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Nutraceutical β-carotene from natural non-conventional sources and its applications

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Abstract

Nutraceutical is a food or food product that reportedly provides health and medical benefits, including the prevention and treatment of diseases. Carotenoids are the most common naturally occurring terpenoid pigmented nutraceuticals. β-carotene is the main source of provitamin A and has widely been used as a food colorant and nutraceutical. Epidemiological evidences and experimental results suggest that β-carotene inhibit the onset of many diseases in which free radicals are thought to play a role in initiation such as arteriosclerosis in cardiovascular diseases, cataracts, age related macular degeneration, multiple sclerosis and most importantly cancer, due to it's free radical and single oxygen scavenging property. The outstanding quencher function of β-carotene advocates dietary supplementation. β-carotene is present at high concentrations in a variety of plant sources. However, the seasonal variations in the carotenoid content and composition of plant sources have been considered as disadvantage. The production of β-carotene can be achieved through biotechnology from non-conventional sources *viz.*, algae, yeasts, bacteria or filamentous fungi.

Key words: Nutraceuticals, β-carotene, Carotenoids, Antioxidants, Anticancer, Dietary supplements, Microbial sources

Introduction

Carotenoids are natural pigments, synthesized as hydrocarbons (carotenes *viz.*, lycopene, α-carotene and β-carotene) or their oxygenated derivatives (xanthophylls, lutein, α-cryptoxanthin and astaxanthin) by plants and microorganisms (Das *et al.*, 2007). The majority are hydrocarbons of 40 carbon atoms which contain two terminal ring systems, joined by a chain of conjugated double bonds or polyene system (Frengova and Beshkova, 2009). Carotenoids are of importance in animals and humans (Table 1) for the purpose of enhancement of immune response, conversion to vitamin A and scavenging of oxygen radicals (Bast *et al.*, 1998; Hughes, 1999; Hughes, 2001; Jimrenez-Escrig *et al.*, 2000; Lee *et al.*, 2003; Kiokias and Gordon, 2004).

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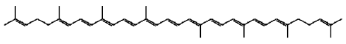
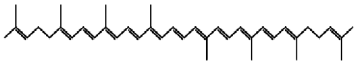
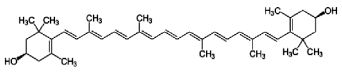
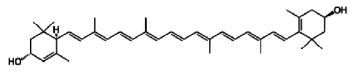
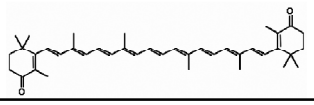
The β-carotene is a carotenoid of increasing demand as precursor of vitamin A, has also been ascribed a central role in cancer prevention and therapy. This is related to the antioxidant property of carotenoids with their conjugated polyene structure, predestined for free radical and singlet oxygen scavenging (Siems *et al.*, 2005). Additionally, β-carotene reduces the risk of cardiovascular diseases, cataract development and macula degeneration (Wong *et al.*, 2011). Moreover, the outstanding quencher function of β-carotene advocates dietary supplementation (Martano *et al.*, 2011). The nutraceutical bloom has also integrated β-carotene, mainly on the claim of their proven antioxidant properties (Delcampo *et al.*, 2007). The beneficial effects of β-carotene on cancer have been found in a number of studies, including epidemiological studies (Block *et al.*, 1992; Ziegler *et al.*, 1989), clinical studies (Holick *et al.*, 2002; Matsuda *et al.*, 2006), animal model studies (Bhosale *et al.*, 2002; Rencuzogullari and Erdogan, 2007) and cell culture studies (Palozza *et al.*, 2002; Prakash *et al.*, 2001; Palozza *et al.*, 2004; Cui *et al.*, 2007; Upadhyaya *et al.*, 2007; Palozza *et al.*, 2008; Shiau *et al.*, 2010; Yurtcu *et al.*, 2011).

Table 1: Structure and applications of important carotenoids

Carotenoids	Chemical structure	Major applications	Reference
Lycopene		Against cardiovascular diseases, prostate cancer and cosmetic preparations	Rissanen *et al.*, 2002
β-carotene		Anticancer agent, nutraceutical as provitamin A source, food colorants, photoprotectant and cosmetic preparations, prevention of age related macular degeneration	Van Keulen *et al.*, 2010; Palozza *et al.*, 2004, Wong *et al.*, 2011
Zeaxanthin		Prevention of age-related macular degeneration and cosmetic preparations	Seddon *et al.*, 1994
Lutein		Used in cosmetic preparations and cancer treatment	Kumaresan *et al.*, 2008
Canthaxanthin		Used in animal feed	Krupa *et al.*, 2010

Along with its medicinal effects, β-carotene has also been used in the cosmetics, food and feed industries, due to its colorant and antioxidant properties (Bhosale and Bernstein, 2004). Owing to their outstanding properties and health related functions, the demand of carotenoids is continuously increasing. The global market of β-carotene was $ 247 million in 2007 and expected to be worth $ 285 million in 2015 with compound annual growth rate of 1.8% (Ribeiro *et al.*, 2011).

Presently, more than 85% of commercially available β-carotene, has been produced by chemical synthesis. However, the increasing importance of β-carotene in the feed, food, cosmetic and pharmaceutical markets and some of other overlapping segments such as nutraceutical and cosmoceutical segments has revamped efforts to produce β-carotene in useful amounts by non-conventional microbial sources (Van Keulen *et al.*, 2010).

