SURYA UNIVERSITY | September 15-16 2014

PROGRAM & ABSTRACT

"Marine natural products and ocean conservation towards sustainable marine development"

Marine Biotechnology

MBOC

Ocean Conservation

Carotenoids: Sources, Medicinal Properties and Their Application in Food and Nutraceutical Industry

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Carotenoids are biosynthesized by bacteria, algae, fungi, and plants, but not by animals, which must obtain them from their food. These compounds are divided into two major classes based on their structural elements; carotenes, constituted by carbon and hydrogen (e.g. β-carotene, α-carotene and lycopene), and xanthophylls, constituted by carbon, hydrogen, and additionally oxygen (e.g. lutein, β-cryptoxanthin, zeaxanthin, astaxanthin and fucoxanthin). Carotenoids have good effect on human health, such as pro-vitamin A, antioxidant, anticancer, antiobesity effect and anabolic effect on bone components. Currently, carotenoids are used commercially as feed additives, animal feed supplements, natural food colorants, nutrient supplement and, more recently, as nutraceuticals for cosmetic and pharmaceutical purposes. These compounds can be produced commercially by chemical synthesis, fermentation or isolation from the small number of abundant natural sources. Furthermore, commercial production of carotenoids from microorganisms competes mainly with synthetic manufacture by chemical synthesis. However, most of the commercially used carotenoids (e.g. β-carotene, astaxanthin and cantaxanthin) are produced by chemical synthesis. Marine carotenoids are considerably new. Our intensive research on fucoxanthin, a type of novel carotenoid, is discussed in this paper.

Keywords: Carotenoid, food industry, nutraceutical, food colorant, feed supplement, pro-vitamin A, antioxidant, anticancer, antiobesity effect.

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INTRODUCTION

Carotenoids, the basic source of yellow, orange, and red plant pigments, are widely distributed in nature (Sugawara et al., 2009). They are present in all living organisms, from bacteria, yeast, algae to higher plants and animals (Basu et al., 2001; Sugawara et al., 2009). These compounds are a group of more than 700 naturally occurring pigments (Godinho and Bhosle, 2008; Konishi et al., 2008) that are biosynthesized *de novo* by plants, algae, fungi and bacteria (Okada et al., 2008). Most of them can be found in higher plants, especially in their leaves, flower and fruits (Mattea et al., 2009). Animals are incapable of producing carotenoids and must obtain them above sources (Okada et al., 2008). On the other hand, animals cannot synthesize carotenoids (Rock, 1997), so their presence is due to dietary intake, e.g., the pink salmon flesh and many birds plumage owe their color to carotenoids (Mortensen, 2006). However, they must ingest carotenoids from food and metabolize them for use in physiological functions (Fujisawa et al., 2008).

Carotenoids are compounds constituted by C_5eight isoprene units (Bonnie and Choo. 1999) joined in a head to tail pattern, most of them have 40 carbon atoms (Mattea et al., 2009). These compounds are derived from phytoene, which is synthesized by a reductive dimerization of geranylgeranyl pyrophosphate (GGPP) (Figure 1), after steps of its dehydrogenation, cyclization, hydroxylation, oxidation and epoxidation (Yano et al., 2005).Carotenoids are structurally divided into two major classes: Firstly, carotenes (*e.g.*, a-carotene, b-carotene, lycopene), which are exclusively hydrocarbons (those without any oxygen molecule) (Aizawa and Inakuma, 2007), and secondly, xanthophylls (*e.g.*, lutein, zeaxanthin, fucoxanthin, astaxanthin) (Pérez-Rodríguez, 2009), which are oxygenated (Britton, 1995) containing hydroxyl, methoxy, carboxyl, keto, or epoxy groups (Basu et al., 2001; de Quiros and Costa, 2006). The chemical structures of carotenes (e.g. lycopene and β-carotene), and xanthophylls (e.g. lutein, β-criptoxanthin, zeaxanthin, astaxanthin and fucoxanthin) are shown in Figure 2.

