based on the link among the initiation and progression of cancerous processes with the presence of free radicals. This association point to the addition of antioxidants, such as carotenoids, to our body as a strategy to positively contribute to risk reduction of cancer. There are evidences about the involvement of free radicals in the initiation and progression of carcinogenesis. Reactive oxygen species act directly as mutagens to oxidize DNA bases, and are also able to activate pre-carcinogens making them reactive substances that modify DNA (Marnett 1987). The continuous decrease of antioxidant defense

levels caused by the activity of reactive oxygen species is another evidence of their involvement in carcinogenesis, as this effect is also produced by the activity of genuine carcinogens (Cerutti 1985; Kensler and Taffe 1986). Consequently, if carotenoids as antioxidants delay the activity of reactive oxygen species, their action may decrease the risk of developing cancer (Malone 1991; Byers and Perry 1992; Voorrips et al. 2000; Holick et al. 2002).

Another beneficial effect derived from the antioxidant capacity of carotenoids is the enhancement of the immune system. UV light produces an increase in the concentration of free radicals that damage the immune system, so that the activity of natural antioxidants limits the negative effect and delays the onset of the reduction of sensitive markers of the immune system. The studies of Fuller et al. (1992) and Herraiz et al (1998) show the positive effect of the intake of β-carotene supplementation on the immune system on volunteers subjected to UV radiation. Nevertheless, carotenoids may change the function of the immune system by non-antioxidant mechanisms which also has a positive significance in the risk of developing cancer, due to the essential role that the immune system in preventing this degenerative process. It has been shown that intake of β-carotene supplementation (50 mg on alternate days for 10–12 years) increased NK activity, natural-killers type cells, which reduce the likelihood of tumor formation (Santos et al. 1996). In this case the mechanism by which β-carotene modulates cellular activity is not known. It has also been suggested that the regulation of the biosynthesis of prostaglandin E2, a recognized immunosuppressant, is due to the intervention of β-carotene in enhancing the immune system (Halevy and Sklan 1987). It can be seen that most of the studies conducted focused exclusively on β-carotene. Experimentation with other carotenoids and their effect on immune function are very infrequent or has remained inconclusive or positive (Hughes et al. 2000).

Improvement of the intercellular communication is an inversely correlated marker with cancer progression. In this case the mechanism of action of carotenoids is not based on the antioxidant action since in most studies focused on the inhibitory effect of carotenoids in the proliferation of neoplastic cells, any correlation among the effect and the antioxidant capacity was found. In fact, the addition of other membrane antioxidants as α-tocopherol did not show inhibitory effect what means the presence of a different mechanism of action. In this case the mechanism of action is based on the ability of carotenoids to increase or re-establishing the intercellular communication that takes place through channels or pores called connexons. Through these various pore cells signaling molecules are exchanged including antiproliferative agents.

Intercellular communication is interrupted in cancer cells because the gene expression of the protein structure forming part of these pores or channels of communication, connexin 43, is decreased. As a consequence communication between cancer cells to normal cells that surround them fails resulting in loss of the control of proliferation of transformed cells (Loewenstein 1979). Conversely, if the intercellular communication between normal and cancer cells is restored, there is a

possibility to delay the proliferation. Several studies demonstrated that carotenoids increase expression of the gene encoding connexin 43, increasing intercellular communication with healthy tissue and decreases proliferation of transformed cells. The mechanism of regulation of gene expression by means of carotenoids is unknown. The prevailing hypothesis is that carotenoids, and particularly some of their metabolic products, are able to activate a series of nuclear receptors (RAR-α and RXR-α) that activate gene expression.