Non-conventional natural sources of β-carotene

β-carotene constitute a multimillion dollar market worldwide but most of the commercially available β-carotene, has been produced by chemical synthesis. Synthetic β-carotene has been produced by Roche (since 1954) and BASF (since 1960). β-ionone is the main precursor for the synthesis of β-carotene and involves either enol-ether condensation or wittig condensation. .

Moreover, the large-scale extraction of carotene from vegetables did not seem feasible, due to economic, environmental and logistic constraints. The majority of the β-carotene (>85%), commercialized in the world, has been obtained by chemical synthesis from β-ionone. The conventional chemical synthesis processes use raw materials derived from fossil fuels that are processed through high temperature, high-intensive operating units, using chemical catalysts and reagents. The chemical industry has increasingly been recognizing the urgent need to diminish its dependence on petroleum based raw materials and fuels to minimize the environmental impact and enhancing the competitiveness. The use of biotechnology to replace existing processes, has expected to make many industries more efficient and environment friendly and contribute towards industrial sustainability (Van Keulen *et al.*, 2010).

Though plants are known to produce a variety of therapeutic molecules (Gautam *et al.*, 2012) including carotenoids; trends in fermentation biotechnology towards the development of processes for the production of high value food additives, have recently been reviewed and they revealed the strong interest towards the β-carotene of microbial origin (Ribeiro *et al.*, 2011).

The natural β-carotene can be produced by biotechnological processes, using filamentous fungi, yeasts, bacteria or microalgae (Table 2).

Table 2: Microbial sources of β-carotene

Microorganism	Reference(s)
Algae	
Dunaliella salina	Gercia-gonzalez *et al.*, 2005; Venkatesh *et al.*, 2005; Zhu and Jian, 2008, Ben Amotz, 2004
Dunaliella bardawil	Mogedas *et al.*, 2009
Haematococcus sp.	Lorenz and Cysewsk, 2000
Murielopsis sp.	DelCampo *et al.*, 2007
Picochlorum oklahomensis	Kviderova and Henley, 2005
Fungi	
Blakeslea trispora	Ciegler *et al.*, 1959; Jeong *et al.*, 1999; Mantzouridou *et al.*, 2002; Roukas *et al.*, 2003; Goksungur *et al.*, 2004; Xu *et al.*, 2007; Chaudhary and Singhal, 2008; Filotheou *et al.*, 2010; Verzakakou *et al.*, 2010
Phycomyces blakesleanus	Mehta *et al.*, 1997; Cerda-Olmedo, 2001; Papp *et al.*, 2009
Mucor circinelloides	Iturriaga *et al.*, 2005; Papp *et al.*, 2009
Fusarium sporotrichoides	Jones *et al.*, 2004
Neurospora crassa	Hansmann and Sandmann, 2000
Phycomyces nitens	Kivanc and Kahyaglu, 2008
Yeast	
Rhodotorula glutinis	Roadjanakamolson and Suntornsuk, 2010; Bhosale and Gadre, 2001; Kim *et al.*, 2004; Wang *et al.*, 2008
Rhodotorula glutinis DBVPG 3853	Buzzini *et al.*, 2000
Rhodotorula glutinis DM28	Malisorn and Suntornsuk, 2008;Malisorn and Suntornsuk, 2009
Rhodotorula graminis	Buzzini *et al.*, 2005
Rhodotorula rubra	Simova *et al.*, 2003
Rhodotorula mucilaginosa	Juckyoung *et al.*, 2009
Xanthophyllomyces dendorhous	An *et al.*, 1991
Sporodiobolus salmonicolor	Valduga *et al.*, 2008 a, b
Sporobolomyces roseus	Maldonade *et al.*, 2008
Cryptococcus sp.	Libkind and Broock, 2006
Rhodospiridium babjevae	Sperstad *et al.*, 2006
Bacteria	
Sphingomonas jaspsi	Asker *et al.*, 2007; Silva *et al.*, 2004
Mycobacterium kansasii	David, 1974
Flavobacterium multivorum	Bhosale and Bernstein, 2004
Flavobacterium sp.	Krubasik and Sandmann, 2000
Micrococcus sp.	Salah and Ibrahim, 2008

However, most of the researchers have reported β-carotene production, using *B. trispora*. Only 2% of the total β-carotene produced worldwide is natural, and has mainly been used as a nutritional supplement (Dufosse *et al.*, 2005).

Production of β-carotene from algae

Microalgae are major source for a vast array of valuable compounds, including a diversity of pigments, for which these photosynthetic microorganisms represent an almost exclusive biological resource. DelCampo *et al.*, (2007) have reviewed the most relevant features of microalgae bitotechnology, related to the production of different carotenoids with a main focus on β-carotene from *Dunaliella salina*. Microalgae combine properties typical of higher plants (efficient oxygenic photosynthesis and simplicity of nutritional requirements) with biotechnological attributes proper of microbial cells (fast growth in liquid culture and ability to accumulate or secrete some metabolites). These particular combinations support the use of these microorganisms for applied processes and represent the basis of microalgal biotechnology.

The most important process for natural production of β-carotene has been developed by the use of the culture of green, unicellular alga, *D. salina*. The extent of β-carotene accumulation in oil globules in the interthylakoid spaces of their chloroplast depends on high salinity, stress temperature, high light intensity and nitrogen limitation. Under these conditions, β-carotene has been synthesized upto 12% of the algal dry weight (Ben-Amotz, 1999). *Dunaliella* production facilities are located in areas where solar irradiance is maximal, cloudiness is minimal, climate is warm and hypersaline water is available. Its cultivation is based on autotrophic growth in medium containing inorganic nutrients with CO_2 as the carbon source (Ben-Amotz and Shaish, 1992).