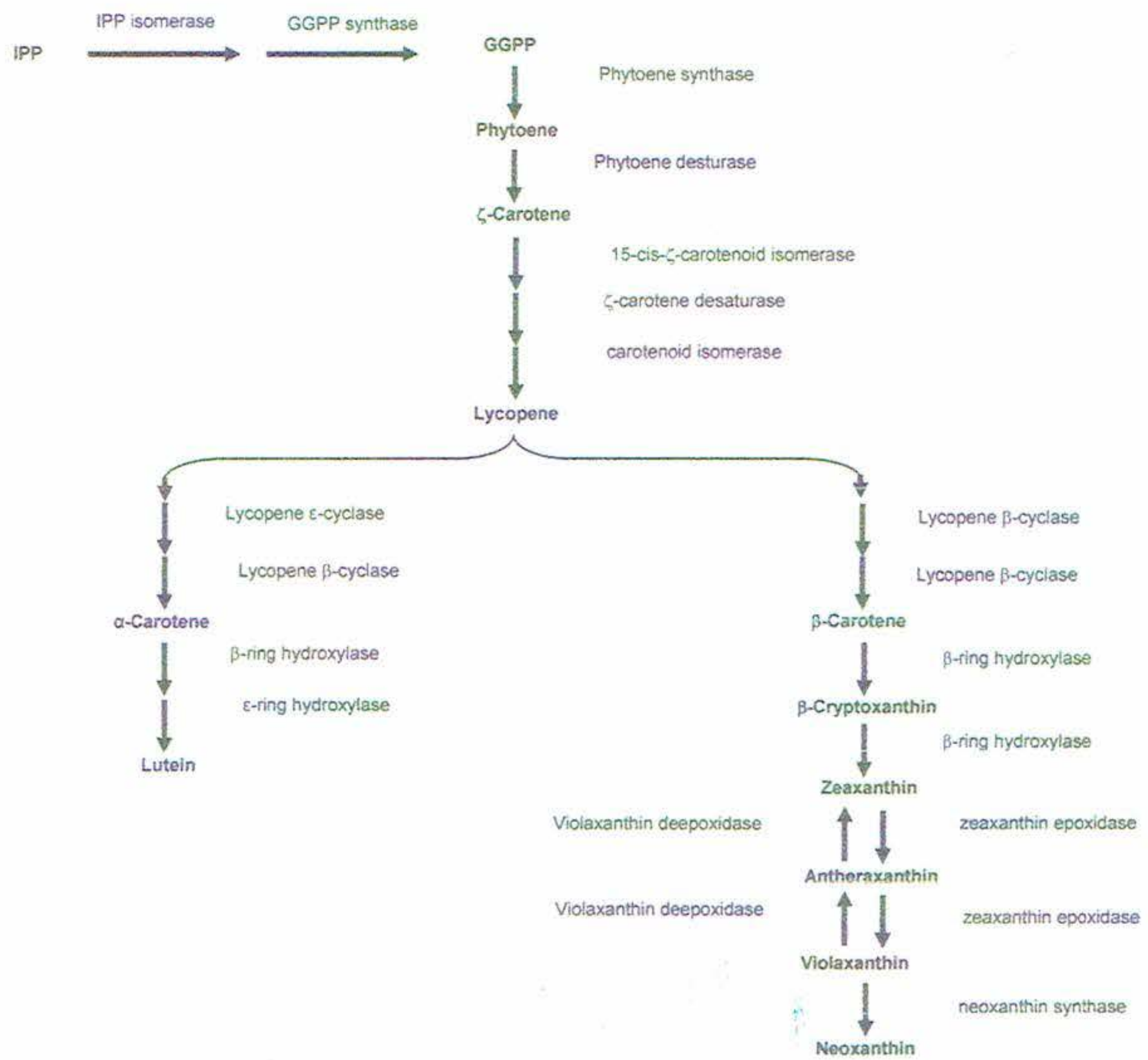

Fig. 1. Carotenoids biosynthesis pathway in plants (Kato et al., 2004; Yano et al., 2005; Diretto et al., 2006; Kato et al., 2007; Yamamizo et al., 2008; Apel and Bock, 2009; Liu et al., 2009; Jaswir and Noviendri, 2010). Note: **IPP**, Isopentenyl pyrophosphate; **GGPP**, geranylgeranyl pyrophosphate.

