In: Carotenoids
Editors: Hanne Leif and Felix Ulrik
ISBN: 978-1-53612-685-3

Chapter 1

CAROTENOIDS AS FUNCTIONAL INGREDIENTS

Jorge Milán Carrillo, PhD[1], Saraid Mora Rochín, PhD[1,*] and Cuauhtémoc Reyes Moreno, PhD[1],

[1]Facultad de Ciencias Químico Biológicas,
Universidad Autónoma de Sinaloa,
Culiacán Sinaloa, México

ABSTRACT

Normal human metabolism produces free radicals, and the over production of these biomolecules leads to increased oxidative stress and contributes to the development of chronic diseases. Recent studies have shown that secondary plant metabolites contain bioactive compounds with functional properties and beneficial effects for human health. The consumption of fruits, vegetables, and grains (wheat and maize) has increased due to their bioactive composition and antioxidant potential, which could mitigate the damage and negative effects of the oxidative stress process. Carotenoids are considered nutraceuticals for their functional properties and their role as antioxidants, acting as physical quenchers of singlet oxygen, and scavengers of other reactive oxygen species. Carotenoids are considered the most important group of pigments with approximately 750 moieties known. Carotenoids such as lutein,

[*]Corresponding Author: Blvd. de las Américas y Josefa Ortiz de Domínguez, Ciudad Universitaria, Facultad de Ciencias Químico Biológicas, Culiacán Sinaloa México. Email: smora@uas.edu.mx.

zeaxanthin, β-cryptoxanthin, carotenes, and their metabolites play a protective role in reactive oxygen species-mediated disorders such as: cardiovascular disease, some types of cancer, and neurological, as well as photosensitive and eye-related disorders. While fruits and vegetables constitute the main source of carotenoids in human diet, recent studies have shown that grains like maize are a functional source of carotenoids, mainly of lutein and zeaxanthin. This chapter is focused on the role of food carotenoids in the prevention of oxidative stress-induced human diseases.

Keywords: carotenoids, antioxidants, chronic diseases, functional ingredients

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by different cellular and metabolic sources, exposure to different physiochemical conditions or pathological states, and their accumulation in the human system is widely considered to cause chronic diseases (Valko et al. 2006; Rao and Rao 2007). ROS and RNS are well recognized for playing a dual role, both deleterious and beneficial on living systems (Valko et al. 2007). The most common ROS include: hydroxil, peroxide anion, and superoxide anion radicals. Under normal physiological conditions superoxide anion and the nitric oxide system are the main radicals generated by cells. These free radicals can alter proteins, lipids, and DNA triggering a number of human diseases (Finkel and Holbrook 2000; Rao and Rao 2007). Hence, the use of antioxidants can help the body tolerate the oxidative stress-induced damage (Dasgupta and Klein 2014; Baiano and Del Nobile 2016).

It has been demonstrated that plants contain secondary metabolites responsible for their bright colors. These bioactive compounds are phytochemicals known to possess antioxidant capacity and functional properties that are physiologically important for protecting human health. Animals and humans are unable to synthesize carotenoids de *novo*, therefore they must include them in a healthy diet (Stahl and Sies 2005; Eldahshan and Singab 2013; Alfieri et al. 2014).

The most abundant types of antioxidants in fruits, vegetables, and grains include: vitamin C, phenolic compounds and carotenoids, whereas tocotrienols and tocopherols are present in lower concentrations compared with grains (Kalt 2005). Epidemiological studies have shown that antioxidant compounds such as vitamin E and carotenoids (β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin) contribute to prevent degenerative disorders, such as cardiovascular disease (CVD), diabetes, and several types of cancer (Rodriguez-Amaya and Kimura 2004; Maughan 2005; Singh and Goyal 2008; Gul et al. 2015).

The carotenoids comprise approximately 750 structurally different compounds of phytonutrients and 50 of them are present in a typical human diet. More than 20 carotenoids have been identified in human blood and tissues (prostate, eye macula and liver) (Jain et al. 1999; Khachik 2006; Prabhashankar-Arathi et al. 2015). Carotenoids are considered of great importance for their biological functions and are among the most abundant families of pigments in nature. They give fruits, vegetables, and grains their distinctive yellow, orange, and red color (Ndolo and Beta 2013; Corrales-Bañuelos et al. 2016). Carotenoids are important not only for coloring food, but as dietary sources of vitamins, and for their reported association with the reduction rates of coronary heart disease and some varieties of cancer (Figure 1). The first correlation between a high intake of carotenoids and health benefits appeared in the literature in the 1970's (Fraser and Bramley 2004). Thus far, the antioxidant and functional properties of carotenoids have been a major focus of research (Rao and Rao 2007; Gul et al. 2015; Corrales-Bañuelos et al. 2016).

