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VEGETABLES AS A SOURCE OF CAROTENOIDS

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Summary: Plants are rich and still unexploited sources of bioactive compounds, attractive for their therapeutic properties and as the raw material for the formulation of pharmaceutical and food products. The colour of the plants, especially comestibles as vegetables or fruits, results from chemical compounds whose metabolic activity is the aim of many studies nowadays. The carotenoids, a group of organic compounds with an unsaturated skeleton of hydrocarbons, results in the colours of yellow, orange and red. Having antioxidant activity and, for some of them, being the precursors to vitamin A in the animal organisms are willingly used in very different medical applications. The article describes the current state of knowledge regarding the types and quantities of carotenoids in vegetables, the advancement of knowledge of their metabolic impact, their possible applications in medicine, as well as the desired directions for further research. Also, the scope of their industrial use and the prospects of carotenoids' market development are included. This work is carried out in the framework of the COST Action – EUROCAROTEN CA15136, with the aim of the exchange and development of knowledge between European researchers working on carotenoids.

Keywords: carotenoids, vegetables, lycopene, bioavailability, carotenoids market.

Streszczenie: Rośliny są bogatymi i wciąż niewykorzystanymi źródłami związków bioaktywnych, atrakcyjnymi ze względu na swoje właściwości terapeutyczne i stanowiącymi surowiec do tworzenia produktów farmaceutycznych i spożywczych. Kolor roślin, zwłaszcza produktów spożywczych, takich jak warzywa czy owoce, wynika z obecności związków chemicznych, których aktywności metaboliczne są obecnie celem wielu badań. Karotenoidy – grupa organicznych związków o nienasyconym szkielecie węglowodorów – powoduje barwy żółtą, pomarańczową i czerwoną. Mają aktywność przeciwutleniającą, a niektóre z nich, będąc prekursorami witaminy A w organizmach zwierzęcych, są chętnie stosowane w medycynie. W artykule przedstawiono obecny stan wiedzy na temat rodzajów i ilości karotenoidów w warzywach, postęp wiedzy na temat ich wpływu metabolicznego, możliwych zastosowań w medycynie, a także pożądanych kierunków dalszych badań. Ponadto omówiono ich wykorzystanie przemysłowe oraz perspektywy rozwoju rynku karotenoidów. Praca ta jest prowadzona w ramach działania COST – EUROCAROTEN CA15136, mającego na celu wymianę i rozwój wiedzy między europejskimi badaczami zajmującymi się karotenoidami.

Slowa kluczowe: karotenoidy, warzywa, likopen, biodostępność, karotenoidy.

1. Carotenoids characteristic

Carotenoids are a group of natural common colorants among which about seven hundred compounds have been identified so far, but only a few have been subject to industrial exploitation, mostly explored among them are α -and β -carotene, lycopene, zeaxanthin, crypthoxanthin, lutein and astaxanthin (Table 1). Carotenoids accumulate in plants, animals and microbial cell providing colours within the range from yellow to red. This colour is related to the number of double bonds appearing in the hydrocarbon skeleton, but only the presence of the seven chromatophore systems makes the compounds exhibit coloration. Cross-linking with some proteins turn carotenes into carotenoproteins and the colour spectra are changed to the blue, green or purple occurring in marine invertebrates. Such compounds include phytoene (3 double-binding) and phytofluene (5 double bonds) [Ötleş 2012; Gryszczyńska et al. 2011; Andersson 2009].

Carotenoids are tetraterpens built mainly from forty carbon atoms chains and can be divided into carotenes – non-oxidized hydrocarbon compounds, and xanthophylls containing an oxygen atom in a hydroxyl, carbonyl or epoxy groups.

Among the carotenoids are also compounds that have less than 40 atoms of carbon and additionally linked four methyl groups which are calleded apo-carotenoids. Their shortened chain results from either degradation or chemical synthesis. Carotenoids may occur in the form of acyclic, monocyclic or bicyclical compounds and are within the group of isoprenoid lipids as they are built from eight isoprene residues whose structure is reversed in the middle of the molecule. They are insoluble in water, but dissolve very well in non-polar or slightly polar solvents [Kączkowski 2009; Sikorski 2006; Świderski 2009].

Carotenoids originate from polyene core in the form of an acyclic structure of $C_{40}H_{56}$ (Figure 1) built with a long chain of conjugated unsaturated bonds. With the increase of conjugated unsaturated bonds the maximum absorption moves to long waves resulting in the change of the compound coloration from yellow to orange-red. High temperatures promote the isomerisation of unsaturated bonds, which finally lightens the colour [Fratianni et al. 2010; Meléndez-Martínez et al. 2010].

