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A Review of the Structure, Biosynthesis, Absorption of Carotenoids-Analysis and Properties of their Common Natural Extracts

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ABSTRACT

Carotenoids are a class of natural pigments familiar to all through the orange-red colours of popular foods like oranges, tomatoes and carrots and the yellow colour of many flowers. They have been studied for a number of years because of their diverse roles in photobiology, photochemistry and photo medicine. Carotenoids are also added as colorants to many manufactured foods, drinks, and animal feeds, either in the forms of natural extracts (e.g annatto, paprika or marigold extracts) or as pure compounds manufactured by chemical synthesis. Carotenoids are often described as provitamins A, as this particular vitamin is a product of carotenoid metabolism. The distribution of carotenoids among the different plant groups shows no obvious pattern. b-Carotene is the most abundant in leafy vegetables, though the colour is masked by its co-existence with chlorophyll, and this carotenoid has the highest vitamin A activity. Zeaxanthin, a-carotene and antheraxanthin are also present in small amounts. In the tomato, lycopene is the major carotenoid, while fruits contain varying proportions of cryptoxanthin, lutein and antheraxanthin.
In this review paper the natural occurrence of carotenoids (with focus on certain natural extracts) is described along with its structure and physicochemical properties. The biosynthesis - industrial synthesis and absorption of carotenoids is also discussed. Finally, a brief overview of analysis and properties of commonly available natural carotenoid extracts (annato, paprika, xanthophylls, lycopene) are also reported.

Key words: Structure, Biosynthesis, Absorption of carotenoids**,** Natural carotenoid extracts (annato, paprika, xanthophylls, lycopene).

INTRODUCTION

Natural occurrence of carotenoids

Carotenoids are lipid secondary metabolites that play essential roles in plants and are also relevant compounds from a nutritional standpoint. (Etoh *et al*., 2000; Grobush *et al*., 2000). They attract much attention due to their proposed antioxidant properties but also as (mainly orange-red) natural pigments abundant in many fruits and vegetables (like oranges, tomatoes and carrots flowers etc.) that constitute an important part of the human diet. (Edge *et al*., 1997; Meléndez-Martínez *et al*., 2014).

Decades of research on carotenoids has improved our understanding of the role of these ubiquitous pigments, which have emerged as important players exerting a protective role against diseases associated with aging, including cancer, cardiovascular disease, cataracts, and age-related macular degeneration (Bowen *et al*., 2015; Bermudez *et al*., 2005; Pantavos *et al*., 2015). They have been studied for a number of years because of their diverse roles in photobiology, photochemistry and photo medicine (Pryor *et al*. 2000). Carotenoids are also added as colorants to many manufactured foods, drinks, and animal feeds, either in the forms

of natural extracts (e.g annatto, paprika or marigold extracts) or as pure compounds manufactured by chemical synthesis (Kiokias *et al*., 2009[a]).

Carotenoids are often described as provitamins A, as this particular vitamin is a product of carotenoid metabolism. The distribution of carotenoids among the different plant groups shows no obvious pattern (Coultate, 1996). b-Carotene is the most abundant in leafy vegetables, though the colour is masked by its co-existence with chlorophyll, and this carotenoid has the highest vitamin A activity. Zeaxanthin, a-carotene and antheraxanthin are also present in small amounts. In the tomato, lycopene is the major carotenoid, while fruits contain varying proportions of cryptoxanthin, lutein and antheraxanthin. (Van den Berg *et al*., 2000). Furthermore, some animals owe their colour to carotenoids, especially birds (yellow and red feathers) and fish (salmon), whereas complex formation with protein may modify their colour to blue or green (Ribayamercado *et al*., 2000). The total carotenoid content of different foods materials is given in *table-2* (Kiokias, 2002).

Although close to 600 carotenoids have been identified in nature only 50 possess provitamin A activity and about 40 are present in a typical human diet, whereas of these only 14 and some of their metabolites have been detected in blood and tissues (Akoh & Min., 1997).

Carrot, tomato and papaya represent important dietary sources of â-carotene and lycopene (Schweiggert *et al*., 2015). A normal and varied food supply provides 1000-4000 mg of carotenoids daily (Mangels *et al.,* 1993). Actually, the daily intake of carotenoids is generally more variable than the intake of protein, fat, and carbohydrates as characterized by large fluctuations. For example, 100g servings of cooked mustard greens, spinach and broccoli, provide very different amounts of carotenoids as shown in Table-1 (Kiokias, 2002).

Carotenoids are believed to play a number of vital roles in the physiology of the plant kingdom, and they are ultimately involved in photosynthesis, the fundamental life-sustaining reaction on the planet (Kritchevsky 1999; Kiokias *et al*., 2008[a]). Moreover, there is a large body of evidence that certain natural carotenoids (e.g. lycopene, lutein and zeaxanthin etc.) possess various antioxidant functions exhibiting both a technological action against lipid oxidation and a protective role in delaying the onset of chronic disease (Krinsky 2001; Mares-Perlman *et al*., 2002).

Structure and biosynthesis

Most carotenoids are 40-carbon terpenoids having isoprene as their basic structural unit (Khachik *et al*., 1997). A general subdivision is into:
(i) "carotenes" which are strictly hydrocarbons (a**-** and b-carotene, lycopene) and
(ii) "xanthophylls" (lutein, bixin, capsanthin etc), which contain polar ends groups reflecting an oxidative step in their formation (Bohm *et al*., 1999;).

The simplest carotene is lycopene, from which a**-** and b-carotene, derive by cyclisation at the end of the chain (Faure *et al*., 1999). The xanthophylls arise initially by hydroxylation of the carotenes, and their subsequent oxidation reactions lead to the formation of epoxides such as antheraxanthin (Kovary *et al*., 2001). The heat treatment of carotenoids sometimes leads to their isomerisation. For instance, a**-** and b-carotene that differ only in the position of a double bond in the cyclic end-group can both show further *cis/trans*-isomerism along the terpene chain. Most naturally occurring carotenoids possess a *trans* configuration for all conjugated double bonds. (Madhavi *et al.*, 1996).

A recent body of scientific evidence focused on biosynthesis of natural carotenoids (Liu *et al*., 2013). The carotenoid biosynthetic pathway serves manifold roles in plants (related to photosynthesis, photoprotection etc.) and also produces compounds that impact human nutrition and metabolic products that contribute to fragrance and flavor of food and non-food crops (Shumskaya and Wurtzel, 2013).