Considering a nutritional approach, carotenoids are considered to be important bioactive compounds, and dietary ingestion as the only source to meet their requirements in humans and animals (Prabhashankar-Arathi et al. 2015). Recent studies indicate that fruits and vegetables are an important source of carotenoids in the human diet (Sommerburg et al. 1998; Rao and Rao 2007; Fiedor and Burda 2014). However, some grains may contain higher, while others lower content of carotenoids compared to fruits and vegetables (Abdel-Aal et al. 2002; Ndolo and Beta 2013). Although grains contain other bioactive compounds that complement those found in fruits and vegetables (Liu 2007).

Cereals such as maize have a complex matrix rich in bioactive compounds such as carotenoids, phenolics, and minerals, all of which have been reported to have antioxidant properties and to be associated with the prevention of chronic diseases (Hänsch and Mendel 2009; Da Silva-Messias et al. 2015). To date, there is a world-wide interest in functional foods. However, more information is needed regarding the levels of carotenoids that have health enhancing properties in fruits, vegetables and grain cereals. The purpose of this review is to discuss the literature focused on the functional properties and the beneficial effects of carotenoids in human health.

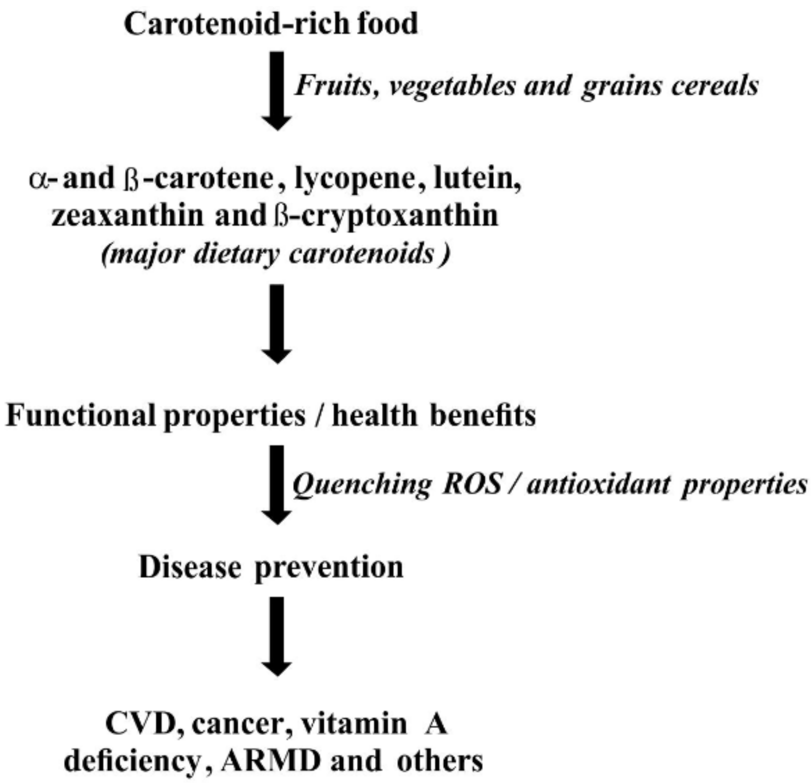

Figure 1. Functional properties of food carotenoids.

Chemical Properties of Carotenoids

Carotenoids are a family of pigmented compounds synthesized by plants and microorganisms (bacteria, yeast, and fungi). However, humans and animals cannot synthesize them (Rao and Rao 2007; Britton et al. 2009). In plants, carotenoids contribute to the photosynthetic machinery and protect them against photo-damage (Rao and Rao 2007; Fiedor and Burda 2014), while in animals, such as insects, fish, crustaceans and birds, carotenoids are responsible for their characteristic color (Stahl and Sies 2005). Carotenoids are intracellular compounds usually synthesized in membranes of mitochondria, chloroplasts, or endoplasmic reticulum (Margalith 1999). Carotenoids are highly hydrophobic and tend to be associated with lipids (oil, fat) or in hydrophobic structures such as membranes (Britton et al. 2008). Carotenoids of fruits, vegetables, and animal products are usually fat-soluble and are associated with lipid fractions, lipid portions of human tissues, cells (El-Qudah 2008), and the lipid core of the bilayer membrane (Britton et al. 2008). The orientation of a particular carotenoid in the membrane and its effect on membrane properties depends on its structural features such as the size and shape of the carotenoid, and the presence of functional groups (Britton et al. 2008).

Carotenoids are tetrapernoids composed by 40 carbon atoms built from four terpene units, each of these containing 10 carbon atoms. Their main chemical functions are determined by the extended system of conjugated double bonds, which is also responsible for their color (Britton 1995). They are categorized into two major groups: 1) those containing oxygen, known as xanthophylls (e.g., lutein, zeaxanthin, α-and β-cryptoxanthin), and 2) those that contain only hydrocarbons and do not contain oxygen, known as carotenes (e.g., α- and β-carotene, and lycopene) (Baiano and Del Nobile 2016) (Figure 2). Therefore, depending on the number of double bonds, a certain array of *cis*/*trans* (E/Z) configurations is possible for a given carotenoid (Britton et al. 1995).