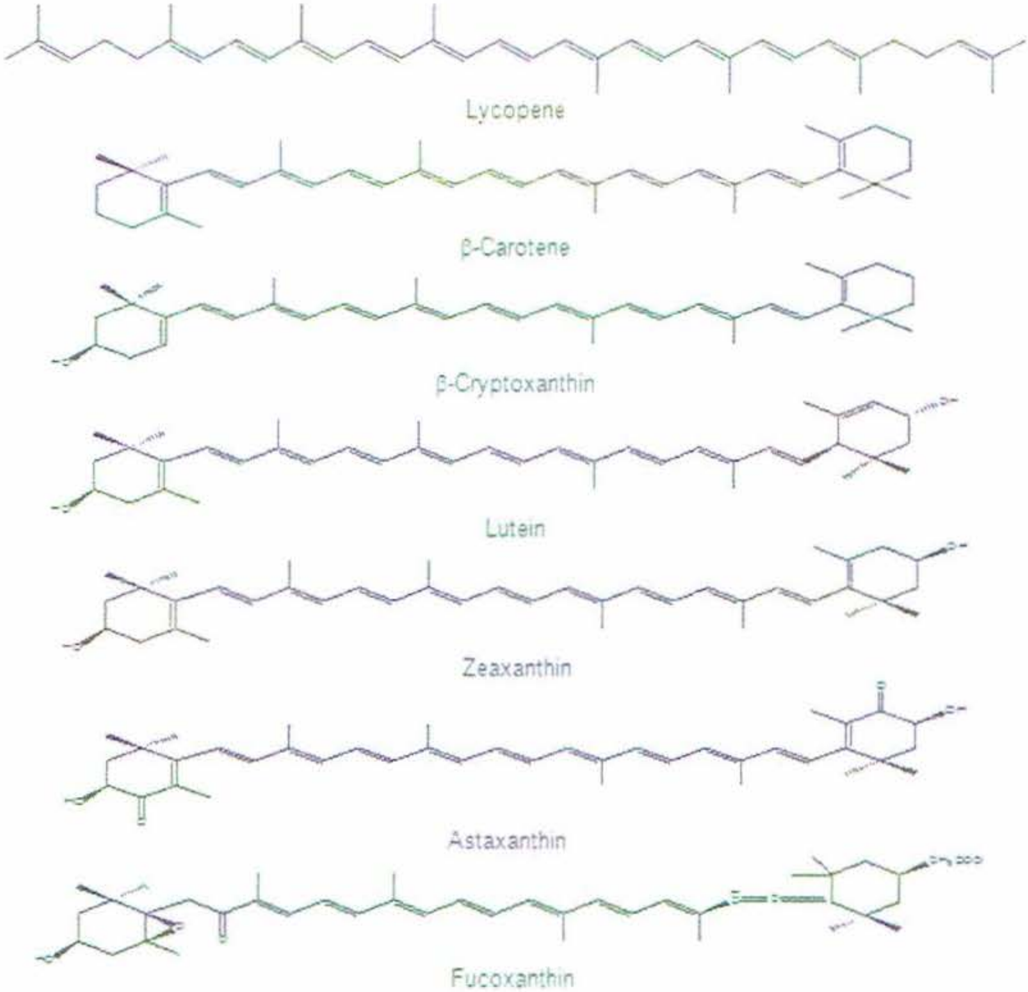

Fig. 2. Chemical structures of several selected carotenoids (Jaswir and Noviendri, 2010; Noviendri et al., 2011).

Carotenoids in nature are responsible for the characteristic colors of various kinds of fruits, vegetables and shellfish (Aoki et al., 2002) and play important biological roles as accessory light-harvesting components of photosynthetic systems, photoprotecting antioxidants, and regulators of membrane fluidity (Umeno et al., 2005). These compounds responsible for color ranging from light yellow through orange to deep red are biosynthesized in all of the photosynthetic organisms containing cyanobacteria, algae and higher plants, and also in some of non-photosynthetic bacteria, yeasts and fungi (Misawa, 2009). Carotenoids as accessory light-harvesting pigments play an essential role in the protection of plants against excess light and photooxidative stress (Demmig-Adams and Adams, 2002). Furthermore, carotenoids exhibit a long central chain of conjugated double bonds carrying acyclic or cyclic substituents (Stahl and Sies, 2007).

SOURCES OF CAROTENOID

Carotenoids are biosynthesized by bacteria, algae, fungi, and plants (Armstrong et al., 1996), but not by animals, which must obtain them from their food. Not only plants, e.g. vegetables (Table 1), fruits, cereals, etc. (Figure 3 and 4), carotenoids also produced by microorganisms are lycopene, β-carotene, astaxanthin, lutein, zeaxanthin (Bhosale, 2004), β-cryptoxanthin and canthaxanthin (Bhosale and Bernstein, 2005). Lycopene, α-carotene, β-carotene, lutein, zeaxanthin, andβ-cryptoxanthin are the most abundant in human plasma (Aizawa and Inakuma, 2007).

Table 1. Common dietary sources of carotenoids in regular vegetable foods (µg/100 fresh weight) Note: *: carotenoid esters present. Source: Southon and Faulks, (2003).

Food	β-Carotene	Lutein	β-Cryptoxanthin	Lycopene	α-Carotene	Zeaxanthin
Carrot	7975	271	-	-	2186	-
Spinach	4489	6265	-	-	-	-
Broccoli	1580	2560	-	-	-	-
Lettuce	890	1250	-	-	-	-
Green peas	548	1840	-	-	-	-
Watercress	5919	10713	-	-	-	-
Tomato	608	77	-	4375	-	-
Orange*	250	120	700	-	200	-
Orange juice*	375	1180	1980	-	-	-
Mandarin*	275	50	1775	-	-	140
Sweet corn	45	520	-	-	60	440
Red pepper*	1700	270	250	-	30	600
Apricot*	3500	70	120	trace	Trace	-
Mango*	3100	-	800	-	-	-
Papaya*	640	-	770	3400	30	-
Watermelon	180	20	300	4750	trace	-

β-cryptoxanthin is a carotenoid pigment found in peach, papaya, and citrus fruits such as orange and tangerine (Sugiura et al., 2002). This carotenoid is abundant in *Satsuma Mandarin* (*Citrus unshiu* MARC) (Yamaguchi et al., 2004; Yamaguchi, 2008), and it is enzymatically converted from β-carotene in plants (Uchiyama et al., 2004a; Yamaguchi et al., 2005). So, Lycopene is responsible for the red color in fruits and vegetables, including tomatoes, red grapes, watermelon, and pink grapefruit. It is also found in papayas and apricots (Zeb and Mehmood, 2004). Lycopene occurs in our diet, predominantly in tomatoes and tomato products (Nishino et al., 2002). Then, zeaxanthin is the dihydroxy-form of β-carotene, and it is distributed in our daily foods, such as corn and various vegetables (Nishino et al., 2002). Zeaxanthin is an oxygenated carotenoid or xanthophyl composed of 40 carmon atoms, yellow in color, and found naturally in corn, alfalfa and marigold flowers (Nelis and DeLeenheer, 1991). Zeaxanthin is also abundant in wolfberry. Zeaxanthin content in wolfberry is very high (Chang et al., 2010).This compound has been used in the cosmetic industry and the feed industry for birds, swine and fish (Masetto et al., 2001).

β-carotene were isolated and identified from peel, pulp and seed fractions of *CanariumodontophyllumMiq* (Prasad et al., 2011). Furthermore, β-carotene has been produced from microalgae, *Dunaliellasalina* (Pisal and Lele, 2005) and zeaxanthin has been produced from *Flavobacteriumsp* (Masetto et al., 2001).Moreover, astaxanthin is a red pigment included in red sea animals such as crab, shrimp and red fish (e.g., red sea bream and salmon).