According to Britton (1995), the conjugated polyene chromophore determines the light absorption properties, and hence colours, having also a strong influence on the physicochemical properties of the molecule. Structural features such as size, shape, and polarity are essential determinants of the ability of a carotenoid to fit correctly into its environment to allow it to function (Kiokias & Gordon 2004;

Mortensen & Skibsted.,1997). Carotenoids in plants are generally water insoluble and are associated with lipids in chloroplast cells. Because of their water-insolubility they do not leach out when the vegetables are prepared and cooked, nor do they change colour markedly with heat or pH, particularly if the chloroplast cells remain relatively intact (Rodriguez-Concepcion and Stange, 2013). On the other hand, they are slightly soluble in oils at room temperature and in non-polar organic solvents, such as chloroform and acetone (Simpson, 1985). In addition, it has been reported that regardless of the food conservation method used, carotenoids undergo a slow degradation on storage, with a loss depending on matrix and storage conditions (Meissonnier, 1983; Bohm *et al.*, 1997).

Carotenoids are synthesized in nature by plants and many microorganisms (Bai *et al.*, 2015). Animals can metabolize them in a characteristic manner, but they are not able to synthesize them. Being terpenoids, carotenoids are synthesized form the basic C5- terpenoid precursor, isopentyl diphosphate (XVII, *Figure-2*, Kiokias, 2002). This compound is converted to geranyl-geranyl diphosphate(XVIII). Its dimerisation leads to phytoene and the stepwise dehydrogenation via phytofluene (X), zeta-carotene (XXI) and neyrosporene (XXII), and gives lycopene (I). Subsequent cyclisations, dehydrogenations, and oxidation reactions, lead to the other naturally occurring carotenoids.Technological advances have made possible the synthesis, at reasonable prices, of carotenoids with well-controlled, reproducible colours, without quality variations, and in a volume that can be scheduled to meet the needs of the food industry (Basu *et al.*, 1999). Carotenoids have been extensively used by the food industry as colorants in the production of many foods. Micropulverised dispersions of carotenoids are used in coloring fat based foods such as margarine, butter, shortenings, cheese, and french dressings. Water-dispersible forms of carotenoids have been developed for the coloring of water-based foods such as orange-type beverages, cake mixes, puddings, dried and canned soups (Boileau *et al.,* 1999).

Absorption and transport of dietary carotenoids

The absorption and transport processes of carotenoids are quite complex and to a large degree not well understood. Absorption is defined as a movement of dietary carotenoids, or their metabolites to the lymphatic or portal circulation (Erdman *et al.*, 1993). Several processes are necessary for optimal absorption to occur: i) sufficient digestion of the food matrix to release carotenoids, ii) formation of lipid micelles in the small intestine, iii) uptake of carotenoids by intestinal mucosal cells, and iv) transport of carotenoids or their metabolic products to the lymphatic or portal circulation (Berni *et al.*, 2015). After absorption through passive diffusion, carotenoids follow the chylomicrons metabolism, they are taken up by the liver and released in the blood stream in lipoproteins (Faure *et al.*, 1999). In the fasted state, the hydrocarbon carotenes are carried by VLDL and LDL lipoproteins, residing in the hydrophobic core of the particles, while the more polar xanthophylls are found mainly in HDL, closer to the surface (Krinsky *et al.*, 1988). As depicted in *figure 3 (*Kiokias *et al.,* 2002*).* carotenoids accumulate or are stored in tissues. It is assumed that at least in the liver, beta-carotene and other provitamin A carotenoids would be available for conversion to Vitamin A (Kopec *et al.*, 2014). The delivery of carotenoids to extra-hepatic tissue is accomplished through the interaction of lipoprotein particles with receptors and their further degradation by extra-hepatic enzymes such as lipoprotein lipase. Adipose tissues and liver appear quantitatively to be the main storage sites, whereas adrenal gland, kidney and testes also contain a high per gram concentration. It is found, that mild cooking and additional ingestion of dietary fats improves carotenoid absorption. This is likely to be due to the release of carotenoids from cellular components of the plants upon heating and the formation of carotenoid-containing micelles from dietary fat, which facilitate carotenoid absorption in the gut (Khachik *et al.*, 1997).

Dietary lipids have been shown to increase bioavailability of provitamin A carotenoids from a single meal, but the effects of dietary lipids on conversion to vitamin A during absorption are essentially unknown. Kopec *et al*. (2014) highlighted the importance of provitamin A carotenoid consumption with a lipid-rich food such as avocado for maximum absorption and conversion to vitamin A, especially in populations in which vitamin A deficiency is prevalent. Berni *et al.*, (2015) has recently examined the bioaccessibility of Provitamin

A carotenoids in home cooked and commercially processed orange fleshed sweet potato.

It is generally accepted that the serum carotenoid concentration reflects the immediate dietary intake. Although, this is a major factor, others may be involved, such as sex, smoking and drinking habits, seasonal variations and geographic origin (Olmedilla *et al.*, 1994).

The concentration of major carotenoids, found in human serum and tissues are given in *table 1.3* (Basu *et al.,* 1999). It has been found that 2 mg of b-carotene is equivalent to 1 mg of retinal in its ability to cure vitamin A deficiency in humans. The RDA (Recommended Dietary Allowances) however, uses conversion factors of 6 to1 and 12 to 1 for b-carotene and other provitamin A active carotenoids respectively, recognizing that compared to more purified forms, dietary carotenes are much more poorly absorbed (Simpson *et al.*, 1985). Therefore for products containing b-carotene and other provitamin A carotenoids:

Total RE (retinal equivalent)

(mg b-car/6) + (mg other provitamins A/12).

The RDA for men is 900 mg/day, and for women 700 mg/day, except in pregnancy (770 mg/day) and lactation (1.300 mg/day).

The US Institute of Medicine (2000) has introduced a new term, “retinal activity equivalent” or RAE, to express the vitamin A activity of carotenoids.

1 RAE = 1 mg dietary or supplemented vitamin A =2 mg b-carotene in oil= 12 mg dietary b-carotene =24 mg other provitamin A carotenoids in diet

The estimated RAE is 625 mg/day for men and 500 mg/day for women.

Consumption of foods rich in beta-carotene is recommended by scientific and government organizations such as the **U.S.** National Cancer Institute (NCI) and the **U.S.** Department of Agriculture (USDA); these dietary guidelines recommend a dietary intake of 3 mg to 6 mg beta-carotene/day, which is associated with a lower risk of chronic diseases.