The all-*trans* configurations are ubiquitously present, while several *cis* isomers of carotenoids are present in blood and tissues such as prostate, breast, adrenal glands, and liver (Jain et al. 1999; Melendez-Martinez et al. 2004; Yahia and Ornelas 2010). However, when fruits and vegetables are processed, it results in an increase from 10 to 39% in *cis*-isomers. The all-*trans* structure is thermodynamically most stable (Yahia and Ornelas-Paz 2010). Nonetheless, the degree of isomerisation is directly correlated with the intensity and duration of the heating process (Eldahshan and Singab 2013). It has been observed that high temperatures and short times are preferred conditions for processing carotenoid-containing foods, yet extreme heat can result in oxidative destruction of carotenoids (Agarwal and Rao 2000; Delgado-Vargas and Paredes-López 2003). Other factors that can influence the stability and nutritional value of matrix foods include temperature, light, and pH (Melendez-Martinez et al. 2004). However, it is important to keep in mind, that food processing and heat treatment cause the mechanical breakdown of the tissue, releasing the carotenoids and improving their absorption (Gärtner et al. 1997). The type of food matrix in which carotenoids are located is a major factor influencing their bioavailability (Haskell 2012; Gul et al. 2015).

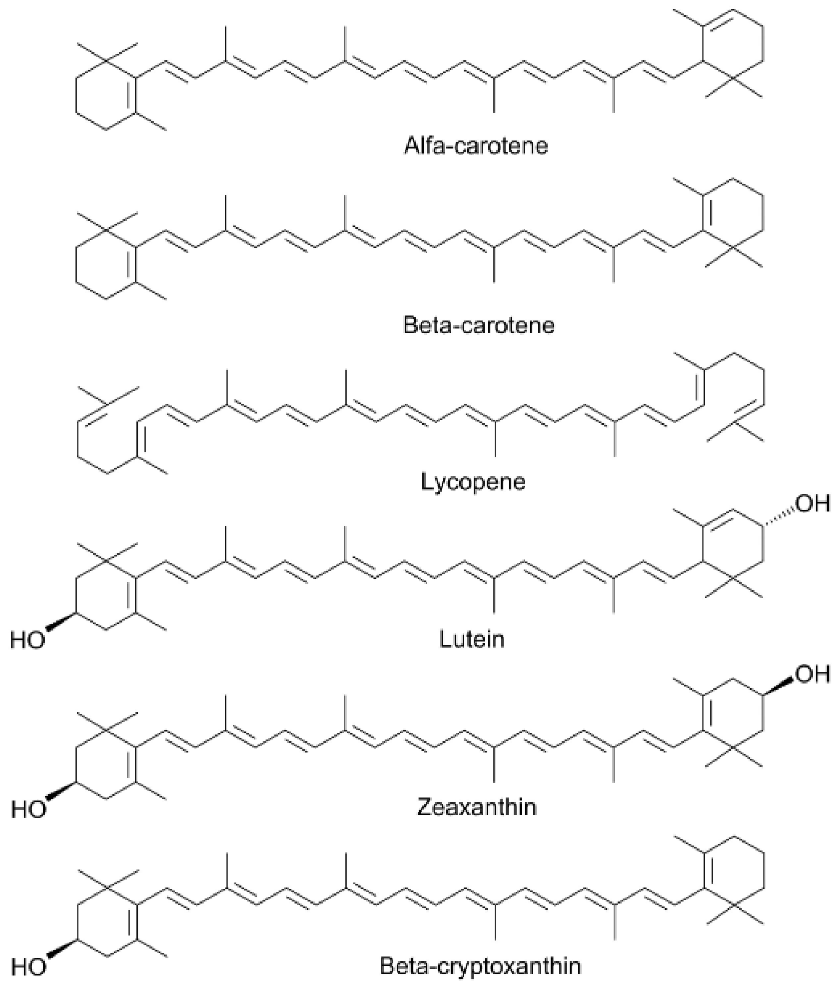

Figure 2. Chemical structure of major dietary carotenoids.

The relatively low bioavailability of carotenoids from natural sources has been attributed to the fact that they exist either as crystals or are located within protein complexes that are not fully released in the gastrointestinal tract during digestion (Williams et al. 1998; Gul et al. 2015).

The mechanism of intestinal absorption of carotenoids occurs by passive diffusion after being incorporated into the micelles, which are formed by dietary fat and bile. They are then incorporated into lipoproteins and released into the blood stream (Olson 1994; Parker 1988; Rao and Rao 2007). It has been reported that only about 5% of the carotenoids are absorbed in the intestine, whereas >50% are absorbed from micellar solution (Olson 1994). Because there are numerous factors affecting the bioavailability, absorption, transport, metabolism, and storage of carotenoids (Fiedor and Burda 2014), the exact mechanism by which carotenes exert their actions *in vivo* are still far from being understood.