Global production of *D. salina* has estimated to be about 1,200 t/years (Pulz and Gross, 2004). Now-a-days, the plants producing β-carotene operate in Israel, China, USA and Australia. The Australian producers use extensive cultivation approach consisting of very large ponds of upto 250 hectares in area. Economic viability of this system seems to rest on low land costs. Production can, thus, be maintained all year round, although β-carotene yield is very low (Ben-Amotz, 2004; Borowitzka, 1999). The productivity of β-carotene under intensive large scale cultivation was claimed to be around 2.0 x 10^{-7} kg/l β-carotene/day on a yearly average (Ben-Amotz, 1999).

The intensive cultivation approach employs two stage technology. In stage one, the *Dunaliella* have been cultivated to obtain maximum biomass containing a low β-carotene to chlorophyll ratio. The culture has been transferred thereafter to stage two with carotenogenesis being further enhanced by N_2 deficiency and exposure of the system to full impringing sunlight (Garcia-Gonzales *et al.*, 2005).

Fabregas *et al.* (2001) studied the freshwater microalga, *Haematococcus pluvaris* for the production of astaxanthin despite of less growth. The microalgae have been cultured in large-scale outdoor ponds, thus, being influenced by environmental constraints, such as rainfall, sunlight and availability of salt water. The production of high levels of β-carotene accumulation requires high salinity, high temperature and high light intensity. Hence, the facilities for microalgal production must be located where there is ample flat land available. Very few locations can be used worldwide for sustained and economic microalgal production of β-carotene due to these specificities. Large scale production of β-carotene is controlled by numerous stress factors like high light intensity, high salinity, temperature and availability of nutrients. Conventional solid-liquid separation operations such as filtration and centrifugation generally shear damage these cells, leading to oxidative loss of β-carotene. In addition, the high salt concentration brine makes corrosion of all metal equipment. Moreover, stringent requirements must be met before the produced β-carotene can be incorporated in human food. Even when performing sophisticated downstream purification, microalgal components remain in final formulation, often conferring an unpleasant fishy taste to the food in which β-carotene has been used. All these reasons explain why the microalgal β-carotene does not provide a viable alternative to the large scale, established chemical synthesis process that currently accounts for more than 85% of the global β-carotene market (Van Keulen *et al.*, 2010).

Production of β-carotene from yeasts

The synthesis of commercially important β-carotene by several yeast species, belonging to the genera *Rhodotorula* and *Phaffia* has led to consider these microorganisms as potential pigment sources. Yeasts have been considered more convenient than algae for large scale cultivation in fermenters, due to their unicellular nature and high growth rate. Some species of the genus *Rhodotorula*, *viz.*, *R. glutinis*, *R. minuta*, *R. mucilaginosa* and *R. graminis* and *Sporobolomyces roseus S. potagonicus* have been used for the production of carotenoids. The major carotenoid producer yeasts, *Rhodotorula* have the capability to produce β-carotene, torulene and torulahodin in various proportions (Frengova *et al.*, 1994; Frengova *et al.*, 1995; Buzzini and Martini, 1999; Bhosale and Gadre, 2001; Frengova *et al.*, 2003; Libkind and Broock, 2006; Azmi *et al.*, 2011a).

Rhodotorula sp. and *Xanthophyllomyces dendrorhous* have been considered as potential source of dietary β-carotene production. However, the high cost of production limits the use of these yeasts. Production cost could be reduced further, by using less expensive substrates as well as increasing the yields of these pigments. The concentrations of individual pigments such as β-carotene, torulene, torularhodin and γ-carotene synthesized from *Rhodotorula* strains depend

upon different substrates used as carbon and nitrogen sources (Frengova and Beshkova, 2009). Buzzini *et al.* (2005) studied the influence of trace elements on carotenogenesis in *R. glutinis* and obtained 803µg/g total carotenoids of which 50.3% correspond to β-carotene. Various factors such as low temperature (H"20°C), high air flow rate and high hydrostatic pressure influence the β-carotene production by *Rhodotorula* sp. (Wang *et al.*, 2008). *Sporodiobolus roseus* produce a carotenoid comprising 50% of β-carotene but with lower yield than *R. glutinis* with about 72 µg/g dry biomass.

R. glutinis has widely been known as a β-carotene producing yeast (Simpson *et al.*, 1971). It is potentially useful for industries, since it has ability to grow on various inexpensive agricultural raw materials such as sugar cane juice, peat extract, whey, grape must, beet molasses, hydrolyzed mung bean waste flour, soybean and corn flour extracts and sugar cane molasses for carotenoid production (Aksu and Eren, 2005; Bhosale and Gadre, 2001; Buzzini and Martini, 1999; Frengova *et al.*, 1994; Matelli *et al.*, 1990; Martin *et al.*, 1993; Tinoi *et al.*, 2005).

Production of β-carotene from bacteria

Bacteria have attracted little interest as potential β-carotene producers as they exhibit low β-carotene content and frequently do not produce carotene of interest. However, bacteria like yeasts are more convenient for large scale production in fermenters due to their unicellular nature and high growth rate. The β-carotene production, using bacteria might, therefore, be advantageous. Silva *et al.*, (2004) have isolated a β-carotene, overproducing soil bacterium *Sphingomonas* sp. which produced 1.7mg carotenoid/g dry cell, out of which β-carotene represent 29% of total carotene. A mutant strain, obtained by treatment with ethyl methanesulfonate, accumulated up to 3.5 mg carotenoids/g dry cell. The accumulation of β-carotene by this strain up to 89% depended on the oxygenation of the growth medium. The β-carotene accumulation could, further be enhanced by incubating the cells in the presence of glycerol and yeast extract which result in an accumulation of 5.7 mg β-carotene/ g dry cell weight. Takano *et al*, (2006) have reported genetic control for the light induced carotenoid production in non-phototrophic bacteria. Bacterial carotenogenesis occurs in a constitutive or light induced manner, which suggests the diversity of the regulatory mechanism. The mechanism for light induced carotene production in non-phototrophic bacteria has been studied in detail in *Myxococcus xanthue*.