At present astaxanthin is manufactured mainly by chemical synthesis, but some processes for the biological production of. Moreover,astaxanthin have been developed using several nature sources such as the green alga *Haematococcuspluvialis* (Misawa, 2009) and the yeast *Xanthophyllomycesdendrorhous*(formerly classified as *Phaffiarhodozyma*) (An et al., 2001; Kim et al., 2005; Gupta et al., 2007; Harada et al., 2009). Astaxanthin, as well as carotenoproteins,is responsible for the red color of red fish and crustaceans (Misawa, 2009). When cooked, the body color of prawns becomes red and the depth of the color depends upon the carotenoid content (Okada et al., 1994). In addition, fucoxanthin is distributed in our daily foodstuff, such as seaweed, and is one of the major carotenoids which distributed in marine organisms (Nishino et al., 2002).

Furthermore, carotenoids are found in colored fruits and vegetables. Apricots, cantaloupe, carrots, pumpkin and sweet potato are sources of α-carotene and β-carotene. Pink grapefruit, tomatoes and watermelon are sources of lycopene, ζ-carotene, β-carotene, phytofluene and phytoene. Spinach isa source of lutein, zeaxanthin, α- and β-carotene; mango, papaya, peaches, prunes, squash and oranges are sources of lutein, zeaxanthin, β-cryptoxanthin, α-, β- and ζ-carotene, phytofluene and phytoene. (Paiva and Russel., 1999).The content of carotenoids (β-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin) have been reported byBritton, and Khachik, (2009). However, the precise values are not given but the content is indicated as a range, as follows. Low: 0 - 0.1 mg/100 g; Medium: 0.1 - 0.5 mg/100 g; High: 0.5 - 2 mg/100 g; Very high: >2 mg/100 g (Table 2).

Table 2. Food sources of the nutritionally important carotenoids. Note: L=Low: 0 - 0.1 mg/100 g; M=Medium: 0.1 - 0.5 mg/100 g; H=High: 0.5 - 2 mg/100 g; VH=Very high: >2 mg/100 g.

Sources	Latin Name	Carotenoids	Content
Apricot	*Prunusarmeniaca*	β-carotene	H-VH
		Lycopene	L
Broccoli	*Brassica oleracea (Italiaca)*	β-carotene	VH
		Lutein	VH
Carrot	*Daucuscarota*	β-carotene	VH
		Lycopene	H
Grapefruit	*Citrus paradisi*	β-carotene	L-M
		Lycopene	M-H
Green leafy vegetables		β-carotene	M-VH
		Lutein	VH
Guava	*Psidiumguajava*	β-carotene	M
Kale	*Brassica oleracea (Acephala)*	β-carotene	V-H
'Karat' banana	*Musa troglodytarum*	β-carotene	H
Lettuce	*Lactuca sativa*	β-carotene	M-H
Loquat	*Eriobotrya japonica*	β-carotene	M
		β-cryptoxanthin	L-M
Mango	*Mangiferaindica*	β-carotene	H-VH
Orange and juice	*Citrus* spp. and hybrids	β-carotene	L-M
Papaya	*Carica papaya*	β-carotene	M
		β-cryptoxanthin	M-H
		Lycopene	M-H
Peach	*Prunuspersica*	β-carotene	H
Pepper (red, orange, green)	*Capsicum annuum*	β-carotene	H
Pepper (red, orange)	*C. annuum*	β-cryptoxanthin	M
Pepper (yellow, green)	*C. annuum*	Lutein	VH
Pepper (orange, red)	*C. annuum*	Zeaxanthin	VH
Red palm oil	*Elaeisguineensis*	β-carotene	VH
Spinach	*Spinaciaoleracea*	β-carotene	VH
Squash/pumpkin.	*Cucurbitaspp*	β-carotene	L-M
		β-cryptoxanthin	M-H
		Lutein	M-VH
		Zeaxanthin	M
Sweet potato	*Ipomoea batatas*	β-carotene	VH
Tomato	*Lycopersiconesculentum*	β-carotene	M
		Lycopene	VH
Persimmon	*Diospyros kaki*	β-cryptoxanthin	H
		Lycopene	L-H
		Zeaxanthin	M
Pitanga	*Eugenia uniflora*	Lycopene	H
Egg yolk		Lutein	M-H
Water melon	*Citrulluslanatus*	Lycopene	H-VH
Sweetcorn	*Zea mays*	Zeaxanthin	M

Source: Britton, and Khachik, (2009).

Carotenoids are intracellular products and are usually located in membranes of mitochondria, chloroplasts or endoplasmic reticulum (Margalith, 1999). Generally, carotenoids are highly hydrophobic compounds which will therefore tend be associated with lipid (oil, fat) or in hydrophobic structure such as membranes (Britton et al., 2008). The carotenoids of fruit, vegetables and animal products are usually fat-soluble and are associated with lipid fractions, lipid portions of human tissues, cells (El-Qudah, 2008), and lipid core of the membrane bilayer (Britton et al., 2008). The orientation of a particular carotenoid in the membrane, and its effect on membrane properties, depend on structural features such as the size and shape of the carotenoid and the presence of functional groups (Britton et al., 2008).

CAROTENOIDS AND THEIR MEDICINAL PROPERTIES

Carotenoids as provitamin A (Tang and Russell, 2009).