Functional Properties of Carotenoids

Antioxidant Capacity of Carotenoids

Reactive oxygen and nitrogen species are generated during aerobic metabolism and pathological processes, such species are highly reactive and can induce the irreversible oxidation of diverse biomolecules such as lipids, proteins, carbohydrates, and DNA (Valko et al. 2006; Dasgupta and Klein 2014). Lipids are the major targets for oxidative damage induced by free radicals. Lipid peroxidation generates cell membrane damage by altering the membrane fluidity and permeability (Aikens and Dix 1991; Dasgupta and Klein 2014). Oxidation of proteins by ROS and RNS involves side chains of all amino acid residues of proteins (Stadtman 2004). Likewise, hydroxyl radicals are known to damage DNA, leading to disorders in purine and pyrimidine bases and the deoxyribose backbone (Halliwell and Gutteridge 1999). DNA damage is implicated in mutagenesis, carcinogenesis, and aging. Oxidative stress can also damage mitochondrial DNA and lead to disorders associated with aging and cancer development in various tissues (Dasgupta and Klein 2014).

Carotenoids and other bioactive compounds provide protection against some types of cancer, coronary vascular disease (CDV), age-related macular degeneration (ARMD), and the risk of vitamin A deficiency (Jaswir et al. 2011; Yahia and Ornelas-Paz 2010).

The way carotenes exert their protective actions most likely involve the scavenging of two of the ROS: singlet molecular oxygen, and peroxyl radicals (Truscott 1990; Valko et al. 2007). They are also effective deactivators of electronically excited molecules involved in the generation of radicals and singlet oxygen (Truscott 1990; Young and Lowe 2001).

The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties, and their ability to trap peroxyl radicals. The quenching activity of a carotenoid is mainly attributed to the number of conjugated double bonds of its structure while is influenced to a lesser extent by carotenoid groups (cyclic or acyclic), or the nature of substituents in carotenoids containing cyclic end groups (Krinsky 1998; Eldahshan and Singab 2013). Until now, it has been concluded that the number of conjugated double bonds is the most effective parameter for quenching properties because the activity of carotenoids increases as the number of the conjugated double bonds in the carotenoids increases. Furthermore, the bioactivity of carotenoids has been associated with their antioxidant capacity *in vivo* and *in vitro* (Delgado-Vargas and Paredes-López 2003; Tanaka et al. 2012). For instance, β-carotene treatment in human dermal fibroblasts (FEK4) exposed to UVA light was able to suppress the heme oxygenase-1 gene upregulation in a dose dependent manner (Elliott 2005). Likewise, in human liver cells (HepG2), carotenoids were able to decrease the oxidant-induced lipid peroxidation (Martin et al. 1996).

β-carotene is a promising potent antioxidant because of its ability as a singlet oxygen quencher. Such antioxidant capacity is due to the structure of conjugated double bonds and ionone rings, with capability of quenching up to 1,000 free radicals per molecule. Thus, it can prevent oxidative damage, alter transcriptional activity and prevent cellular damage (Abdel-Aal and Akhtar 2006; Singh and Goyal 2008; Qian et al. 2012; Gul et al. 2015). A study by Sindhu et al. (2010) reported lutein as a potent antioxidant *in vivo*. The authors gave oral lutein to mice for a month and observed an increase in the activity of the antioxidant defense enzymes: superoxide dismutase, catalase, glutatione reductase, and glutatione in blood and liver when compared to controls. Likewise, liver glutatione peroxidase and glutatione-S-transferase also increased their activity. On the other hand, it is important to note that high carotenoid concentrations may also induce a pro-oxidative effect, which could be modified by interactions with other nutrients (Eldahshan and Singab 2013).

Health Benefits of Carotenoids

Provitamin A Carotenoids and Prevention of Vitamin A Deficiency and Eye-Related Disorders

Carotenoids are divided in two classes: provitamin A and non-provitamin compounds. The contribution of provitamin A carotenoids to the daily vitamin A intake depends on dietary habits and available food sources. It has been estimated that carotenoids from fruits and vegetables provide more that 70% of the vitamin A intake in third world countries. However, intake must be corrected for bioequivalence, evaluating what portion of the ingested provitamin A carotenoids is absorbed, metabolized, and available as retinol or retinyl ester (Het-Hof et al. 2005; Stahl and Sies 2005).

Several studies have shown that the bioequivalence of plant-derived provitamin A carotenoids is much less than anticipated from studies using dietary supplements. As previously discussed, bioavailability and metabolism of carotenoids are affected by several factors including food matrix properties, preparation of the food, co-ingestion of fat and fiber, gastrointestinal tract diseases, vitamin A status, and malnutrition (Stahl and Sies 2005).