Carotenogenesis in prokaryotes *viz.*, *Myxococcus*, *Streptomyces*, *Mycobacterium*, *Agromyces* and *Sulfolobus* occur in a constitutive or light induced manner. Maximum concentration of total carotenoids was obtained under submerged conditions by *Myxococcus xanthue* after light induction (Takano *et al.*, 2006). In another study, Valduga *et al.*, (2009) have optimized the cell disruption process for carotenoid extraction from *Spridiobolus salmonicolor* CBS 2636. Different solvents like dimethyl sulfoxide, petroleum ether, hexane, ethyl acetate, chloroform, acetone along with liquid nitrogen and diatomaceous earth were used to disrupt the cell and release the intracellular carotenoids. This study showed that the synergistic effect on the extent of carotenoids recovery was obtained with multiple solvents. The maximum concentration of total carotenoids was extracted when the cells were treated with liquid nitrogen and dimethylsulfoxide for disruption, followed by the extraction with a mixture of acetone/methanol. Asker *et al.*, (2007) isolated some carotenoid producing bacteria (TDMA-16) from freshwater samples collected at Misasa, a region known for high radioactivity. The result of polyphasic taxonomic analysis suggests that strain TDMA-16 represents a novel *Sphingomonas jaspsi*. β-carotene production by *Flavobacterium multivorum* has been reported in the presence of inorganic salts and urea by Bhosale and Bernstein (2004). Among natural carotenogenic, bacterial sources *Brevibacterium* sp. (Hsieh *et al.*, 1974), *Micrococcus roseus* (Cooney and Berry, 1981), *Mycobacterium* sp. (David, 1974) have reported to produce β-carotene as their minor product.

Several carotenogenic bacterial strains *viz.*, cyanobacteria, *Erwinia uredovora*, *Erwinia herbicola*, *Flavobacterium*, *Rhodomicrobium vannielai*, *Protaminobacter rubber*, halophilic bacteria and *Mycobacteria* have also been reported by Van Keulen *et al.*, (2010). These organisms, however, produce mixture of carotenoids whereas a method, using *Paracoccus* strain was reported with the claim of producing β-carotene at 100% purity. Moreover, a novel process using a new naturally occurring strain *Sphingomonas* M63Y has also been reported by the same workers. This strain constitutively overproduce the β-carotene which could be isolated in one step and produced easily under controllable and scalable fermentation using inexpensive and renewable raw materials. The conditions for the production of β-carotene by various microorganisms have been summarized (Table 3).

Production of β-carotene from fungi

The fungal carotenoids have been synthesized by the isoprenoid pathway with isopentenyl pyrophosphate as the general precursor. They are found in all divisions of the fungal realm, and several are at the edge of being exploited at an industrial scale for satisfying an increasing demand of carotene pigments in food and feed additives and as components of cosmetics and pharmaceuticals. Fungi as carotene source are highly appealing and the genes for carotene synthesis were cloned from different fungi in order to stimulate further functional studies on genetic pathways for internal and

environmental regulation of carotene synthesis (Kim *et al.*, 2006). The filamentous fungi, *Phycomyces blakesleanus* and *B. trispora* have been considered as potential industrial sources of β-carotene and lycopene. The filamentous zygomycetes *B. trispora* and *P. blakesleanus* have been converted into attractive sources of carotenes by protracted improvements of strains and culture conditions (Cieglel, 1959; Mehta *et al.*, 1997; Cerda-Olmedo, 2001; Avalos and Cerda-Olmedo, 2004). Many patents have been filed or granted for production of β-carotene from fungal strains. A method of production of β-carotene from *B. trispora* has been described in one European patent (Javier *et al.*, 2004). Further, β-carotene production in synthetic medium by *B. trispora* has also been reported by many workers (Mantzouridou *et al.*, 2004).

Mantzouridou *et al.*, (2004) in a study also applied the mathematical modeling to different physical and chemical parameters and their interaction on β-carotene production by *B. trispora*. The maximum β-carotene was obtained with addition of linoleic acid, kerosene and antioxidant in the production medium. In another study, Mantzouridou *et al.* (2002) have studied the effect of aeration rate and agitation, speed on β-carotene production and morphology of *B. trispora* in a stirred tank reactor. It has been observed that aeration and agiation both significantly affect the β-carotene and biomass production. The highest β-carotene concentration was obtained at lower impeller speed (150 rpm) and high aeration rate (1.5vvm). The agitation creates shear forces, which effect microorganisms in several ways, causing morphological changes, variation in their growth and product formation and also damaging the cell structure. The aeration could be beneficial for the growth and performance of microbial cells by improving the mass transfer with respect to substrates, productivity, product and oxygen. The morphology of the microorganism can strongly influenced the product formation, since it affect broth rheology and consequently the mass and heat transfer capabilities of the fermentation broth (Atkinson and Mavituna, 1985). Papp *et al.*, (2009) have reported β-carotene production by mucoralean fungi. Further, *Mucor azygosporus* has been reported to grow on waste whey and produce β-carotene (Azmi *et al.*, 2011b).