14.5 In vivo Antioxidant Action of Carotenoids. Epidemiological and Intervention Studies

Several prospective an retrospective studies of diet and some types of cancer suggest that dark green and yellow orange vegetables rich in carotenoids reduce cancer risk, particularly linking this correlation with an increased consumption of β-carotene food sources and with increased plasma levels in this carotene. The study of Stähelin et al. (1991) associated bronchus and stomach cancers with a low mean plasma carotene level. Population-based cohort studies have found inverse associations of plasma carotenoids with risk of chronic diseases. The studies of Eichholzer et al. 1996 and Yuan et al. 2001 showed that increased levels of carotenoids other than β-carotene obtained through a high intake of fruit and vegetables are associated with a lower incidence of lung cancer. Association seems to be clear in the case of carotenoids and lung cancer but less consistent results are obtained in the case of other types of cancer and other carotenoids. Indeed, when the positive correlation observed from observational epidemiologic studies among carotenoid intake and cancer risk has been tested with intervention trials, no association or even unexpected negative and harmful effects have been obtained. Three large intervention trials were conducted in 1980s providing harmful effects of high dose (20 mg) β-carotene supplementation (Heinonen and Albanes 1994; Omenn et al. 1996) or no effect (Hennekens et al. 1996). Several questions arose from the results of these intervention trials including possible differences between supplemental and dietary β-carotene, health status of the population receiving the supplementation, high dose effects and different metabolism of β-carotene derivatives in smokers, etc (Upritchard et al. 2003). It has been suggested that excluding of other dietary antioxidants (vitamin C) from intervention trials could have an impact in the results even they could be the source(s) of the cancer prevention potential of fruits and vegetables (Zhang and Omaye 2001). Results of literature survey are definitively confusing and either positive or negative association must be considered with caution. Sufficient evaluation of antioxidant supplements should be provided but this should not point diets rich in fruit and vegetables as harmful. They must be considered as a source of nutritional antioxidants with many health benefits.

14.6 Dietary Sources of Carotenoids

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 six 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 14.2 (for structures see Fig. 14.2, Chaps. 1, 2 and 3). 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 et al. (1993) only included fruits and vegetables as carotenoid-containing foods. Later on, a new database was developed, published by Holden et al. (1999),

Table 14.2 Common fresh fruits and vegetables contributing to the major carotenoids described in human tissues

Lutein/zeaxanthin	Lycopene	α-carotene	β-carotene	β-cryptoxanthin
			Apricot	
Beans			Beans	
Beet			Beet	
			Blueberry	
Broccoli			Broccoli	
Brussel sprouts			Brussel sprouts	
Carrots		Carrots	Carrots	
Celery			Celery	
Coleslaw		Coleslaw	Coleslaw	
Courgette			Courgette	
Cucumber			Cucumber	
Kiwi				
Leeks				
Lettuce			Lettuce	
			Mango	
				Oranges
Parsley			Parsley	
Peas			Peas	
Pepper			Pepper	Pepper
			Plum	
Pumpkin			Pumpkin	
			Spinach	
Sweetcorn				
	Tomatoes			
			Watermelon	

Minimum content of the carotenoid in the food item is 0.1 mg/100 g of food
For a complete compilation of carotenoid content in food sources see O'Neill et al. (2001)