Provitamin A carotenoids such as α-, β-carotene, and β-cryptoxanthin, provide the primary dietary source of vitamin A. While α-carotene and β-cryptoxanthin produce one molecule of vitamin A, two molecules are generated from β-carotene. Hence, the consumption of provitamin A carotenoids can prevent vitamin A deficiency. Vitamin A deficiency is a serious global health problem that affects many people, especially children. In humans, this deficiency is associated with impaired iron mobilization, growth retardation, blindness, depressed immune response, increased susceptibility to infectious disease, and increased childhood mortality (Wurtzel et al. 2012). Carotenoids, particularly lutein and zeaxanthin, offer protection against eye diseases such as cataract and ARMD (Botella-Pavia and Rodríguez-Concepción 2006; Alfieri et al. 2014). Most likely, the mechanism of action of carotenoids for reducing the incidence of these diseases is quenching ROS (Fraser and Bramley 2004). Interestingly, it has been shown that the addition of lycopene to cell cultures prevents vacuolization in human lens epithelial cells (Mohanty et al. 2002). Likewise, other reports have shown the presence of lutein and zeaxanthin in the human macula, suggesting a role of these biocompounds in the macula protection from light-induced damage and scavenging of free radicals formed in the photoreceptors. However, it is still challenging to understand the presence of these specific carotenoids in the eye macula (Bone et al. 1997).

Carotenoids and Prevention of Coronary Vascular Disease

Carotenoids, as lipophilic compounds, are efficient scavengers of ROS within the hydrophobic parts of cell membranes and lipoproteins (Agarwal et al. 2012). ROS-mediated damage in atherosclerosis (hardening of the arteries) is the result of oxidative modification of low-density lipoproteins (LDL) in the arterial walls leading to coronary heart disease (Vogiatzi et al. 2009; Eldahshan and Singab 2013). This hypothesis is supported by observational and epidemiological studies that report that carotenoids, antioxidant vitamins, and the consumption of processed-tomato products can reduce LDL oxidation and oxidative stress at the site of the plaque formation (Mayne 1996; Hadley et al. 2003).

β-carotene supplementation (with or without aspirin) results in a significant reduction of vascular accidents in individuals with ischemic heart disease (Hennekens et al. 1996). However, clinical and intervention studies are contradictory. In some cases, it can be clearly observed a positive effect of β-carotene on cardiovascular disease, while in others, little or no correlation between them can be found, and sometimes an inverse relationship has been reported (Fiedor and Burda 2014).

Due to the abundance of CVD, studies on factors that prevent or delay its development are of special importance. The results of reports of the association of carotenoids (mainly β-carotene, α-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin) with the risk of CVD and atherosclerosis have been summarized by Mayne (1996) and more recently by Voutilainen et al. (2006). These authors pointed out that there is a positive correlation between a high intake of carotenoid-rich fruits and vegetables and the reduction of CVD morbidity and mortality (Fiedor and Burda 2014). However, the biological mechanisms for such protection are currently unclear.

Carotenoids and Cancer Chemoprevention

Carotenoids by themselves or in combination with other bioactive compounds could protect from cancer risk due to their antimutagenic properties and their well-known ability to scavenge free radicals, delay tumor development, and improve the immune response (Tanaka et al. 2012).

Carotenoids have been extensively studied using *in vitro* experiments. These studies have shown that carotenoids can inhibit cell proliferation, transformation and micronucleus formation, and can modulate gene expression (Fraser and Bramley 2004). Likewise, epidemiological studies have demonstrated an association between a diet rich in β-carotene and the reduction of risk of cancer in stomach, lung, and other organs (Le-Marchand et al. 1993; van Poppel 1993; van Poppel and Goldbohom 1995; Eldahshan and Singab 2013). Similarly, *in vivo* studies have demonstrated that β-carotene can delay or prevent the induction of sarcomas and skin cancer in mice exposed to carcinogens (Smigel 1990; Eldahshan and Singab 2013).

Lycopene has been shown to be the main bioactive compound in tomatoes. Since 1995 Giovannuci and colleagues revealed an inverse relationship between the consumption of tomatoes and the risk of prostate cancer. Interestingly, in humans there is an increase in lycopene in serum and prostate when they consume tomatoes processed by heat treatment and oil vs.

when they consume raw tomatoes (Stahl and Sies 1992; Gärtner et al. 1997; Van Breeman et al. 2002; Giovannucci 2005). It has been also reported that tomato-based lycopene is more effective than lycopene supplementation (Agarwal and Rao 1998; Giovannucci 2005). Thus, suggesting that foods naturally rich in lycopene can be used in the prevention of prostate cancer. Besides prostate cancer, the protective effect of lycopene has been reported in other varieties of cancer including cervical, ovarian, breast, lung, gastrointestinal, and pancreatic cancer (Giovannucci 1999). Lycopene has also shown to decrease breast cancer risk. The mechanism of action of lycopene in the inhibition of breast cancer is associated with inhibition of cell cycle progression at the G_1 phase (Nahum et al. 2001). Similarly, *in vivo* studies have showed that β-carotene can delay or inhibit the development of sarcomas and skin cancer in mice exposed to carcinogens (Smigel 1990).

To date, α- and β-carotene, β-cryptoxanthin, lutein, and zeaxanthin are the major carotenoids with functional properties, and have been vigorously evaluated for their potential chemopreventive ability (Tanaka et al. 2012).