Table 3: The conditions for the production of β-carotene by various microorganisms

Microorganism	Production medium	Mode of fermentation	Incubation temperature (°C)	Initial pH	Improvement fold	Reference
Dunaliella salina	High salt medium containing potassium chloride	Batch at flask level	32°C	7.5-8.5	1.2 fold	Venkatesh *et al.*, 2005
Rhodotorula mucilaginosa	Semi synthetic medium containing malt extract	Batch at flask level	25°C	4.5	2.0 fold	Libkind and Broock, 2006
Dunaliella salina	High salt medium	Batch at flask level	30°C	7.0	4.9 fold	Orset and Young, 2000
Sporodiobolus salmonicolor	Yeast malt extract medium	Batch in bioreactor	25°C	4.0	1.2	Valduga *et al.*, 2008a
Blakeslea trispora	Semi synthetic medium containing soyabean meal and corn hydrolysate with n-dodecane and span 20 as oxygen vectors	Batch in bioreactor	28°C	6.5	1.8	Xu *et al.*, 2007
Phycomyces blakesleeanus	Malt extract medium with dextrose	Batch at shake flask	30°C	6.0	4.0	Papp *et al.*, 2009
*Rhodotorula glutinis**	Synthetic medium	Batch at shake flask	25°C	6.0	2.72	Wang *et al.*, 2008
Blakeslea trispora	Cheese whey treated with beta glucosidase	Batch at shake flask	26°C	7.5	0.8	Verzakakou *et al.*, 2010
Flavobacterium multivorum	Synthetic medium with urea	Batch at shake flask	28°C	6.0	7.0	Bhosale and Bernstein, 2004
Blakeslea trispora	Beet molasses and linoleic acid	Batch, in stirred tank reactor	26°C	6.5	0.5	Goksungur *et al.*, 2004
Blakeslea trispora	Synthetic medium	Batch in bioreactor	26°C	6.0	1.0	Mantzouridou *et al.*, 2002
Rhodotorula glutinis	Fermented radish brine	Continuous in bioreactor	30°C	5.5	2.1	Malisorn and Suntornsuk, 2009

* Barotolerant strain, high hydrostatic pressure was applied

Fang *et al.* (2002) have improved production of lycopene and β-carotene from *B. trispora* by 1.8-fold with use of oxygen vectors (n-dodecane and Span 20). Oxygen vectors are hydrophobic liquids in which oxygen has a higher solubility than in water (Wang, 2001). The production of ubiquinone associated with β-carotene was reported in various strains of *Blakeslea* and *Phycomyces* by Kuzina *et al.*, (2008). The production of β-carotene by *B. trispora* is dependent upon sexual mating of two compatible strains, during the fermentation, which have to be independently grown for about 48 h prior to mating. Moreover, the broth of *B. trispora* cultures becomes viscous and needs considerable energy input to keep it well mixed and at the required levels of dissolved oxygen (Van Keulen *et al.*, 2010).

Applications of β-carotene

The β-carotene has variety of applications *viz.*, food and feed additives, as components of cosmetics and pharmaceuticals *etc*. The major applications of β-carotene have been summarized in Figure 1.

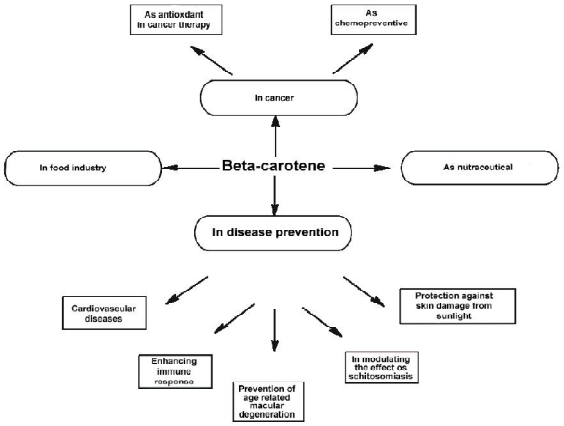


Figure 1: The major applications of β-carotene

Role of β-carotene in prevention and treatment of cancer

β-carotene as a chemopreventive agent

Chemoprevention involving the use of phytochemicals such as β-carotene, flavonoids and oxidative vitamins to suppress, block or reverse the process of carcinogenesis has received considerable attention over several decades (Keloff *et al.*, 1999; Lu *et al.*, 2005). There has been expanding source of information on the potential application of synthetic non-steroidal, anti-inflammatory drugs as chemopreventives but the rate of prevention was limited to 40% only as the drug efficacy was found to be associated with side effects to human body (Schreinemachers and Everson, 1994). Therefore, much attention has been devoted to identify cancer chemopreventive agents of dietary origin such as carotenoids and especially β-carotene which are more effective in preventing and reducing the risk of cancer growth (Upadhyaya *et al.*, 2007; Palozza *et al.*, 2008).