It should be noted, however, that the European Food Safety Authority (2006) have decided that the existing evidence is insufficient to establish a recommended dietary allowance (RDA) or adequate intake (AI) for beta-carotene and other carotenoids. In most European countries, the recommended intake is based on the assumption that 4.8 mg beta-carotene is needed to meet the requirement of 800 micrograms vitamin A (conversion factor 6). From epidemiological studies it can be concluded that a plasma level of 0.4 micromole/liter beta-carotene should be aimed at in order to benefit from the preventive health potential. This can be achieved with 2–4 mg/day (Biesalski *et al.*, 1997).

Table 1: Carotenoid content of selected foods and vegetables (mg/100g) (Kiokias et al., 2002)

Food item	b-carotene	a-carotene	lutein	b-cryptoxanthin
Mustard greens, ckd	2.7	0	9900	0
Spinach, ckd	5500	0	12600	0
Broccoli, ckd	1300	1	1800	0
Carrots, ckd	9800	3700	260	0
Corn, yellow	51	50	780	0
Acorn squash	2400	110	1300	0
Mangoes, raw	1300	0	0	54
Papaya, raw	99	0	0	470
Cantaloupe raw	3000	35	0	0

ckd=cooked

Natural Plant Extracts and Carotenoid Analysis

A certain body of literature has focused on the analysis and functional properties certain natural extracts of carotenoids such as paprika, lycopene, lutein-rich extracts (Kiokias & Gordon, 2003[b]; Kiokias *et al.*, 2008[b]).

A few of the most common -idustrially manufactured-extracts of natural Carotenoids are listed below:

(i) Natural extract of carotenes

Carrot extracts, carrot oil, and palm oil related extracts are available in the market and their main components are a- and b-carotenes (Kiokias and Gordon, 2003[a]). Purified crystalline products, dispersions of microcrystals in oil and carrot oil are also commercially produced.

(ii) Natural tomato Lycopene extract

Lycopene is a tetraterpenic C40 carotenoid that absorbs light in the red region being thereby responsible for the color of tomato and watermelon (Gann *et al*, 2012). For industrial uses natural lycopene is readily purified from tomato processing wastes (Wandai & Shaikly, 1985, Silva *et al.*, 2013).

Table 2: Total carotenoid content of selected food items(Kiokias, 2002)

Food	(mg/100g of edible portion)
Red palm oil	30000
Carrots	15000
Leafy vegetables	685
Tomatoes	100
Apricots (fresh)	250
Bananas	30
Sweet potatoes (white)	50
Sweet potatoes(yellow)	670
Orange juice	8

(iii) Refined Marigold extracts

Refined marigold extract is a natural yellow food colorant produced from marigold flowers (Targeta Erecta) and a stable source of the carotenoid lutein, which is a normal constituent of human plasma and retina (Vargas & Lopez, 1996).

(iv) Paprika oleoresin extract

Extracts of paprika (Capsicum annuum), rich in capsanthin carotenoids, are the oldest and most important natural carotenoid food colours, used as dry powder or oleoresin of paprika which is the oil extract of coloring and flavoring components from the pods (Pérez-Gálvez and Mínguez-Mosquera, 2004; Giuffrida *et al.*, 2013) Oleoresin of paprika, unlike ground paprika, is a liquid product completely soluble in oils and therefore does not impart specks or vegetable tissues to the food product. (Mordi, 1993). A great advantage of the oleoresin is the possibility of standardizing the color of the oil extract since the paprika depends on various factors such as drying temperature, moisture content and storage conditions (Deli *et al.*, 1992). Topuz *et al.* (2011) have recently examined the influence of different drying methods on carotenoids and capsaicinoids of paprika.

(v) Natural preparations of Annatto seeds

The term annatto includes a series of coloring preparations consisting of carotenoid-type pigments; all based on extracts of the seed of the tree Bixa orellana which grows abundantly in the tropics (Chisté *et al.*, 2011). Bixin is the main component of oil-soluble preparations, and norbixin of the water-

Table 3: Concentration of Selected Carotenoids in Human Serum and Tissues (Basu *et al.,* 1999)

Carotenoid	Serum (mmol/L)	Liver (mmol/g)	Kidney (mmol/g)	Lung (mmol/g)
lycopene	0.13-0.82	0.20-17.2	0.093-2.4	0.1-1.0
b-carotene	0.09-0.91	0.039-19.4	0.093-2.8	0.1-1.6
lutein	0.16-0.72	0.10-3.0	0.037-2.1	0.1-2.3
b-cryptoxanthin	0.05-0.38	0.037-20.0	0.019-3.9	0.1-2.5
a-Carotene	0.02-0.22	0.075-10.8	0.037-1.5	0.1-1.0

soluble products. Norbixin powder is obtained from fresh annatto seeds by means of alkaline extraction following by a precipitation of the colorants with a mineral acid. The extract is then filtered pressed and dried at controlled temperature in order to permit its preservation (Scotter *et al.*, 1998). It produces orange solutions, suitable to color foods including cheese products, butter, margarine and salad dressing. The major coloring component of annatto is the apo-carotenoid 9-cis-bixin, the solubility of which