CONCLUSION

Carotenoids are important bioactive compounds known for their role as antioxidants and scavengers of free radicals. Carotenoids are found ubiquitously in fruits, vegetables, and grains, and to include these carotenoid-rich foods in a regular diet has been reported to be beneficial for human health. Evidence from epidemiological and clinical studies support a strong correlation with dietary intake of carotenoids and the reduction of vitamin A deficiency, age-related macular degeneration, certain types of cancer, and cardiovascular disease. Although, the antioxidant properties of some varieties of carotenoids have been reported *in vivo* and *in vitro*, these studies are mainly focused on provitamin A carotenoids. Thus, more studies that include non-provitamin compounds are necessary. In addition, future studies using dietary supplements should consider the stage of oxidative damage in order to establish the appropriate antioxidant supplementation. Only through such studies, our understanding of the role played by carotenoids and other bioactive compounds will be enhanced. Then, we would be able to develop complementary strategies for the prevention, treatment and management of human diseases.

REFERENCES

Abdel-Aal, E. S. M. and Akhtar M. H. (2006). Recent advances in the analyses of carotenoids and their role in human health. *Current Pharmaceutical Analysis, 2*, 195-204.

Abdel-Aal, E. S. M., Young, J. C., Wood, P. J., Rabalski, I., Hucl, P., Falk D., Frégeau-Reid, J. (2002). Einkorn: A potential candidate for developing high lutein wheat. *Cereal Chemistry, 79*, 455-457.

Agarwal, M., Parameswari, R. P., Vasanthi, H. R., Das, D. K. (2012). Dynamic action of carotenoids in cardioprotection and maintenance of cardic health. *Molecules, 17*, 4755-4769.

Agarwal, S. and Rao, A. V. (2000). Carotenoids and chronic diseases. *Drug Metabolism and Drug Interaction, 17*, 189-210.

Agarwal, S. and Rao, A. V. (1998). Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. *Lipids, 33*, 981-984.

Aikens, J. and Dix, T. A. (1991). Perhydroxyl radical (HOO•) initiated lipid-peroxidation-the role of fatty-acid hydroperoxides. *Journal Biological Chemistry, 266*, 15091-15098.

Alfieri, M., Hidalgo, A., Berardo, N., Redaelli, R. (2014). Carotenoid composition and heterotic effect in selected Italian maize germplasm. *Journal of Cereal Science, 59*, 181-188.

Baiano, A. and Del Nobile, M. A. (2016). Antioxidant compounds from vegetable matrices: Biosynthesis, occurrence, and extraction systems. *Critical Review in Food Science and Nutrition, 56*, 2053-2068.

Bone, R. A., Landrum, J. T., Friedes, L. M., Gomez, C. M., Kilburn, M. D., Menendez, E., Vidal, I., Wang, W. (1997). Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Experimental Eye Research, 64*, 211-218.

Botella-Pavia, P. and Rodríguez-Concepción, M. (2006). Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum, 126*, 369-381.

Britton G. (1995). Carotenes by their chemical structure. *American Journal of Nutrition*, *47*, 1990-2001.

Britton, G., Liaaen-Jensen, S., Pfander, H. (1995). *Carotenoids,* Vol. 1B*: Spectroscopy*, Basel, Switzerland: Birkhäuser Verlag.

Britton, G., Liaaen-Jensen, S., Pfander, H. (2009). Carotenoids. In: *Nutrition and health*, Vol. 5. Ch. 1. Basel, Switzerland: Birkhäuser Verlag.

Britton, G., Liaaen-Jensen, S., Pfender, H. (2008). *Special Molecules*: *Special properties*. *In*: *Carotenoids: Natural Functions,* Vol. 4 Ch. 1. Britton, G., Liaaen-Jensen, F., Pfander, H. (Eds.). Basel, Switzerland: Birkhäuser Verlag, pp. 1-6

Corrales-Bañuelos, A., Cuevas-Rodríguez, E. O., Gutiérrez-Uribe, J. A., Milán-Noris, E. M., Reyes-Moreno, C., Milán-Carrillo, J., Mora-Rochín, S. (2016). Carotenoid composition and antioxidant activity of tortillas elaborated from pigmented maize landrace by tradicional nixtamalization or lime cooking extrusion process. *Journal of Cereal Science, 69*, 64-70.

Dasgupta A. and Klein K. (2014). Antioxidants in food, vitamins and supplements: *Prevention and treatment of disease* (1rs ed.). San Diego, CA: Elsevier.

Da Silva-Messias, R., Galli, V., Delmar-Dos, S., Silva, A., Artigas-Schirmer, M., Valmor-Rombaldi, C. (2015). Micronutrient and Functional Compounds Biofortification of Maize Grains. *Critical Reviews in Food Science and Nutrition*, *55*, 123-139.

Delgado-Vargas, F. and Paredes-López, O. (2003). *Natural Colorants for Food and Nutraceutical Uses* (1rs ed.). Boca Raton, FL: CRC PRESS.