Nutraceuticals such as β-carotene from microorganisms, algae and plant have attained immense importance in cancer prevention (Khachik *et al.*, 1997; McCarty and Block, 2006). β-carotene was also found to influence the cellular differentiation, apoptosis programme and cellular antiproliferation potential with different molecules as target points (Neuhauser *et al.*, 2003). There was accumulating evidence that β-carotene may exert a marked protective effect against carcinomas of oral, skin, colon, urinary bladder, mammary and salivary gland in animal models as well as cell line studies (Williams, 2000; Palozza *et al.*, 2002; Russell, 2004; Aggarwal and Shishodiya, 2006). One epidemiological study (Holick *et al.*, 2002) showed that intake of fruits and vegetables, carotenoids, folate and vitamins A, C and E lowers the risk of bladder cancer among women of USA. Similarly, β-carotene of palm oil has also been reported to have immue modulatory effects, in particular enhancement of natural killer cell activity and tumor necrosis factor-alpha production by macrophages on breast tumorigenicity in nude mice (Nesaretnam *et al.*, 2002). As the breast cancer has been considered the most common cancer and cause of death due to cancer in women. This study suggested that β-carotene can induce anticancer and antimetastatic effects. The effect of β-carotene induced growth retardation of lung cancer cells was reported by Prakash *et al*. (2001). The results demonstrated that the treatment of small lung cancer cells with β-carotene induced morphological changes in the cells concomitant with a reduction in their proliferation. Similarly the effect of β-carotene on *in vitro* growth rates, as well as the conversion of β-carotene to retinol were investigated in three human prostate adenocarcinoma cell lines (William *et al.*, 2000). These experiments showed the reduction of growth rates *in vitro* in all three cell lines with application of β-carotene. In another study, apoptotic/growth inhibitory effect of trichostatin A was studied on human lung carcinoma cell line (Schiau *et al.*, 2010).

Moreover, high-dose of β-carotene supplementation was found to increase the lung cancer in case of cigarette smokers and asbestos-exposed workers (Van Poppel *et al.*, 1993; Solerno *et al.*, 2011). Although the pharmacological role of β-carotene in the prevention and treatment of colon cancer has received increasing attention, little is known about the molecular mechanisms of action of β-carotene (Palozza *et al.*, 2002). Moreover, the genotoxic and cytotoxic effects of ascorbic acid and β-carotene on human hepatocellular carcinoma cell line was evaluated by Yurtcu *et al.*, (2011). The results showed that ascorbic acid and β-carotene both caused DNA damage which were also concordant to increased apoptosis and necrosis of cells.

Cervical dysplasia is a premalignant lesion that can progress to cervical cancer, a common epithelial cancer and second most common cancer in women. Several mechanisms have been proposed to explain the putative role of β-carotene in

the modulation of cell growth including its provitamin A activity, its ability to act as a redox agent, to enhance immune response and gap junction communication (Muto *et al.*, 1995). However, little is known about the growth inhibitory effects of β-carotene and other carotenoids. Palozza *et al.*, (2004) have reviewed the available evidences for a modulatory action of carotenoids on apoptosis and found the main molecular pathways involved in the process. Proapoptotic effects of β-carotene and other carotenoids have been reported previously in cancer cells and different mechanisms have been implicated, including caspase cascade activation (Palozza *et al.*, 2003; Prasad *et al.*, 2006), effect mitochondrial functions (Palozza *et al.*, 2003), modulate the expression of apoptosis related proteins (Prasad *et al.*, 2006) and levels of transcription factors involved in apoptosis induction (Palozza *et al.*, 2008).

β-carotene as an antioxidant in cancer therapy

The study of the use of antioxidants such as β-carotene in cancer treatment is a rapidly evolving area. The antioxidants have extensively been studied for their ability to prevent cancer in humans (Singh and Lippman, 1998). Lamson and Brignall, (1999) reviewed the use of antioxidants as therapeutic intervention in cancer patients and potential interactions with radiation and chemotherapy. The cancer patients can be divided into three groups: those receiving standard or experimental therapy, those who have become unresponsive to these therapies and those in remission at risk for recurrence or a second new cancer. There has been no strategy to reduce the risk of recurrence of the primary tumors or of a second cancer among survivors at present. Therefore, the additional approaches such as the use of antioxidants can be helpful and increase the efficacy of cancer management. It has been reviewed that an active nutritional protocol which includes high doses of multiple dietary antioxidants and derivatives such as vitamin C, β-carotene and β-tochopheryl succinate as an adjunct to radiation therapy, chemotherapy or experimental therapy was found to improve the treatment efficacy by increasing tumor response and decreasing toxicity (Prasad, 2004).

Prasad *et al.*, (2001) discussed the scientific rationale for a micronutrient protocol as well as effective concentrations of doses of dietary antioxidants to be used adjuctively with radiation therapy. The findings support the use of retinoids concomitantly with radiotherapy. In a human study, daily supplementation of 75mg β-carotene during radiation treatment for advanced squamous cell carcinoma of the mouth significantly reduced the incidence of severe mucositis reactions. The remission rate was unchanged by β-carotene treatment (Kennedy *et al.*, 1994). Other *in vitro* studies, using human cell lung cancer lines demonstrated the positive effect of β-carotene when used with chemotherapy (Doyle *et al.*, 1989). These findings, further, strengthened the proposed use of β-carotene as adjunct to enhance the effectiveness of radiotherapy as well as chemotherapy.

β-carotene as nutraceutical

The definition of nutraceutical that appears in the latest edition of the Merriam-Webster dictionary is as food stuff as a fortified food/dietary supplements that provides health benefits. Vitamin A or retinol is an essential nutrient for man and all mammalian species but it cannot be synthesized within the body. The deficiency of vitamin results in adverse effects on growth, reproduction and resistance to infection. The most important infestation of severe vitamin A deficiency is xerophthalmia and irreversible blindness which may eventually occurs in one or both eyes. The carotenoid in diet has been used as major source of vitamin A by many communities in developing countries (Simpson, 1983; Siong, 1995; Palozza, 1998; Palozza, 2005). β-carotene is known precursor of vitamin A (provitamin A). The role of β-carotene as nutraceutical, has become very important as hypovitaminosis is still one of the major nutritional problems in least developed regions of the world (Ribeiro *et al.*, 2011).