Carotenoids are formed in the reaction of hydrogenation, dehydration, cyclisation, oxidation, or a combination of these processes. Depending on the number of unsaturated bonds there are many isomeric forms of cis/trans (E/Z). These compounds easily isomerise and combinations of mono-and poly-cis-isomers combine easily with all-trans forms which abound in nature [Baranski, Cazzonelli 2016].

Name	Structural formula	Molecular weight	Molecular formula
β-carotene β,β-carotene		536,88	C ₄₀ H ₅₆
α-carotene β,ε-carotene		536,87	$C_{40}H_{56}$
lycopene ψ,ψ-carotene		536,85	$C_{40}H_{56}$
astaxanthin (3S,3'S)-3,3'- dihydroxy-β,β- carotene-4,4'- dione	OH OH	596,84	$C_{40}H_{52}O_{4}$
zeaxanthin (3R,3R)- β,β- carotene-3,3'- diol	OH OH	568,88	$C_{40}H_{56}O_2$
crypthoxanthin (3 <i>R</i>)-β,β- carotene-3-ol	HOWER	552,85	$C_{40}H_{56}O$
lutein (3R,3'R,6')-β,ε- carotene-3,3'- diol	он	568,88	$C_{40}H_{56}O_2$

Table 1. Carotenoids formula and their molecular weight

Tabela 1. Wzory sumaryczne i strukturalne karotenoidów oraz ich masa molowa

Source: own study based on [Ötleş 2012; Schieber, Weber 2016].

Źródło: opracowanie własne na podstawie [Ötleş 2012; Schieber, Weber 2016].

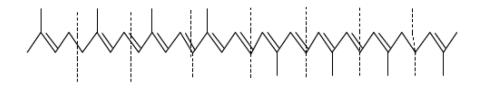


Fig. 1. Acyclic structure $C_{40}H_{56}$ **Rys. 1.** Acykliczny wzór C40H56

Source: [Ötleş 2012]. Źródło: [Ötleş 2012].

2. Bioactivity of carotenoids

The bioavailability of carotenoids, which reflects the extent to which the compound is released from food in the digestive tract and then absorbed and distributed to tissues and organs, depends on the operations used in the preparation of food. For example, lycopene from raw tomatoes is poorly absorbed but the use of edible fats in preparing these vegetables for consumption and their cooking or slicing facilitates transport and increases its absorption.

Carotenoids, whose structure is identical to the molecule of retinol, reveals vitamin A activity. Of all the carotenoids, the β -carotene has the highest bioactivity as a provitamin A compound. β -carotene molecule contains two β -ionone rings whose breakage in the position – C15 = C15 ' – in theory provides two retinol molecules. The conversion of β -carotene to retinol is on the path of passive diffusion in the mucosa of the small intestine, where the carotenoproteins are created under the action of the enzyme 15:15 ' – dioxygenase enzyme in the form of retinal aldehyde. The conversion of β -carotene to vitamin A is incomplete and this compound shows only 1/6 of retinol activity, which means that 1 mg of retinol is equivalent to 6 mg β -carotene. This compound released during the food preparation is dissolved in the lipid phase, since carotenoids exhibit lipophilic properties and accumulate in the cell membranes and lipoproteins [Tunamihardjo 2013; Stachowiak, Czarnecki 2006; Fernández-García et al. 2012].

 β -carotene is partially converted to vitamin A and the remaining quantity of unconverted β -carotene and retinyl esters are embedded in chylomicrons and excreted into the lymph, and then transported to the liver. Carotenoids molecules that do not possess the β -ionone ring do not show the pro vitamin activity [Sikorski 2006; Harrison 2012].

Due to the solubility in fats, carotenoids influence many biological processes, such as photosynthesis, the vision process, free radicals scavenging and singlet oxygen trapping [Geens et al. 2009; Odriozola-Serrano et al. 2009; Widomska et al. 2009].

The characteristic feature of carotenoids is the absorption of light. As a result of photon energy, the basic singlet state S_2 is formed which exists only by the 200 fs

and converts to singlet state S_1 . The energy can be transferred from the triple photo sensibiliser or singlet oxygen onto the carotenoid [Krinsky, Johnson 2005].

In plants, carotenoids play an important role in the bright stage of photosynthesis during the conversion of light energy, as secondary pigments absorb light that is not absorbed by chlorophyll, and then transmit energy to chlorophyll molecule [Ohmiya 2013]. Carotenoids protect the lipids contained in cell membranes from oxidative stress. In this way they maintain communication signals between cells and receptors in the cell wall, which ensures the normal functioning of cells and improving the resilience of the human body [Zhuo et al. 2016].