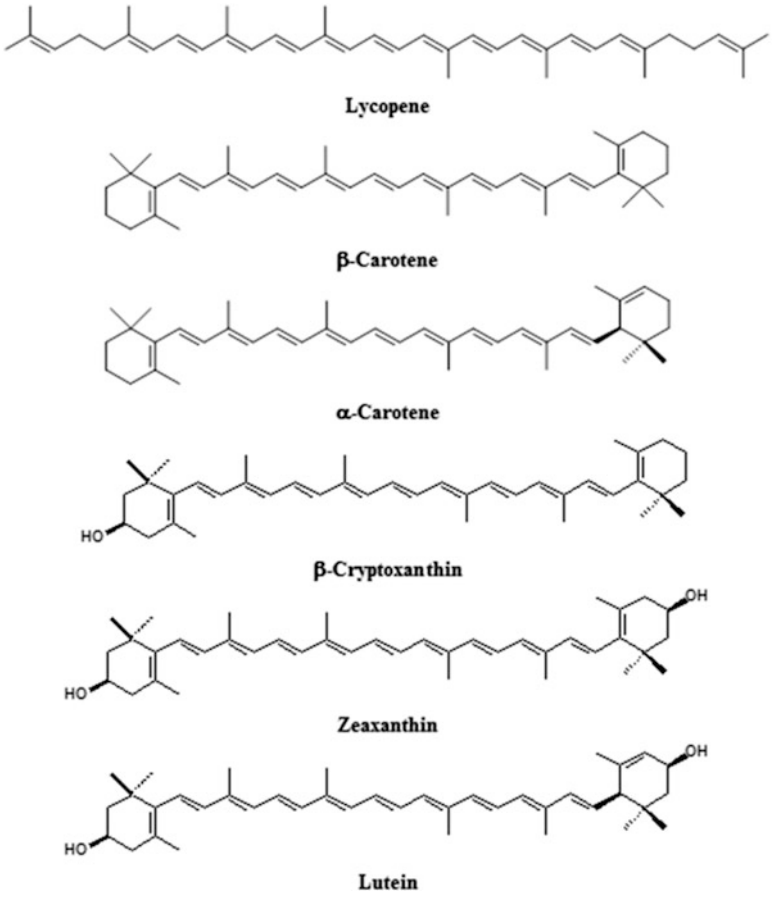

Fig. 14.2 Structure of the main carotenoids described in human tissues

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: http://www.ars.usda.gov/Services/docs.htm?docid=8964 (last accessed 22/12/2014). 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 Linus Pauling Institute Micronutrient Information Center (http://lpi.oregonstate.edu/infocenter/phytochemicals/carotenoids/index.html, last accessed 22/12/2014) including information on α- and β-carotene, lycopene, β-cryptoxanthin, zeaxanthin and lutein. Another complete database is published in the work of O'Neill et al. (2001) with information on the five main dietary carotenoids content in various food

sources consumed in five European countries. 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 ingested along with carotenoids. Neither the health-promoting biological actions that these compounds may have in our bodies (antioxidant capacity, immune enhancement, increased intercellular communication) or the fact that some of them exhibit provitamin A activity have 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. There is currently no recommendation for daily intake of carotenoids, even though a reference value of 6 mg/day has been proposed, based on the contribution of carotenoids with provitamin A activity. Mammals rely on diet to incorporate those carotenoids that develop provitamin A activity as they are metabolized to retinol. It has been shown that 14 μg of β-carotene are necessary to produce 1 μg of retinol, that is, 1 retinol activity equivalent. FAO and WHO recommendations for vitamin A intake are 700–900 μg of retinol per day what should mean a daily intake of 10–13 mg of β-carotene (if this carotenoid would be the only vitamin A source). The importance of other physiological functions (antioxidant and non-antioxidant activities) demands further study of them and of the dose-effect correlation in order to set daily intake values for these compounds.