Indeed, the provitaminA activity of carotenoids has been known for a long time (Rodriguez-Amaya, 1997). More than 600 natural carotenoids have been identified (Aizawa and Inakuma, 2007; Rao and Rao, 2007). Of the approximately 700 carotenoids found in nature, only about 50 have provitamin A activity (Rodriguez-Amaya, 1997; Okada et al., 2008), Among them, only three are most important precursors of vitamin A in humans: α-carotene, β-carotene and β-cryptoxanthin (Thane and Reddy, 1997; Park et al., 2009; Carrillo-Lopez et al., 2010) (Table 3)which are converted into vitamin A or retinol in the body (Zeb and Mehmood, 2004). The β-carotene is the major pro-vitamin A component of most carotenoid-containing foods and is found in fruits and vegetables, is a weel known nutrient exhibiting pro-vitamin A activity (Imamura et al., 2006). This compound is precursor of vitamin A, which is well known to capable of preventing serious eye diseases, such as night blindness (Takashi et al., 2006). Moreover, in order to function physiologically as vitamin A, carotenoids containing food must be well digested to release the carotenoids from the food matrix (Carrillo-Lopez et al., 2010).

Table 3. The sources of carotenoids in fruit and vegetables and their medicinal properties to humans

Carotenoid type	Example	Abundant sources	Medicinal Properties
Provitamin A	α-carotene	Green leafy vegetables and	Antioxidants, anticarcinogenic
	β-carotene	Yellow-orange fruit and vegetables, e.g. carrot, broccoli, spinach, parsley, celery, tomatoes.	Antioxidant boosts activity of Natural Killer immune cell, Gives cornea protection against UV light.
	β-cryptoxanthin	Peaches, oranges, tangerins, mangoes, papayas	Antioxidants, anticancer
Non-provitamin A	Lycopene	Tomatoes, pink-red, grapefruit, red-fleshed papayas, water melon	Powerful antioxidant, protects against formation of cancers (Prostate, Bladder, Cervical, Leukemia, reduces cholesterol levels.
	Lutein	Green and dark green, leafy vegetables-spinach, parsley, kale, broccoli, corn, avocado, Brussels sprouts, beans, peans.	Protects the eyes against the development of Age-related Macular Degeneration (AMD), Cataracts, Anticancer (colon)
	Zeaxanthin	As for lutein, plus some fruits, e.g. mandarins, peaches, oranges	Protects eye from macular degeneration and cataracts

Sources: Thane and Reddy, (1997); Dharty et al., (2010); Keservani et al., (2010).

Carotenoids as antioxidant/pro-oxidant (Yeum et al., 2009).

In all organisms carotenoids may function as antioxidants and promote oxidative stress resistance (Tian et al., 2007). In the human organism, carotenoids are part of antioxidant defense system (Stahl and Sies, 2003). Carotenoids can quench single oxygen in a similar manner to tocopherols. Carotenoids, as well as, and tocopherols, are known to be efficient antioxidants and capable of scavenging reactive oxygen species generated during photooxidative stress (Stahl et al., 2000)Furthermore, carotenoids can scavenge oxidizing free radicals via at least three primary reactions (equation 1-3), by its addition, electron transfer, addition and hydrogen atom transfer (El-Agamey and McGarvey, 2008). They are also able to react directly with superoxide and other free radicals. Carotenoids (CAR) can form resonance-stabilised carbon-centred radicals, e.g. by reaction with lipid peroxyl radicals ($ROO^{\cdot}$) (Smirnoff, 2005):

$CAR + ROO^{\cdot} \rightarrow ROO\text{-}CAR^{\cdot}$ *Addition* (1)

They can transfer electrons forming a radical cation ($CAR^{\cdot+}$):

$CAR + ROO^{\cdot} \rightarrow CAR^{\cdot+} + ROO^{-}$ *Electron transfer* (2)

Allylic hydrogen abstraction, e.g. from lipid peroxyl radicals, could also occur:

$CAR + ROO^{\cdot} \rightarrow CAR^{\cdot} + ROOH$ *Hydrogen atom transfer* (3)

Alternatively, they can accept electrons, e.g. from superoxide, forming a radical anion, as in the case of lycopene:

$Lycopene + O_2^{\cdot-} \rightarrow Lycopene^{\cdot-} + O_2$

Lycopene can function as an antioxidant by several mechanisms (Erdman et al., 2008).One of the best documented mechanisms is through the quenching singlet oxygen ($^1O_2$), that occurs as follows (Krinski, 1992):

$Lycopene + {}^1O_2 \rightarrow {}^3Lycopene + {}^3O_2$

Lycopene in the excited state (^{3}lycopene) has insufficient energy to cause excitation of other molecules and generate reactive species. Therefore, more than one free radical can be quenched by a single lycopene molecule (Krinski, 1992). Unlike some other carotenoids, lycopene does not have pro-vitamin A properties. Because of the unsaturated nature of lycopene it is considered to be a potent antioxidant and a singlet oxygen quencher (Rao and Rao, 2007). In photosensitized oxidation, carotenoids, especially lycopene and β-carotene, are effective quenchers of singlet oxygen ($^1O_2$) (Bonnie and Choo, 1999).

More than 80% of lycopene consumed in the United States (Table 4) is derived from tomato products, although apricots, papaya, pink grapefruit, guava, and watermelon also contribute to its dietary intake. Lycopene from tomatoes now is consumedin approximately the same amount as β-carotene (Paiva and Russel, 1999). Lycopene content of tomatoes can vary significantly, depending on type of tomato and ripening (Stahl and Sies, 2007). Lycopene, a predominant carotenoid in tomatoes, exhibit highest antioxidant activity among all dietary carotenoids (Rao and Agarwal, 1999).