β-carotene in prevention of diseases

Epidemiological studies have shown that people who consume diets with a high content of vegetables, have a reduced risk of degenerative diseases such as cardiovascular disease, age related macular degeneration and cataracts (Basu *et al.*, 2001).

Prevention of cardiovascular diseases

β-carotene, vitamin C and vitamin E have been implicated as cardioprotective nutrients (Basu *et al.*, 2001). Their potential benefits have been attributed to their antioxidant activities on the oxidation of low density lipoprotein (LDL), which has been considered important step in the development and progression of atherosclerosis and the main cause of cardiovascular diseases (Dagenais *et al.*, 2001). These findings were supported with some epidemiological studies. Dougas *et al.*, (1998) reported that the formation of lipidhydroxyperoxides by LDL was inhibited when LDL was incubated with human endothelial cells in a β-carotene enriched culture.

β-carotene action on the immune response

The early studies have demonstrated the ability of dietary carotenes to prevent the infections (Chew and Park, 2004). It has been reported that β-carotene enhances the cell-mediated and humoral immune responses in animals and humans. Similar result of immuno-enhancement by provitamin A carotenoids which act as antioxidants and can potentially reduce the toxic effects of reactive oxygen species has also been observed. These ROS and, therefore, β-carotene have been implicated in the etiology of diseases such as cancer, cardiovascular and neurodegenerative diseases and aging. These findings suggest that β-carotene can influence immune function through their ability to regulate membrane fluidity and gap junctional communication. The action of β-carotene on immune response hangs on a delicate balance with intra and

extracellular milieu, the outcome of which depends not only on the concentration of β-carotene but also on the cell type and animal species involved.

β-carotene as protective against skin damage from sunlight

The exposure of the skin to UV light leads to chemical and biological reactions, denoted as photo-oxidative stress (Wenk *et al.*, 2001). In the presence of oxygen, secondary reactive oxygen species have been generated which extend the range of photodamage. Photo-oxidative damage effects cellular lipids, proteins and DNA and has been involved in the photobiochemistry of erythema formation, premature aging of the skin, development of photodermatoses and skin cancer (Boelsma *et al.*, 2001).

Sunburn is a visible dermal reaction, following excessive exposure to sunlight, called UV-induced or solar erythema and has been characterized by tenderness, sometimes painful blistering and second degree burns (Clydesdale *et al.*, 2001). Stahl and Sies (2007) have reviewed the role of available micronutrients as photoprotectant. The β-carotene and lycopene have been distributed into light exposed tissues such as skin or the eye as micronutrients where they provide systemic photoprotection and also prevent UV-induced erythema formation. β-carotene supplements have also been given in diet as sun protectant. Data from human studies on the photoprotective effects of dietary β-carotene is dependent upon the duration of treatment before light exposure and on the dose (Garmyn *et al.*, 1995). However, it was found that the application of moderate doses of β-carotene, alone is not sufficient to obtain sustained photoprotection (McArdle *et al.*, 2004). Moreover, retinoids have been used in dermatological treatment such as for the elimination of acne (Rucker *et al.*, 2001; Ribeiro *et al.*, 2011).

β-carotene in modulating the effect of Schistosomiasis

Schistosomiasis is a common parasitic disease, affecting millions of people, mostly in tropical and developing countries. One of the causative agent of the disease is a trematode worm *Schistosome mansoni* (Bos *et al.*, 2009). *S. mansoni* infects over 83 million people in Africa and the Middle east (Criscione *et al.*, 2009). Schistosomiasis has been suspected as a risk factor for various types of cancers *e.g.*, bladder cancer, colorectal cancer and hepatic cancer as it is known to have a mutagenic effect (Aboul-Ela, 2002). The protective role of β-carotene and vitamin E in minimizing these genotoxic effects was studied by Khaled *et al.*, (2010). The β-carotene was found to have antimutagenic effect due to antioxidant protection by scavenging DNA damaging free radicals or by acting as a modulator of the metabolism selectively, inhibiting certain forms of mixed function oxidases through modulation of DNA repair mechanisms (Glei *et al.*, 2002). The schistosomiasis has a potential mutagenic effect, causing the chromosomal aberrations decreased the level of mitotic indices and caused cell cycle delay. The β-carotene could be recommended on regular basis to minimize the genotoxic hazards that may occur as a sequel of infection (Khaled *et al.*, 2010).

β-carotene in prevention of age related macular degeneration

Age related macular degeneration (AMD) is one of the leading causes of blindness in developed countries, affecting 11.5% of the population of the USA (Evans and Wormald, 1996; Vannewkirk *et al.*, 2001). The exact mechanism of AMD remains unknown (Bressler, 2000), however, one of the main components which have been thought to be responsible is oxidative stress of the retina (Perlman *et al.*, 1995; Beatley *et al.*, 2000; Zarbin, 2004). Its high oxygen concentration and intense light exposure was found to be susceptible to damage by oxidative stress. The use of nutritional supplements such as β-carotene was found to prevent age related macular degeneration (Wong *et al.*, 2011).