The antioxidant properties of carotenoids are determined using a variety of techniques *in vitro*, *ex vivo* in LDL (Low Density Lipoprotein) and *in vivo* [Yaqub et al. 2016]. The *in vitro* study on antioxidant properties of carotenoids became the basis for the understanding of the mechanism of photo protection, which involves the exchange of an electron and energy transfer between singlet oxygen (${}^{1}O_{2}$) and carotenoid to form the triple state carotenoid (${}^{3}KAR$), and oxygen in the base state.

In such a way the carotenoids as β -carotene can act as catalysts inactivating the reactive oxygen species especially in liposomes. Some studies have shown that they are not always strong enough to protect the cell from oxidative stress and free radical damage. The weakening of these properties is associated with the activity in different fatty fractions of the body. Additionally, β -carotene may also act as an oxidant in conditions of high vapour pressure of oxygen (0.1 MPa) [Rodriguez-Amaya 2015; Chen, Djuric 2001; Chanda, Dave 2009].

Ex vivo studies on the antioxidant potential of β -carotene in LDL-cholesterol transport fraction in the human body confirmed the protective effect of this compound. However, some studies reveal the increasing LDL oxidation in the presence of β -carotene and other carotenoids like lutein or zeaxanthin. In addition, supplementation experiments on a group of healthy people do not always result in an increase of protection of LDL during exposure to oxidative agents. The effects were dependent on the duration of the diet, different types and size of the population as well as other unknown factors. Studies *in vivo* have shown that the presence of malondialdehyde, which is the end product of lipid oxidation, decreases with the long term intake of β -carotene [Li et al. 2016; Hininger et al. 2001].

The studies revealed that the lycopene has the largest reducing properties while astaxanthin, acting as donor acceptor, is the weakest regulator. It was noted thatlutein and zeaxanthin are reduced by lycopene while β -carotene does reveal such activity [Igielska-Kalwat et al. 2015].

Also, the synergy effect was observed for the antioxidant activity between vitamins and phytochemicals. Small amounts of vitamin E are sufficient to protect carotenoids, which greatly increases their antioxidant activity [Amitava, Kimberly 2014].

3. The sources of carotenoids

The occurrence of carotenoids is very common. Carotenoids are synthesized in plants and photosynthesizing and some non-photosynthesizing bacteria, yeast and moulds. Microorganisms synthesizing carotenoids are present on the surface of plants, water and soil. Animals cannot synthesize carotenoids but must absorb them from food, thus obtaining the coloration of feathers, scales or skin, as for example flamingos, salmon or storks (legs). The widespread occurrence of carotenoids in nature tends to look for methods and technologies to receive these extremely valuable colorants [Amitava, Kimberly 2014; Geens et al. 2009; Liang et al. 2009].

Carotenoids are found in various green parts of the plant as well as in flowers, fruits, seeds, roots and tubers. In the plant cells, carotenoids are located in the membranes of tylacoids which are chloroplast organelles. Large quantities occur in vegetables e.g. carrots, spinach, tomatoes, and fruit such as watermelon and grapefruit [Gryszczyńska et al. 2011]. Table 2 shows the content of carotenoids in selected vegetables. Analysis of the data has shown that vegetables are richer in carotenoids compared to fruit.

Plant material	carrots Daucus carota L.	tomato Solanum lycopersicum L.	Lamb's lettuce Valerianella locusta (L.) Laterr	spinach Spinacia oleracea L.	kale Brassica oleracea L.	parsley leaf Petroselinum crispum (Mill.) Fuss	broccoli Brassica oleracea L.	pumpkin <i>Cucurbita maxima</i> Duchesne	red paprica <i>Capsicum annuum</i> L.
		Carote	enoids co	ntent [mg	g/100 g p	roduct]			
β-carotene	9.02	0.89	3.22	3.25	7.28	5.50	0.28	0.20	3.25
&-carotene	4.89	0.15	0.08	0.09	0.15	0.17	_	-	0.51
lutein	0.36	0.21	9.65	9.54	18.63	13.78	0.80	1.33	-
zeaxanthin	_	_	-	0.35	-	0.34	-	_	2.20
lycopene	-	11.44	-	_	_	_	-	-	0.13
β-crypthoxanthin	_	_	0.10	_	0.12	0.11	0.011	0.011	1.01

Table 2. Carotenoids content in vegetables
Tabela 2. Zawartość karotenoidów w warzywach

Source: own study based on [Solovchenko 2010; Tiwari et al. 2013; De la Rosa et al. 2010; Belter et al. 2011; Chilczuk et al. 2014].

Źródło: opracowanie własne na podstawie [Solovchenko 2010; Tiwari et al. 2013; De la Rosa et al. 2010; Belter et al. 2011; Chilczuk et al. 2014].