Table 4. Examples of major contributor of carotenoids in United States diets

Carotenoids	Food sources	Amount (μg/100g)
β-carotene	Apricot, dried	17600
	Carrots, cooked	9771
	Green Collard	5400
	Spinach, cooked	5300
	Canteloupe	3000
	Beet Green	2560
	Broccoli, cooked	1300
	Tomato, raw	520
α-carotene	Carrots, cooked	3723
Lycopene	Tomatoes paste	36500
	Tomatoes sauce	13060
	Tomatoes ketchup	12390
	Tomatoes juice	10000
	Tomatoes, raw	3100
Lutein	Green collard	16300
	Spinach, cooked	12475
	Beet, green	7700
	Broccoli, cooked	1839
	Green peas, cooked	1690
β-cryptoxanthin	Tangerine	1060
	Papaya	470

Sources: Mangels et al., (1993); Rao and Rao, (2007).

Cooking and food processing enhance the bioavailability of lycopene (Gärtner et al., 1997), and improving accessibility of the lipophilic compound for the formation of lipid micelles together with dietary lipids and bile acids (Stahl and Sies, 2007). Thus, lycopene uptake is higher after ingestion of processed tomatoes (tomato paste) as compared to fresh tomatoes (Gärtner et al., 1997). Furthermore, besides of lycopene, β-carotene and others carotenoids have antioxidant properties *in vitro* and in animal models. The use of animal models for studying carotenoids is limited since most of the animals do not absorb or metabolize carotenoids similarly to humans (Paiva and Russel, 1999). Then, Nishino et al., (2002) reported that antioxidative activity of lycopene and α-carotene were proven to be higher than that of β-carotene. In addition, mixtures of carotenoids or associations with others antioxidants (*e.g.*, vitamin E) can increase their activity against free radicals.

Carotenoids as anticancer (Nishino, 1998)

Worldwide, about 10 million cancer diagnoses occur each year, and the number is increasing rapidly. It has been projected that if people were to eat plant-based diets rich in a variety of vegetables (broccoli, carrots, arugula, pumpkins, sweet potatoes, squash, tomatoes, watercress) and fruits (apricots, cantaloupes, mangos, papayas, peaches and persimmons, legumes) and minimally processed starchy staple foods each day, the overall cancer rates could decline by as much as 20% (Basu et al., 2001). A diet that includes a sufficient amount of vegetables and fruits, including those that are rich in carotenoids, is a scientifically supportable low-risk strategy that would enable the potential beneficial effects of carotenoids on the risk and progression of cancer to be realized (Rock, 2009).

Carotenoids (α-carotene, lutein, zeaxanthin, lycopene, β-cryptoxanthin, fucoxanthin, astaxanthin), as well as β-carotene, may be useful for cancer prevention (Nishino et al., 2002). Scientific interest in dietary carotenoids has increased in recent years because of their beneficial effects on human health, such as lowering the risk of cancer and enhancement of immune system function, which are attributed to their antioxidant potential (Das et al., 2007) (Table 5).

A diet that includes a sufficient amount of vegetables (broccoli, carrots, arugula, pumpkins, sweet potatoes, squash, tomatoes, watercress) and fruits (apricots, cantaloupes, mangos, papayas, peaches and persimmons, legumes), including those that are rich in carotenoids, is a scientifically supportable low-risk strategy that would enable the potential beneficial effects of carotenoids on the risk and progression of cancer to be realized (Rock, 2009). In this sense, the scientific interest in dietary carotenoids (α-carotene, lutein, zeaxanthin, lycopene, β-cryptoxanthin, fucoxanthin, astaxanthin), as well as β-carotene has increased in recent years because of their beneficial effects on human health, such as lowering the risk of cancer and enhancement of immune system function, which are attributed to their antioxidant potential (Das et al., 2007) (Table 5).

Table 5. Incidence of medical condition treated with carotenoids (nutraceutical)

Medical conditions	Incidence of condition (%)	Nutraceuticals used
Enlarge prostate	16.9	Lycopene
Degenerative eye conditions	9.9	Lutein
Lactose intolerance	7.2	Lycopene
Diabetes	5.8	Lycopene
Prostate cancer	5.3	Lycopene

Source: Gunther et al.,(2004).

Among the carotenoids, lycopene is major component found in the serum (Rao and Agarwal, 1999). Interestingly, it was shown that carotenoid (lycopene) was shown to enhanced the expression of connexin43, a gene encoding major gap junction protein, and thereby upregulated gap junction communication.and acted as anticarcinogeneic (Zhang et al., 1991; Zhang et al., 1992) (Figure 6). Lutein, zeaxanthin, and cryptoxanthin are major xanthophyll carotenoids in human plasma. The consumption of these xanthophylls is directly associated with reduction in the risk of cancers, cardiovascular disease, age-related macular degeneration, and cataract formation (Bhosale and Bernstein, 2005).