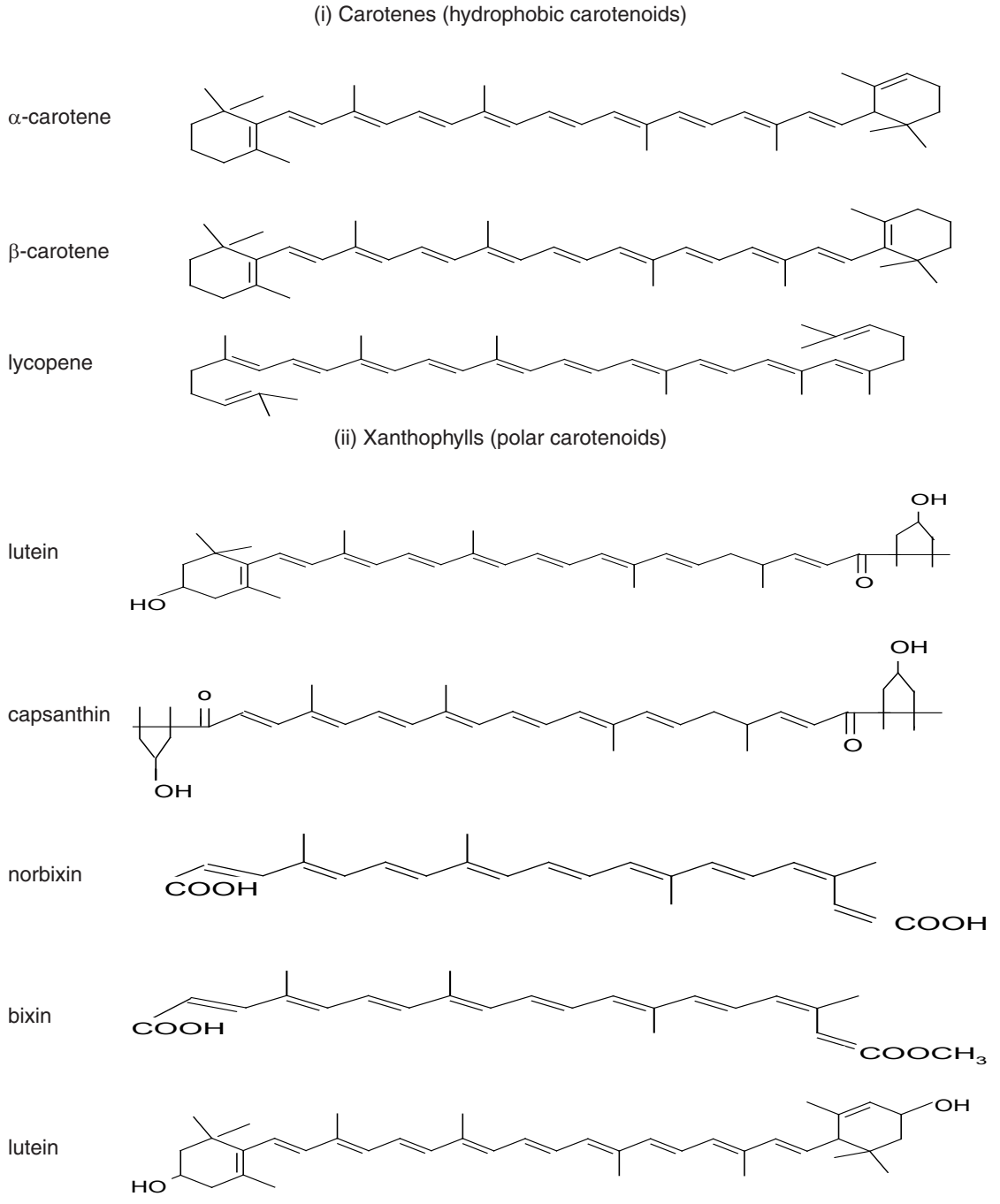

Fig. 1: Structure of the most common natural carotenoids (Kiokias & Oreopoulou, 2006)

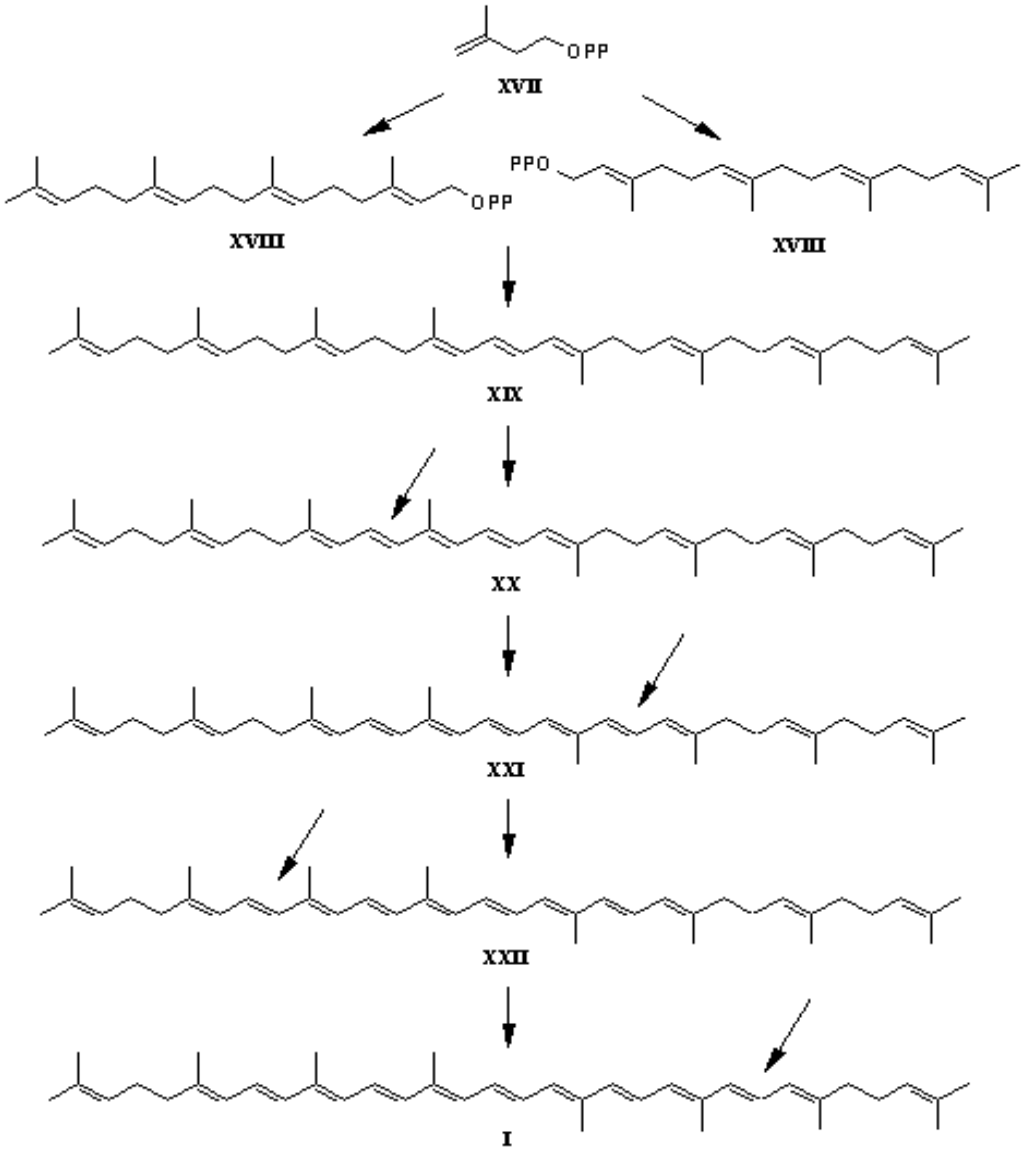

Fig. 2: Biosynthesis of natural carotenoids (Kiokias, 2002)

Table 4: Common natural carotenoid extracts (Kiokias, 2002)

Natural extract	Natural plant source	Main carotenoids ($+l_{max}$ values in nm)
Carotenes	Carrot root, palm oil	a-b, Carotenes (446-450)
Lycopene extract	Tomatoes	Lycopene (472), a,b-carotene
Xanthophylls	Marigold flowers (Targeta Erecta)	Lutein, lutein esters and Zeaxanthin (450)
Paprika extract	Capsicum annuum	Capsanthin (450), capsorubin (445)
Annatto	Bixa Orellana	Norbixin, Bixin (456)

is commercially achieved by heating a preparation of the seeds in oil to a maximum temperature of 130ÚC in vacuo. Under these conditions, 9 cis bixin undergoes isomerisation to produce oil-solutions containing variable proportions of the pigment dependent on extraction temperature and time (Scotter,1995).