Role of β-carotene in food industry

Pigment is vital constituent of food and probably one of the first characteristics perceived by sense. However, with the increasing awareness of toxicity of synthetic dyes or colours, demand of pigments from natural sources has increased (Babu and Shenoliker, 1995). The scrutiny and negative assessment of synthetic food dyes by modern consumer have given rise to a strong interest in natural colouring alternatives. There has been an increasing demand of natural colours in food, pharmaceuticals, cosmetics, textile and in printing dye industry. Natural pigments are extracted from fruits, vegetables and microorganisms. The 20 to 30 % oil suspensions of micro crystals of β-carotene have been used for the colouring of fats, oils and cosmetic products. The β-carotene has been used as food colorant in concentrations between 2 and 50 ppm, hence, its contribution for the color of food ranges from yellow to orange. It has also been used as a nutritional supplement being, the main source of provitamin A. According to the study of Britton (1983), 100% of β-carotene can be converted to vitamin A. In order to be traded, carotenoids must be formulated for application in hydrophilic (juices and drinks) or lipophilic (butter, margarine and cheese) matrices, and for this reason, the crystals sizes have to be reduced (Ribeiro *et al.*, 2011).

β-carotene in photostability of biopolymers

The steady increase in worldwide plastic consumption has promoted a shift of interest to the development of bioplastics, obtained from renewable resources. Polyactide (PLA) is polyester obtained from the fermentation of corn starch or other carbohydrates. The PLA is the bioplastic that has achieved the greatest market penetration in the packaging sector, being used as a food packaging polymer for short shelf-life products (Auras *et al.*, 2006). However, some properties such as its brittle character and poor impact strength have limited its applications (Byrne *et al.*, 2009).

Polycaprolactone (PCL), a thermoplastic, is biodegradable polyester synthesized by chemical conversion of crude oil. The PCL has good water, oil, solvent and chlorine resistance, a low melting point and low viscosity and can be easily processed, using conventional melt blending technologies (Gross *et al.*, 2002). These bioplastics have to be improved to meet the specific processing and application requirements and this can be done through the incorporation of additives, stabilizers, processing aids, colorants *etc*. (Guilbert *et al.*, 1997). Many bioplastics have been mixed or blended with synthetic components such as polymers and additives, to improve the functional properties of the finished product and to expand the range of application. If the additives and pigments used are based on renewable resources, one can obtain a polymer with approximately 100% weight of biodegradable compounds. Amongst natural antioxidants, β-carotene has been chosen for its excellent antioxidant claims. This compound would prove to be a good candidate as an additive for food packages as it can quench the singlet oxygen and, therefore, it is expected to exert significant light protection and can operate as UV absorber (Lopez-Rubio and Lagaron, 2010). A study was carried out for the improvement of stability against UV light and mechanical properties of biopolyesters by the addition of β-carotene by Lopez-Rubio and Lagaron, (2010). β-carotene addition to the biopolyesters resulted in significant increase in the strength due to its plasticizer effect and increased its stability against UV.

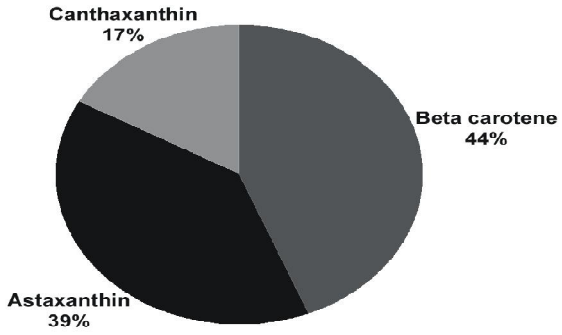


Figure 2: The estimated market share of β-carotene ($285 million), astaxanthin ($ 253 million) and canthaxan-thin ($110 million) by year 2015

Market analysis of β-carotene

An expanding carotenoids market is witnessing increase in its overall demand. The global strategic business report analyzes the worldwide markets for carotenoids in US and the major end use segments analyzed are animal feed, food and pharmaceuticals. According to the research report, the global market for carotenoids was worth $ 766 million in 2007 and expected to increase to over $ 919 million by 2015 with a compound annual growth rate of 2.3% (Ribeiro *et al.*, 2011). Out of all the major carotenoids (*viz.*, astaxanthin, β-carotene, canthaxanthin and others) the β-carotene has the largest share of market, valued at $247 million in year 2007 (Figure 2). The estimated market of β-carotene has expected to be worth $ 285 million by 2015 with a compound annual growth rate of 1.8%. Currently, the international trade is dominated by private companies such as Roche, BASF, Merck, Rhone-Poulene, algatechnologies, cyanotech and DSM (Fraser and Bramley, 2004; global market for carotenoids from BCC research, 2008 and Natural product insider, 2010).

Future Prospects of β-carotene

The β-carotene is playing an ever-increasing role in human health in the developed world. The nutritional value of β-carotene has been known for many years. Its antioxidant activity in the prevention of certain human diseases such as cancer has also been claimed. Consequently interest in this compound from nutraceutical aspect has increased substantially and a multimillion dollar market has established this fact. Moreover, the chemical synthesis has been used to fulfill most of this demand. However, some of the by-products resulting from such chemical processes may have undesirable side effects on human. Hence, the production of β-carotene from microbial sources has become the focus of extensive research. Many genetic engineering techniques have been used to enhance the production of β-carotene in microorganisms. However, these methods resulted in the limited enhancement in the production of β-carotene in cmparison to commercial carotene levels produced by chemical synthetic technology. Modern systems biology tools, together with the development of genomics and metabolomics databases, may facilitate the advancement of our knowledge in gene to metabolite networks in microorganisms. Metabolomics accompanying genomics, transcriptomics and proteomics as well as bioinformatics aided metabolic engineering efforts towards designing superior biocatalysts in cell factories might result in increase in the production of β-carotene.

Conflict of interest

The authors declare no conflict of interest.

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