Fucoxanthin is one of the most abundant carotenoids found in *Undariapinnatifida* (Liu et al., 2009) and it distributed on the earth as abundantly as β-carotene and its anticancerogenic effecthas been studied. Thus, it seems worthy to evaluate its biological activity (Nishino, 1998). There are many researches about this compound on anticancer activities. The apoptosis-inducing effect of fucoxanthin on human leukemia HL-60 cells was investigated.Fucoxanthininhibited the proliferation of the HL-60 cells and induced DNA fragmentation, a typical characteristic feature of apoptotic cells (Hosokawa et al., 1999). This carotenoid also showed Fucoxanthin has been shown tumor proliferation *in vitro*the inhibition of tumor proliferation of human hepatoma SK-Hep-1 cells and murine embryonic liver BNL CL.2 cells (Liu et al., 2009). Furthermore, it was reported that it induces cells cycle arrest at G0/G1 phase in human colon adenocarcinoma WiDr cells (Das et al., 2005), and human hepatocarcinoma HepG2 cells (Das et al., 2008). In addition, fucoxanthin remarkably reduced the viability of human colon cancer cell lines, such as Caco-2, HT-29 and DLD-1 (Hosokawa et al., 2004).

Carotenoid as antiobesity effect (Maeda et al., 2005)

Obesity is an abnormal condition produced by lipid accumulation in adipose tissue (Ikeuchi et al., 2007).Especially, the accumulation of fat around the internal organs is a major risk factor causing many kinds of diseases (Miyashita, 2006). A great deal of interest has been focused on adaptive thermogenesis by uncoupling protein (UCP) families (UCP1, UCP2, and UCP3) as a physiological defense against obesity, hyperlipidemia and diabetes (Jezek, 2002). Mitochondrial uncoupling protein 1 (UCP1), usually expressed only in brown adipose tissue (BAT) (Maeda et al., 2007a), is a key molecule for antiobesity (Miyashita, 2006) as its dysfunction contributes to the development of obesity (Lowel et al., 1993). Maeda et al., (2007a) hadreported that feeding with fucoxanthin (carotenoid) significantly reduce white adipose tissue (WAT) in rats and mice with a clear expreesion of UCP1 protein and mRNA in WAT, while there was little expression of UCP1 in WAT in mice fed control diet. In addition, the combination of fucoxanthin and fish oil was more effective for attenuating the weight gain of WAT than feeding with fucoxanthin alone (Maeda et al., 2007b). Then, the daily intake of fucoxanthin in mice also caused a significant reduction of body weight (Maeda et al., 2008).

Furthermore, Ikeuchi et al., (2007) have been reported that astaxanthin inhibits the increaseof body weight and adipose tissues weight caused by a diet high in fats. In addition, astaxanthin reduced liver weight, liver triglyceride, plasma triglyceride, and total cholesterol. These results indicated that astaxanthin might be of value in preventing obesity.

Carotenoid as anabolic effect on bone components (Uchiyama et al., 2004)

β-cryptoxanthin is a kind of carotenoid that has a potential effect in maintaining bone health and in preventing osteoporosis (Yamaguchi, 2008). The presence of β-cryptoxanthin (10^{-7} or 10^{-6} M) caused a significant increase in calcium content and alkaline phosphatase activity in the femoral-diaphyseal and femoral–metaphysial tissues (Yamaguchi, 2008) in 50 weeks old female rats (Uchiyama et al., 2004a). This compound may have a stimulatory effect on bone mass loss that induces osteoporosis leading to bone fracture, as Uchiyama et al., 2004b reported that its oral administration induced an anabolic effect of bone components in femoral tissue of aged female rats *in vivo*. In addition, this carotenoid has a potent anabolic effect on bone calcification in femoral-diaphysial (cortical bone) and femoral-metaphysial (trabecular bone) tissues of rats in vitro (Yamaguchi and Uchiyama, 2003), and *in vivo* (Uchiyama et al., 2004c). Moreover, Yamaguchi et al., (2006) reported that the intake of reinforced juice, which contains more β-cryptoxanthin than regular juice, has a preventive effect on bone loss over age. Furthermore, they reported that the prolonged intake of juice fortified with β-cryptoxanthinhas a stimulatory effect on bone formation and an inhibitory effect on bone reabsorption in humans, which is beneficial in menopausal woman.

APPLICATION OF CAROTENOIDSIN FOOD AND NUTRACEUTICAL INDUSTRY

Carotenoids are widely used in food applications (Bonnie and Choo, 1999). Carotenoids such as β-carotene and lycopene have plenty of scientific and commercial value (Liu et al., 2009b). Traditionally, carotenoids have been used in the feed, food and nutraceutical industries. The recent discoveries of health-related beneficial properties attributed to carotenoids have spurred great interest in the production of structurally diverse carotenoids for pharmaceutical applications (Lee and Schmidt-Dannert, 2002). Currently, carotenoids are used commercially as natural food colorants, nutrient supplement (Bramley, 2003), feed additives (Bhosale and Bernstein, 2005), animal feed supplements and, more recently, as nutraceuticals for cosmetic and pharmaceutical purposes (Schmidt-Dannert, 2000; Lee and Schmidt-Dannert, 2002) (Table 6). Industrially, carotenoids are used in pharmaceuticals, neutraceuticals, and animal feed additives, as well as colorants in cosmetics and foods (Das et al., 2007; Mortensen, 2009) (Table 6).

Table 6. Global market for Carotenoids in 2004 and 2009 (US$ Million)

	β-carotene		Lycopene		Lutein		Cantaxanthin		Astaxanthin		Zeaxanthin	
	2004	2009	2004	2009	2004	2009	2004	2009	2004	2009	2004	2009
Supplements	125.0	128.0	50.5	68.7	54.0	85.0	3.0	3.0	3.5	4.8	22.0	35.0
Food	98.0	103.0	3.5	9.3	18.0	20.0	7.0	8.0	0	0	0	0
Cosmetics	6.0	7.0	0	3.0	0	0	0	0	0	0	0	0
Feed	13.0	15.0	0	0	67.0	82.0	138.0	145.0	230.5	252.2	0	0
Total	242.0	253.0	54.0	81.0	139.0	187.0	148.0	156.0	234.0	257.0	22.0	35.0

Source: Mortensen, (2009).