The main carotenoid pigments that are present in a range of common natural extracts are given in Table-4 (Kiokias *et al*., 2002).

Classical method of analysis of carotenoid pigments using column chromatography, paper chromatography and TLC potentiate isomerisations and transformations during the separation (Philip & Chen, 1988). Carotenoid extracts from natural sources are usually saponified to remove chlorophylls and unwanted lipids and to hydrolyse carotene esters (Khachik & Beecher, 1988). Organic solvents extract carotenoids, whereas fatty acids that precipitate as soaps and glycerol are retained in the aqueous phase. In addition, High Performance Liquid Chromatography (HPLC) is the analytical method of choice for separation, quantification and structural characterization of the naturally occurring and synthetic carotenoids as well as for their most important metabolic products (Rodriguez-Amaya, 2015). A body of research (Kiokias & Gordon 2003[a]; Kiokias & Oreopoulou 2006) has focused on the analysis of natural extracts by Reversed Phase HPLC for the identification of the

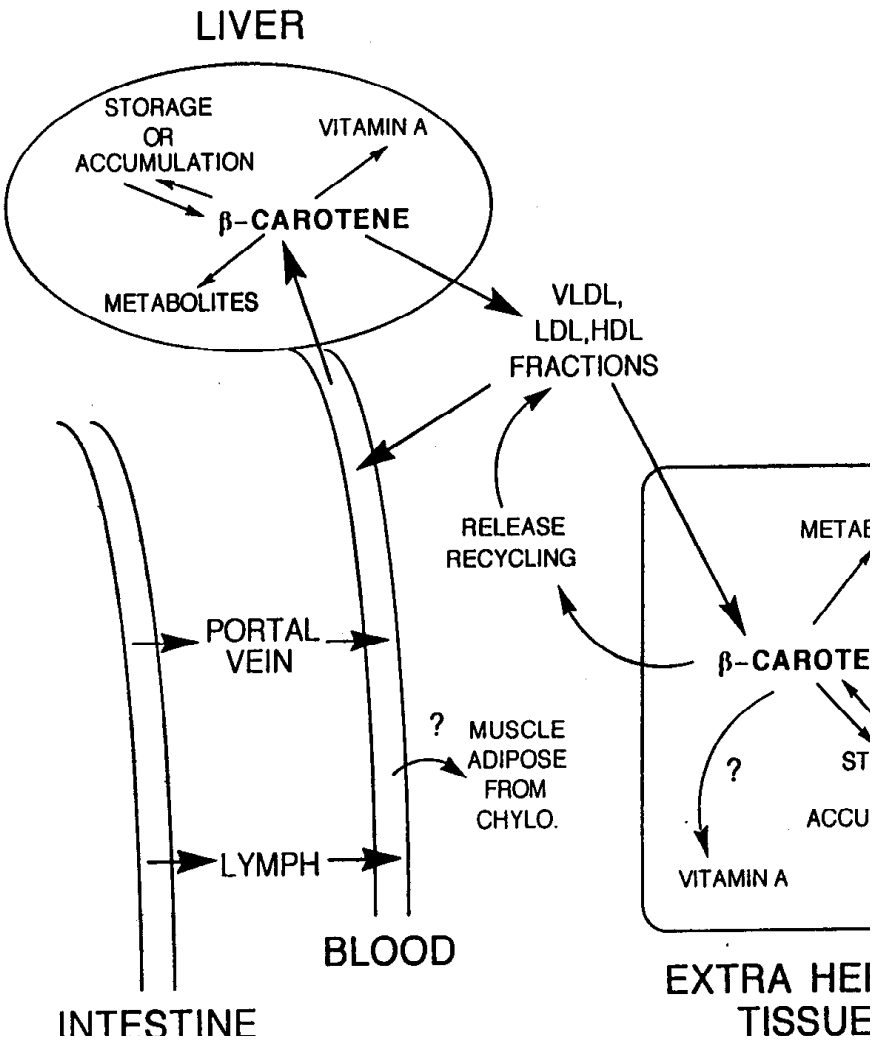

Fig. 3: Post-absorption transport of b-carotene to liver and extrahepatic tissues (Kiokias, 2002)

containing pigments. Additionally, the introduction of the photodiode array detector has facilitated the identification of carotenoids after HPLC separation by using spectral characteristics (Shoefs *et al.*, 1995).

In food analysis spectrophotometry has been widely applied to the determination and quantification of carotenoids following the removal of interfering substances by liquid chromatography. The total carotenoid content of specific natural extracts (paprika, annatto, marigold etc.) has been determined by visible spectroscopy measuring total absorption at a specific wavelength and using absorptivity values reported in the literature (Kiokias *et al.*, 2009[a]). Solutions of the various carotenoid extracts (1mg/100 ml) were prepared after saponification and a spectrum of each one (200-500 nm) in a selected solvent was obtained. The specific absorbance, at the l_{max} of the major carotenoid for each extract was calculated, and compared with absorptivity values reported in the literature. (Hart & Scott, 1995). The l_{max} values of the major natural carotenoids (in n-hexane), with their absorptivity are also provided in table-4. A large body of scientific evidence suggests that carotenoids scavenge and deactivate free radicals both *in vitro* and *in vivo,* whereas a few researchers claimed that they can also act as prooxidants by accelerating rather than retarding the oxidative process (Khachik *et al.*, 1997; Bub *et al.*, 2000, Matos *et al.*, 2000; Kiokias & Varzakas, 2014). Carotenoids can also act as chemical quenchers undergoing irreversible oxygenation. The molecular mechanisms underlying these reactions are still not fully understood, especially in the context of the anti- and pro-oxidant activity of carotenoids, which, although not synthesized by humans and animals, are also present in their blood and tissues, contributing to a number of biochemical processes (Tessa *et al.*, 1995; Fiedor, and Burda, 2014). The antioxidant activity of carotenoids is a direct consequence of the chemistry of their long polyene chain (Farombi & Burton 1999; Boileau *et al.*, 1999): a highly reactive, electron–rich system of conjugated double bonds susceptible to attack by electrophilic reagents, and forming stabilized radicals (Mortensen & Skibsted, 1997; Bast *et al.*, 1998; Kiokias *et al.*, 2008[b]). Therefore, this structural feature is mainly responsible for the chemical reactivity of carotenoids towards oxidizing agents and free radicals, but also other factors such as oxygen pressure (Jorgensen and Skibsted, 1993) and synergistic effect with other natural compounds may determine their antioxidant action or even prooxidant character in certain model systems (Mordi *et al*; 1993; Kiokias & Gordon 2004, Krinsky 2001; Palozza, 1998). In the last decade, there is an increasing interest of a few researchers in investigating the effect of natural carotenoid extracts both in vivo following dietary supplementation (Kiokias and Gordon 2003; Stahl & Sies, 2005; Hofer *et al.*, 2014) and in vitro in oil based systems (Dimakou and Oreopoulou, 2012; Viuda-Martos *et al.*, 2012; Kiokias *et al.*, 2009[b]). Further experimental evidence of the antioxidant potential of certain carotenoid extracts following their incorporation in olive oil and olive oil based oil-in-water emulsions along with aspects of analysis and identification of their carotenoid pigments, are discussed by Kiokias and Varzakas (2014).