Eldahshan, O. A. and Singab, A. N. B. (2013). Carotenoids. *Journal of Pharmacognosy and Phytochemistry, 2*, 225-234.

Elliott, R. (2005). Mechanisms of genomic and non-genomic actions of carotenoids. *Biochimica et Biophysica Acta*, *1740,* 147-154.

El-Qudah, J. M. (2008). Dietary intake of selected common vegetable foods and their total carotenoids determination. *American Journal of Agricultural and Biological Sciences*, *3*, 729-733.

Fiedor, J. and Burda, K. (2014). Potential role of carotenoids as antioxidants in human health and disease. *Nutrients, 6*, 466-488.

Finkel, T. and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of aging. *Nature, 408*, 239-247.

Fraser, P. D. and Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research, 43,* 228-265.

Gärtner, C., Stahl, W., Sies, H. (1997). Lycopene is more biovailable from tomato paste than from fresh tomatoes. *American Journal of Clinical Nutrition, 66*, 116-122.

Giovannucci, E. (2005). Tomato products, lycopene, and prostate cancer: a review of the epidemiological literature. *Journal of Nutrition*, *135*, 2030-2031S.

Giovannucci, E. (1999). Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *Journal of the National Cancer Institute, 91*, 317-333.

Gul, K., Tak, A., Singh, A. K., Singh, P., Yousuf, B., Wani, A. A. (2015). Chemistry, encapsulation, and health benefits of β-carotene- A review. *Cogent Food and Agriculture, 1*, 1-12.

Hadley, C. W., Clinton, S. K., Schwartz, S. J. (2003). The consumption of processed tomato products enhances plasma lycopene concentrations in association with a reduced lipoprotein sensitivity to oxidative damage. *Journal of Nutrition, 133,* 727-732.

Halliwell, B. and Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine* (3rd ed.). Oxford, UK: Oxford University Press.

Hänsch, R. and Mendel, R. R. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology, 12,* 259-266.

Haskell, M. J. (2012). The challengue to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion-evidence in humans. *American Journal of Clinical Nutrition, 96*, 1193S-1203S.

Hennekens, C. H., Buring, J. E., Manson, J. E., Stampfer, M., Rosner, B., Cook, N. R., Belanger, C., LaMotte, F., Gaziano, J. M., Ridker, P. M., Willet, W., Peto, R. (1996). Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal Medicine, 334*, 1145-1149.

Het-Hof, K. H., West, C. E., Wetstrate, J. A., Hautvast, J. G. (2005). Dietary factors that affect the bioavailability of carotenoids. *Journal of Nutrition, 130*, 503-506.

Jain, C. K., Agarwal, S., Rao, A. V. (1999). The effect of dietary lycopene on bioavailability, tissue distribution, in-vivo antioxidant properties and colonic preneoplasia in rats. *Nutrition Research, 19*, 1383-1391.

Jaswir, I., Noviendri, D., Hasrini, R. F., Octavianti, F. (2011). Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry. *Journal of Medicinal Plants Research, 5*, 7119-7131.

Kalt, W. (2005). Effects of production and processing factor on major fruit and vegetable antioxidants. *Journal of Food Science, 70*, R11-R19.

Khachik, F. (2006). Distribution and metabolism of dietary carotenoids in humans as a criterion for developments of nutritional supplements. *Pure and Applied Chemistry, 78,* 1551-1557.

Krinsky, N. I. (1998). Overview of lycopene, carotenoids, and disease prevention. *Proceeding of the Society for Experimental Biology and Medicine, 218*, 95-97.

Le-Marchand, L., Hankin, J. H., Kolonel, L. N., Beecher, C. R., Wilkens, L. R., Zhao, L. P. (1993). Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiology Biomarkers and Prevention, 2*, 182-187.

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science, 46*, 207-219.

Margalith, P. Z. (1999). Production of ketocarotenoids by microalgae. *Applied Microbiology and Biotechnology, 51*, 431-438.

Martin, K. R., Failla, M. L., Smith, J. C. (1996). Beta-carotene and lutein protect HepG2 human liver cells against oxidant-induced damage. *Journal of Nutrition, 126*, 2098-2106.

Maughan, R. (2005). Basic metabolism II: Carbohydrate. *Surgery (Oxford), 23*, 154-158.

Mayne, S. T. (1996). Beta-carotene, carotenoids, and disease preventions in humans. *FASEB Journal*, *10*, 690-701.

Melendez-Martinez, A. J., Vicario, I. M., Heredia, J. (2004). Stability of carotenoid pigments in foods. *Archivos Latinoamericanos de nutrición, 54*, 2009-2015.

Mohanty, I., Joshi, S., Trivedi, D., Srivastava, S., Gupta, S. K. (2002). Lycopene prevents sugar-induced morphological changes and modulates antioxidant status of human lens epithelial cells. *British Journal of Nutrition, 88*, 347-354.