Fruits and vegetables constitute the major sources of carotenoid in human diet. About 40 are present in a typical human diet (Rao and Rao, 2007). Although carotenoids are present in many common human foods, deeply pigmented fruits, juices and vegetables constitute the major dietary sources with yellow-orange vegetables and fruits providing most of the β-carotene and α-carotene, orange fruits providing α-cryptoxanthin, dark green vegetables providing lutein and tomatoes and tomato products lycopene (Mangels et al., 1993). Table 4 shows typical food sources and amounts of carotenoids present (Rao and Rao, 2007).

In most countries, the using of food additives (including colorants) is governed by strict regulation. The legislation specifies which colorants may be used the sources of the colorant, the purity of the colorant, to which foods the colorant may be added, and at what level the colorant may be added to a specific food (Mortensen, 2006). For examples, the natural and nature-identical colorants shown in Table 7 are allowed in the EU and the USA (in this case, colorants only allowed in fish or chicken feed for pigmenting flesh and/or eggs are not included).

In food application, carotenoid namely, lycopene is allowed in the EU as a food colorant and has recently (July 2005) become allowed in the USA as well. The only allowed source is tomatoes (*Lycopersiconesculentum*).lycopersicon meaning wolf peach). Besides lycopene, a tomato oleoresin also contains appreciable amounts of β-carotene, phytoene, and phytofluene (Mortensen, 2006). This compound is hardly used as a colorant because it is a rather expensive pigment and is very prone to oxidative degradation (much more so than β-carotene) (Mortensen, 2006). Food colorants has always been target of complains of the food industry consumers, mainly because of the bad fame of the initial synthetic pigments that only have a cosmetic value and were associated with health damage (Mattea et al., 2009).

Table 7. Approved carotenoids for food and feed industries by different organisms

Color	EU	FDA	WHO
Mixed carotenes (carrot oil)	Yes(Non-legislated)	Yes	Not specified
β-Carotene	Yes (E 160a)	Yes	Yes
Annatto extract, bixin, norbixin	Yes (E 160b)	Yes	Yes
Canthaxanthin	No (E 161g)	Yes	Not-allocated
β-Apo-8'-carotenal	No (E 160e)	Yes	Yes
Citranaxanthin	No	Yes	Yes
Xanthophylls, flavoxanthin, rubixanthin, zeoxanthin, and other natural products with some of these carotenoids	Not all	Not all	Not specified

EU (European union); FDA (food and drug administration) and WHO (world health organization). Sources: Mortensen, (2006);Mattea et al., (2009).

Presently however, only a few carotenoids (β-carotene, lycopene, astaxanthin, canthaxanthin, capsanthin, lutein, annatto, β-apo-8-carotenal, β-apo-8-carotenal-ester) can be produced commercially by chemical synthesis,fermentation or isolation from the small number of abundant natural sources (Johnson and Schroeder 1995). Commercial production of carotenoids from microorganisms competes mainly with synthetic manufacture by chemical procedures. Efficient stimulation of carotenoid biosynthesis is expected to promote accumulation of carotenoid by microbes (Bhosale, 2004). Canthaxanthin and astaxanthin also have considerable importance in aquaculture for salmonid and crustacean pigmentation, and are of commercial interest for the pharmaceutical and food industries (Bhosale and Bernstein, 2005). The global market of astaxanthin that is extracted from an unicellular green alga *Haematococcuspluvialis* producing this pigment (ester forms) has been extended as a food supplement or nutraceutical (Misawa, 2009). However, most of the commercially used carotenoids (e.g. β-carotene, astaxanthin and cantaxanthin) are produced by chemical synthesis (Johnson and Schroeder, 1995) (Table 8).

Table 8. Examples of plant extracts (contain carotenoids) used commercially

Plant	Carotenoids	Use
Bixaorellana seeds	Bixin, norbixin (annatto)	Food colouring
Carrot root	Carotenes, mainly β-carotene	Dietary supplement
Capsicum annum fruit	Capsanthin, capsorubin (paprika)	Food colouring
Crocus sativus petals	Crocin, crocetin (saffron)	Food colouring
Marigold petals	Lutein, zeaxanthin	Dietary supplement
Tomato fruit	Lycopene	Dietary supplement
Palm oil	Carotenes	Dietary supplement, colouring

Source: Bramley, (2003).

CONCLUSION

Carotenoids, the basic source of yellow, orange, and red plant pigments, are widely distributed in nature. Carotenoids have been known have some medicinal properties. They are widely used in food and nutraceutical applications. Presently however, only a few carotenoids can be produced commercially by chemical synthesis. Currently, commercial production of carotenoids from microorganisms competes mainly with synthetic manufacture by chemical synthesis. However, most of the commercially used carotenoids (e.g. β-carotene, astaxanthin and cantaxanthin) are produced by chemical synthesis. Furthermore, in most countries, such as EU and USA the application of carotenoid, namely as food additives (including colorants) is governed by strict regulation.

ACKNOWLEDGMENT

This work was financially supported by a research grant (IIUM RMGS 09-05) from Research Management Center (RMC), International Islamic University Malaysia.

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