REFERENCES

1. Akoh, C.C. and Min, B.D. In Food Lipid Chemistry, Nutrition and Biotechnology, Marcel Dekker, New York. (1997).
2. Bai, C., Capell, T., Berman, J., Medina, V., Sandmann, G., Christou, P., and Zhu, C. Bottlenecks in carotenoid biosynthesis and accumulation in rice endosperm are influenced by the precursor–product balance. *Plant Biotechnology Journal* (2015), (published online, DOI: 10.1111/pbi.12373)
3. Bast, A., Haanen, G.R., and VandenBerg, H. Antioxidant effects of carotenoids. *Int.J.Vit. Nutr.Res.* **68**: 399-403 (1998).
4. Basu, K.T., Temple, J.N., and Gerg, L.M. (1999). Antioxidants in human health and disease. CABI Publishing, New York. pp. 160.
5. Bermudez, I.O., Ribaya-Mercado, D.J., Talegawkar, A.S., Tucker, L.K. Hispanic and Non-Hispanic White Elders from Massachusetts Have Different Patterns of Carotenoid Intake and Plasma Concentrations.

Journal of Nutrition, **135**: pp. 1496-1502 (2005).

6. Berni, P., Chitchumroonchokchai, C., Canniatti-Brazaca,G.S., De Moura,F.F., Failla, L.M. Comparison of Content and *In vitro* Bioaccessibility of Provitamin A Carotenoids in Home Cooked and Commercially Processed Orange Fleshed Sweet Potato (*Ipomea batatas* Lam). *Plant Foods for Human Nutrition*, 70, pp. 1-8 (2015).
7. Biesalski H.K,, Böhles H., Esterbauer H., Furst. P., Gey. F., Hundsdörfer, G., Kasper H *et al.*: Antioxidant vitamins in prevention. *Clin Nutr,* **16**: 151–155 (1997).
8. Bohm, F., Edge, R., Lange, L., McGarvey, J., & Truscott, T.G. Carotenoids enhance vitamin E antioxidant activity. *Amer.Chem.Soc.,* **119**: 621-622 (1997).
9. Bohm, F., and Bitsch, R. Intestinal absorption of lycopene from different matrices and interactions to other carotenoids to the lipid status and antioxidant capacity of human plasma. *Europ.J.Nutr.* **38**: 118-125 (1999).
10. Boileau, W.M., Moore, C., and Erdman, W.J. Carotenoid and Vitamin A. In: Antioxidants in human health and disease. Basu, K.T., Temple, J.N., and Gerg, L.M. CABI Publishing, New York (1999).
11. Bowen, Å.P., Sapuntzakis, M.S., and Navsariwala, V.D. Carotenoids in Human Nutrition. *Pigments in Fruits and Vegetables*, 31-67 (2015).
12. Britton G. UV/visible spectroscopy. In Britton G, Liaaen-Jensen S, Pfander H (eds), Carotenoids: Spectroscopy, vol 1B, pp 13-63 (1995).
13. Bub, A., Waltz, B., Abrahamse, Z., Adam, S., Wever, J., Muller, H.S., and Rechenmmer, G. Moderate intervention with carotenoid rich vegetable products reduces lipid peroxidation in men. *Amer.Soc.Nutr.Sci.* **18***:* 2200-2206 (2000).
14. Chisté, C.R., Yamashita, F., Gozzo, C.F., and Mercadante, Z.A. Simultaneous extraction and analysis by high performance liquid chromatography coupled to diode array and mass spectrometric detectors of bixin and phenolic compounds from annatto seeds. *Journal of Chromatography*, **1218**: pp. 57–63 (2011).
15. Coultate, T.P. *Food-The chemistry of its components-2*nd edit. The Royal Society of Chemistry, Cambridge (1996).
16. Deli, J.; Matus, Z.; Szabolcs, J. Carotenoid composition in the fruits of black paprika (*Capsicum annuum* variety *longum nigrum*) during ripening. *J. Agric. Food Chem.*, **40**: 2072 (1992).
17. Dimakou, C and Oreopoulou,V. Antioxidant activity of carotenoids against the oxidative destabilization of sunflower oil-in-water emulsions. *LWT - Food Science and Technology*, **46**: pp. 393-402 (2012).
18. Edge, R, Truscoot, T,G., McGarvey, D.J. The carotenoids as antioxidants- a review. *Journal of Photochemisrty and Photobiology*, **41**: 89-200 (1997).
19. Etoh, H., Utzunomiga, Y., Komori, A., Murakami, Y., Oshima, S., and Inakuma, T. Carotenoids and human blood plasma after ingesting paprika juice. *Biosc.Biot.Bioph.* **64**: 1096-1098 (2000).
20. European Food Safety Authority, Scientific Committee on Food. Tolerable Upper Intake Levels for Vitamins and Minerals.: ISBN: 92-9199-014-0 (2006).
21. Farombi, E.O., and Burton, G. Antioxidant activity of palm oil carotenes in organic solution-effect of structural and chemical reactivity. *Food Chemistry*, **11**: 315-321 (1999).
22. Faure. H., Galabert, G., Le Moel, G., and Nabet, F. Carotenoids: metabolism and physiology. *Annales de Biologie Clinique.*, **57**: 169-183 (1999).
23. Fiedor, J and Burda, K. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. *Nutrients*, **6**(2), 466-488 (2014).
24. Gann, H.P., Deaton, R., Enk, E., van Breemen, B.R., Han, M., Lu, Y., and Ananthanarayanan, V. A Phase II randomized trial of lycopene-rich tomato extract among men with high-grade prostatic intraepithelial neoplasia (HGPIN). *Cancer Res*, **72**; 3564 (2012).
25. Giuffrida, D., Dugo, P., Torre, G., Bignardi, C., Cavazza, A., Corradini, C., and Dugo, G. Characterization of 12 *Capsicum* varieties by evaluation of their carotenoid profile and pungency determination. *Food Chemistry*,

140, 794-802 (2013).