Thus, individuals who eat a diet rich in fruits and vegetables 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 3–6 mg/day of carotenoids is sufficient to maintain plasma levels of these components (Micozzi et al. 1992; Yong et al. 1994; Zino et al. 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. Bearing in mind the six most representative carotenoids mentioned above, Table 14.2 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 found in minor concentration in some vegetables (Table 14.2), although in ripe red peppers and tropical fruits like papaya in 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. In the last decades an increased interest has been focused on the inclusion of information concerning nutritional content of foodstuffs in the food labeling. Legislation concerning this issue has aimed food producers to offer valuable information, from which the consumer can adapt the selection of food items to cover requirements for key nutrients and to maintain health. The consideration of recommended daily intake values theoretically should promote an increase in nutritional quality of food because manufacturers have to analyze in detail the nutritional composition of their products, assuring that key nutrients are present in the levels indicated on the label and controlling the effect of processing on those compounds in order to restore adequate levels if necessary. Similarly, introducing the term "bioaccessibility" would theoretically promote further changes. Bioaccessibility has been defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract, and thus becomes available for intestinal absorption (i.e. enters the blood stream). Studies concerning health benefits based on functionality of nutrient or bioactive compounds (carotenoids) would be significant if the required amount to achieve the in vitro bioactivity is compared with the bioaccessible amount that can be reached from some natural sources or food formula where the bioactive compound is present. In vitro digestion procedures have been applied to carotenoid-rich meals or formulations with different experimental conditions. Garrett et al. (1999) developed a complete in vitro digestion procedure in combination with Caco-2 cell-culture model to assess bioaccessibility of carotenoids from meals. Effect of various factors on incorporation of carotenoids to mixed micelles was studied (impact of gastric phase, presence or absence of bile extract and pancreatin, and lipid composition). Any source of interaction at the micellization process will reduce efficiency of the digestion step and affects final bioaccessibility. Carotenoids as lipophilic compounds need to be incorporated to mixed micelles to reach epithelial cells so that, the carotenoid amount incorporated to the micellar fraction gives an estimation of the efficiency of the digestion step, and it is usually expressed in terms of percentage with respect to the total initial carotenoid amount. The method of Garret et al. (1999) has been applied to assess bioaccessibility of different test meals and supplements in other studies (Ferruzzi et al. 2001; Garrett et al. 2000). Other procedures just apply the in vitro digestion model and did make use of the assimilation stage with a cell culture-based model. The experimental approach developed by Granado-Lorencio et al. (2007) is an optimization of the validated method of Oomen et al. (2003) and includes a complete in vitro digestion procedure (simulation of mastication and saliva solution, gastric and intestinal phases) with the addition of human pancreatic lipase and other specific enzymes (cholesterol esterase, phospholipase A2) to achieve more physiological conditions. The method applied by Hedrén et al. (2002) applies an in vitro digestion method to estimate the maximum amount of carotenoids released from food matrix without isolation of micellar fraction and determination of cellular uptake. The latter studies and others (Breithaupt et al. 2002; Fernández-García et al. 2007, 2008; Serrano et al. 2005) have been performed with the aim of establishing in detail the factors involved

on the micellization process and its efficiency, including the effect of composition of the lipid environment and the activity of pancreatic lipases on hydrolysis of xanthophylls esters. Strength of the in vitro protocols arises from their consistency with the results obtained from in vivo studies. Thus, most of the carotenoid bioaccessibility values obtained through in vitro assessment and predictions of changes in bioaccessibility due to dietary factors or physiological modifications, outlined from those procedures, are similar to the in vivo observations. However it must be stressed that an in vitro vs. in vivo validation process should be carried out to delimitate reliability of the in vitro models.

14.7 Conclusions

One of the factors setting the cause and progress of some chronic diseases like cancer is the imbalance in tissues between free radical species and antioxidants of either endogenous or diet origin. The wide array of free radicals structures, their different sites of formation and activity and the reactions chain they initiate are clearly compensated by the high diversity of antioxidants found in common dietary sources from which fruits and vegetables are of outstanding interest. Epidemiological and case–control trials clearly point to protective effects regarding the onset of degenerative diseases. In vitro studies of the antioxidant activity with the use of different analytical approaches obtain valuable comparative information about influence of structures features and characteristics of reaction environment. A more complicated picture is obtained from the application of ex vivo experiments as different factors might affect results although a close approximation to the antioxidant action of the substance in biological systems is obtained. Controversial results appear when in vivo measurements of the effect of dietary antioxidants are measured through intervention trials or case–control studies. In these cases conclusions either positive or negative must be considered with caution as several aspects deserve thoughts when dissection of the results is made. Carotenoids do not escape from these considerations. It has been demonstrated that they are good antioxidants through a wide arrange of methodologies suitable to their lipophilic nature, and the application of ex vivo tools of measurement also pinpoint this family of pigments as compounds with potential benefits to health. These features joined to their wide distribution in fruits and vegetables consumed in our diet make carotenoids as one group of possible dietary chemopreventive agents. However with data obtained so far it is not advisable to recognize one single family of compounds as responsible of health benefits of dietary compounds but the complex and cooperative cocktail of phytochemicals that fruits and vegetables contribute to our body.

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