Nahum, A., Hirsch, K., Danilenko, M., Watts, C. K. W., Prall, O. W. J., Levy, J., Sharoni, Y. (2001). Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells associated with reduction in cyclin D levels en retention of p^{27KipI} in the cyclin E-cdk2 complexes. *Oncogene, 20*, 3428-3436.

Ndolo, V. U. and Beta, T. (2013). Distribution of carotenoids in endosperm, germ, and aleurone fractions of cereal grain kernesl. *Food Chemistry, 139*, 663-671.

Olson, J. A. (1994). Absorption, transport, and metabolism of carotenoids in humans. *Pure and Applied Chemistry, 66*, 1011-1016.

Parker, R. S. (1988). Carotenoid and tocopherol composition in human adipose tissue. *American Journal of Clinical Nutrition, 47*, 33-36.

Prabhashankar-Arathi, B., Raghavendra-Rao, P., Sowmya, Vijay, K., Baskaran, V., Lakshminarayana, R. (2015). Metabolomics of carotenoids:

The challenges and prospect. *Treds in Food Science and Technology*, *45*, 105-117.

Qian, C., Decker, E. A., Xiao, H., McClements, D. J. (2012). Physical and chemical stability of β-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chemistry*, *132*, 1221-1229.

Rao, A. V. and Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research*, *55*, 207-216.

Rodriguez-Amaya, D. and Kimura, M. (2004). *HarvestPlus Handbook of Carotenoid Analysis* (1rs ed.). Washington, DC: HarvestPlus.

Sindhu, E. R., Preethi, K. C., Kuttan, R. (2010). Antioxidant activity of carotenoid lutein *in vitro* and *in vivo*. *Indian Journal of Experimental Biology*, *48*, 843-848.

Singh, P. and Goyal, G. K. (2008). Dietary lycopene: Its properties and anticarcinogenic effects. *Comprehensive Reviews in Food Science and Food Safety*, *7*, 255-270.

Smigel, K. (1990). Beta-carotene didn´t prevent cancer: what´s up, doc? *Journal of the National Cancer Institute*, *82*, 899-900.

Stadtman, E. R. (2004). Role of oxidant species in aging. *Current Medical Chemistry*, *11*, 1105-1112.

Stahl, W. and Sies, H. (1992). Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *Journal of Nutrition*, *122*, 2161-2166.

Stahl, W. and Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta*, *1740*, 101-107.

Summerburg, O., Keunen, J. E. E., Bird, A. C., van Kuijk, F. J. G. M. (1998). Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *British Journal of Ophthalmology*, *82*, 907-910.

Tanaka, T., Shnimizu, M., Moriwaki, H. (2012). Cancer chemoprevention by carotenoids. *Molecules*, *17*, 3202-3242.

Truscott, T. G. (1990). The photophysics and photochemistry of the carotenoids. *Journal of Photochemistry and Photobiology B: Biology*, *6*, 359-371.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology*, *39*, 44-84.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, *160*, 1-40.

van Breeman, R. B., Xu, X., Viana, M. A., Chen, L., Stacewicz-Sapuntzakis, M., Duncan, C., Bowen, P. E., Sharifi, R. (2002). Liquid chromatography-mass spectrometry of cis-and all-trans-lycopene in human serum and prostate tissue after dietary supplementation with tomato sauce. *Journal of Agricultural and Food Chemistry*, *50*, 2214-2219.

van Poppel, G. (1993). Carotenoids and cancer: and update with emphasis on human intervention studies. *European Journal of Cancer, 29*, 1335-1344.

van Poppel, G. and Goldbohom, R. A. (1995). Epidemiologic evidence for beta-carotene and cancer prevention. *American Journal of Clinical Research, 62,* 1393S-1402S.

Vogiatzi, G., Tousoulis, D., Stefanadis, C. (2009). The role of oxidative stress in aterosclerosis. *Hellenic Journal of Cardiology, 50,* 402-409.

Voutilainen, S., Nurmi, T., Mursu, J., Rissanen, T. H. (2006). Carotenoids and cardiovascular health. *American Journal Clinical Nutrition, 83*, 1265-1271.

Williams, A. W., Boileau, T. W., Erdman, J. W. (1998). Factors influencing the uptake and absorption of carotenoids. *Experimental Biology and Medicine, 218*, 106-108.

Wurtzel, E., Cuttriss, A. J., Vallabhaneni, R. (2012). Maize provitamin A carotenoids current resources and future metabolic engineering challenges. *Frontiers in Plant Science, 3*, 1-12.

Yahia, E. M. and Ornelas-Paz, J. J. (2010). Chemistry, stability, and biological actions of carotenoids. In: de la Rosa, L. A., Alvarez-Padilla, E., Gonzalez-Aguilar, G. A., editors. *Fruit and Vegetable Phytochemicals*: *Chemistry, Nutritional value, and Stability* (1rs ed., pp. 177-222). Ames, Iowa: Wiley-Blackwell.

Young, A. J. and Lowe, G. M. (2001). Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics, 385*, 20-27.