26. Grobush, K., Lanner, L.J., Geleinjinse, J.M., Boeing, H., Hofman, A., & Witteman, J.C. Serum carotenoids and atherosclerosis. The Roterdam Study. *Atherosclerosis*, **148**: 49-56 (2000).
27. Hart, J.D., & Scott, K., Development and evaluation of HPLC methods for the analysis of carotenoids in foods and the measurement of carotenoid content of vegetables commonly consumed in UK. *Food Chemistry*, **54**, 101-111 (1995).
28. Hofer, T., Jørgensen, Ø.T., and Ragnar L. Olsen, L.R. Comparison of Food Antioxidants and Iron Chelators in Two Cellular Free Radical Assays: Strong Protection by Luteolin. *J. Agric. Food Chem.*, **62**: pp 8402–8410 (2014).
29. Jorgensen, K., and Skibsted, L.H. Carotenoid scavenging of radicals-effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Zeitshrift fur lebensmitteln.* **196**: 423-429 (1993).
30. Khachik, F., & Beecher, GR. Separation & quantitation of carotenoids in foods. *Journal of Chromatography*, **449**: 118 (1988).
31. Khachik, F., Beecher, G.R., and Smith, J.C. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J.Cell.Biochem.* **22**: 236-246 (1997).
32. Kiokias, S. *In vitro* and *in vivo* antioxidant properties of natural carotenoid mixtures. Faculty of Life Sciences, School of Food Biosciences, The University of Reading. p. 77 (2002).
33. Kiokias, S. & Gordon, M. Antioxidant properties of annatto carotenoids. *Food Chemistry*, **83**: 523-529 (2003[a]).
34. Kiokias, S and Gordon, M. Dietary supplementation with a natural carotenoid mixture decreases oxidative stress. *Europ. J. Clin. Nutr.* **57**: 1135-1140 (2003[b]).
35. Kiokias, S. and Gordon M. Properties of carotenoids in *vitro* and in *vivo*. *Food Reviews International*, **20**: 99-121 (2004).
36. Kiokias, S., and Oreopoulou, V. Antioxidant properties of natural carotenoid preparations against the AAPH-oxidation of food emulsions. *Innovative Food Science & Emerging Technologies,* **7**: 132-139 (2006).
37. Kiokias, S., Dimakou, C., Varzakas, T., & Oreopoulou,V. Activity of natural carotenoid extracts, individually or mixtured with olive oil phenolics during thermal autoxidation of olive oil systems. *Proccedings of 4th Central European Congress on Food (Cavtat, Croatia)*, p. 381-387 (2008[a]).
38. Kiokias, S., Varzakas, T., and Oreopoulou,V. In vitro activity of vitamins, flavonoids, and natural phenolic antioxidants against the oxidative deterioration of oil-based systems. *Critical Reviews in Food Science and Nutrition*, **48**: 78–93 (2008[b]).
39. Kiokias, S., Dimakou, C., and Oreopoulou, V. Effect of natural carotenoid preparations against the autoxidative deterioration of sunflower oil-in-water emulsions. *Food Chemistry,* **114**: 1278-1284 (2009[a]).
40. Kiokias, S., Dimakou, C., and Oreopoulou,V. *In vitro antioxidant activity of synthetic beta-and carotene and natural carotenoid extracts against the oxidatative degradation of food emulsions.* In beta-carotene, Dietary sources, Cancer and Cognition, (chapter-5) p.231-262. Nova Science (2009[b]).
41. Kiokias, S., & Varzakas, T. Activity of flavonoids and beta-carotene during the auto-oxidative deterioration of model food oil-in water emulsions. *Food Chemistry,* **150C**: 280-286 (2014).
42. Kopec, E.R., Jessica L. Cooperstone, L.J., and Schweiggert, M.R., Young, S.G., Harrison, E.H., Francis, M.D., Steven K. Clinton, K.S., and Schwartz, J.S. Avocado Consumption Enhances Human Postprandial Provitamin A Absorption and Conversion from a Novel High–â-Carotene Tomato Sauce and from Carrots. *Journal of Nutrition*, **8**: 1158-1166 (2014).
43. Kovary, K., Lourain, T., Silva, C., Albano, F., Pires, M.B., Lage, S.L., and Felzenswalb, I. Biochemical behaviour of norbixin during *in vitro* DNA damage induced by reactive oxygen species. *British Journal of Nutrition.* **85**: 431-440 (2001).
44. Krisky, N.I., Cornwell, D.G., and Oncley, J.L. The transport of carotenoids in human plasma. *Archive of Biochemistry and Biophysics.,* **73**: 233-246 (1988).
45. Krinsky NI: Carotenoids as antioxidants.

Nutrition **17**: 815-817 (2001).

46. Kritchevsky, S.B. Beta-carotene, carotenoids and the prevention of coronary heart diseases. *Journal of Nutrition*, **129**: 1:5-8 (1999).
47. Liu., G., Gerken,H., Huang, J. and Chen, F. Engineering of an endogenous phytoene desaturase gene as a dominant selectable marker for *Chlamydomonas reinhardtii* transformation and enhanced biosynthesis of carotenoids. *Plant Science,* **208**: pp 58-63 (2013).
48. Madhavi, L.D., Singhal, S.R., and Kulkavni, R.P. Technological aspects of foods antioxidants. In: Food antioxidants: technological toxicological and health perspectives. Madhavi *et al*. Editors, Marcel Dekker, New York, pp. 242–246 (1996).
49. Mangels, G.A., Holden, M.J., Beecher, G.R., & Lanza, E. The carotenoid content of fruits and vegetables on evaluation of analytical data. *J.Amer.Diet.Assoc.,* **93**: 284-296 (1993).
50. Mares-Perlman JA., Millen AE., Ficek TL., and Hankinson SE: The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. *J.Nutr*. **132** (3), 518S-524S (2002).
51. Matos, H.R., Di Mascio, P., and Medeiros, M.H.G. Protective effect of lycopene on lipid peroxidation and oxidative damage in cell culture. *Archives of Biochemistry and Biophysics.* **383**: 56-59 (2000).
52. Meissonnier, E. The supply of vitamins to dairy cattle. F. Hoffmann-La Roche and Co. Ltd, Basle, Switzerland (1983).
53. Meléndez-Martínez, A.M,; Carla M. Stinco, C.M., Brahm, P.M., and Vicario, I.M. Analysis of Carotenoids and Tocopherols in Plant Matrices and Assessment of Their In Vitro Antioxidant Capacity. *Plant Isoprenoids-Methods in Molecular Biology*, **1153**: pp 77-97 (2014).
54. Mordi, R.C. Carotenoids-function and degradation. *Chemistry and Industry.* **110**: 79-83 (1993).
55. Mortensen, A., and Skibsted, L.H. Relative stability of carotenoid radical cations and homologues tocopheroxyl radicals. *FEBS Letters.* **417**: 261-266 (1997).
56. Olmedella, B., Granado, F., & Blanco, I. Seasonal and sex related variations in sex serum carotenoids, retinol and a-tocopherol. *Amer.J.Clin.Nutr.*, **60**: 106-110 (1994).
57. Palozza, P. (1998). Pro-oxidant actions of carotenoids in biological systems. *Nutrition Reviews*, **56**: 257-265.
58. Pantavos, A., Ruite, R., Feskens, F.E., de Keyser, E.C., Hofman, A., Stricker, H.B., Franco, H.O., and Kiefte-de Jong, C.J. Total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk: The Rotterdam study. *International Journal of Cancer*, **136**: pp. 2178-2186 (2015).
59. Pérez-Gálvez A., Mínguez-Mosquera M.I. Degradation, under non-oxygen-mediated autooxidation, of carotenoid profile present in paprika oleoresins with lipid substrates of different fatty acid composition, *J. Agric. Food Chem.,* **52**: 632–637 (2004).
60. Philip T.S., & Chen, T.S. Separation and quantitative analysis of some carotenoid fatty acid esters by HPLC. *Journal of Chromatography,* **435**: 113-126 (1988).
61. Pryor, W.A., Stahl, W., and Rock, C.L. Beta-carotene: From biochemistry to clinical trials. *Nutrition Reviews.,* **58**: 39-53 (2000).
62. Ribayamercado, J.D., Solon, S.F., Tang, G., Cabal-Borza, M., Perfecto, S.C., and Russel, R.M. Bionconversion of plant carotenoids to Vit-A in Filipino school-aged children varies inversely with Vit-A status. *Amer.J.Clin.Nutr.* **72**: 455-465 (2000).
63. Rodriguez-Concepcion, M., and Stange, C. Biosynthesis of carotenoids in carrot: An underground story comes to light. *Archives of Biochemistry and Biophysics*, **539**, pp. 110-116 (2013).
64. Rodriguez-Amaya, D.B. Status of carotenoid analytical methods and *in vitro* assays for the assessment of food quality and health effects. *Current Opinion in Food Science*, **1**, pp 56–63 (2015).
65. Schweiggert, R.M.,. Kopec, R.E., Villalobos-Gutierrez, G.M., Högel, J., Silvia Quesada, S., Esquivel, P., Schwartz,J.S., and Reinhold Carle, R. Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised cross-over study. *British Journal of Nutrition,* **111**: pp 490-498 (2015).

66. Scotter, M.J. Characterisation of the coloured thermal degradation products from annatto, and a revised mechanism for their formation. *Food Chemistry*, **53**: 177-185 (1995).
67. Scotter, J.M., Wilson, L.A., and Appleton, G.P. Analysis of annatto food color formulation. Determination of colour components and thermal degradation products by HPLC. *J.Agric.Food.Chem.*, **46**: 1301-1308 (1998).
68. Shoefs, B., Bertram, M., and Lemoine, Y. Separation of photosynthetic pigments and their precursors by reversed phase HPLC (1995).
69. Shumskaya, Ì., and Wurtzel, E.T. The carotenoid biosynthetic pathway: Thinking in all dimensions. *Plant Science*, **208**: pp 58-63 (2013).
70. Silva, F.A., Marcelo M.R., and Silva, M.C. Supercritical solvent selection (CO_2 *versus* ethane) and optimization of operating conditions of the extraction of lycopene from tomato residues: Innovative analysis of extraction curves by a response surface methodology and cost of manufacturing hybrid approach. *The Journal of Supercritical Fluids*, **95**: pp 618–627 (2013).
71. Simpson, K.L., Tsou, C., & Chisester, C.O. Carotenes. In "*Methods of vitamins Assays*" 4th edition, by Augustin *et al.*, John Wile, New York, pp 185-200.using photodiode array detectors. *Journal of Chromatography,* **692**, 239-245 (1985).
72. Stahl, W., and Sies, H. Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease,* **1740**(2): 30 pp 101–107 (2005).
73. Tessa. J., Land, J., Shcalch, W., Truscott, G., and Tinkler, H.J. Interactions between carotenoids and the CCl_3O_2 radical. *Amer. Chem.Soc.,* **117**: 8322-8326 (1995).
74. Topuz, A., Dincer, C., Özdemir, S.K., Feng, H., Kushad, M. Influence of different drying methods on carotenoids and capsaicinoids of paprika (Cv., Jalapeno). *Food Chemistry,* pp. 860–865 (2011).
75. Van den Berg, Ç., Faulks, R., Granado, F., Hirscheberg, J., Olmedilla, Â., Sandn1anç, G, Southon, S. and Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systematic effects. *Journal of the Science of Food and Agricultire*, **80**: 880-912 (2000).
76. Vargas. D.F., and Lope, D.O. Correlation of HPLC and AOAC methods to assess all-trans lutein content in marigold flowers. *J.Sci.Food. Agric.*, **72**; 283-290 (1996).
77. Viuda-Martos, M., Ciro-Gomez, L.G., Ruiz-Navajas,Y., Zapata-Montoya, E.Z., Sendra, E., José A. Pérez-Álvarez, A.J. and Fernández-López. J. *In vitro* Antioxidant and Antibacterial Activities of Extracts from Annatto (*Bixa orellana* L.) Leaves and Seeds. *Journal of Food Safety*, **32**: pp 399-406 (2012).
78. Wandai, A.L., and Shaikly, A. Tomato process wastes as essential raw materials. *J.Agric. Food Chem.*, **33**: 804-807 (1985).
79. Yamamoto.S., Enzymatic lipid peroxidation, reactions of mammalians lipoxyganases, *Free Rad Biol Med.*, **10**: 149 (1991).