The concentration of β -carotene in vegetables range from 0.2 mg/100 g of pumpkin to 9.02 mg/100 g of carrots while in fruit the concentration of β -carotene reaches up to 3 mg/100 g of biomass. A similar relationship can be observed for α -carotene, though its average content in vegetables is not high and amounts to 0.86 mg/100 g of product. The highest concentration divergence of 18.609 mg/100 g for edible parts of fruits and vegetables is observed for lutein. Large amounts occur in kale and parsley leaves but also a significant amount is found in lamb's lettuce and spinach. The lowest concentrations for both fruit and vegetables was observed for β -crypthoxanthin which averages in fruit at 0.054, and in vegetables at 0.227 g/100 g of product. The content of lycopene in both tomato and watermelon is comparable at level of 11.44 mg/100 g and 11.39 mg/100 g respectively.

4. Carotenoids recovery methods

Carotenoids are a valuable source of pigments and vitamins, therefore, the development of effective methods of their recovery is the subject of many studies. For their production both plant and microbiological raw materials are used. The methods of chemical synthesis were discovered in the 1950s and carotenoids production on an industrial scale was provided based on it. For a long time, chemical synthesis was the main source of carotenoid colouring compounds.

Studies on the biological functions of carotenoids revealing differences in bioactivity between naturally and chemically obtained carotenoids have resulted in greater interest in natural sources of this group of chemicals. As a result, a number of methods for carotenoids recovery were elaborated which can be classified as physico--chemical, chemical and biotechnological involving microorganisms.

The oldest way of obtaining carotenoids was extraction from plant material, based on the physicochemical processes. Carotenoids are extracted from the green parts of plants, flowers, fruits, seeds, roots, and tubers [Grajek 2007]. In terms of the efficiency of the carotenoids production process, the main role is played by the operation of extraction. An extraction mixture of petroleum ether and acetone is commonly used as well, as are the known methods of extraction with supercritical carbon dioxide. Also, the carotenoids are obtained from the by-products of tomato processing, palm oil, the Amazon region fruit *Mauritia flexuosa* – buriti and *Rosa canina* fruit [Dasgupta, Klein 2014; Szterk et. al 2008; Tozzi et al. 2008; Vagi et al. 2007].

The main drawbacks of carotenoid production from plant material are the high cost as well as the geographical and seasonal restrictions. To obtain a few grams of carotenoids, dozens of kilograms of material have to be used. Only 2 g of α - and β -carotene in crystal form is produced from about 50 kg of carrots. Because of this, a lot of work is being done to improve the efficiency of carotenoid biosynthesis by some plants by genetic modifications [Dasgupta, Klein 2014].

Carotenoid formulation	Plant material	Extraction method	Solvent	Carotenoids formula	Application
	Palm oil unrefined				
	Carrot				
	Daucus carota subsp. sativus		1:0	Mainly B-carotene (85%),	Pigment: Non-alcoholic drinks,
Carotene mixture CI Food Orange 5	Alfa alfa Medicago sativa	Solvent extraction	Eat Fat	-carotene (15%) and trace amount of γ -carotene	
	Grass		пехапе	$C_{40}H_{56}$	Pastry; Ice-cream
	Stinging nettle Urtica dioica				
Oleoresins Natural Yellow 27	Tomato Solanum lycopersicon	Solvent extraction	Fat	Lycopene C ₄₀ H ₅₆	Pigment: Tomato products; Jam; Marmalade
		Solvent extraction		Bixin C ₂₆ H ₃₃ O ₃	
Annatto	Annatto – <i>Bixa orleana</i> ,	Water alkali hydrolyse of extracted bixin	Oil	Norbixin C ₂₄ H ₂₈ O ₄	Pigment: Fruit drink; Juice; Tomato products; Butter;
CI Natural Orange 4	annatto seed extract	Extraction of the external seed cover with NaOH and KOH	Water	Norbixin C ₂₄ H ₂₈ O ₄	Margarine; Ice-cream; Noodles; Instant soup
		Extraction of the external seed cover with edible plant oil	Oil	Mainly bixin	
Oleoresins form	Annual paprica, (Capsicum	Solvent extraction	Fat	Capsanthin	Pigment and taste additive:
paprica	annuum L. without seeds – crushed or ground)			C ₄₀ H ₅₆ O ₃ Capsorubin	Cold meat; Delicatessen food Cheese; Canned vegetables;
Lutein	I eaves Transtas practa			C_{40} ¹¹ 56 O_4	Calification incation that
Mivhire of	Traves jugeres erein		LiO	Lutain	cloudy citrus drinks. Dins:
carotenes	Alfa alfa	Solvent extraction	Ethanol	C_{ACH}	Ice-creams; Diary products;
and xanthophylls	Medicago sativa			7 × +C 0+	Confectionary

Source: own study based on [Świderski 2009; Rozporządzenie Ministra Zdrowia... 2010]. Źródło: opracowanie własne na podstawie [Świderski 2009; Rozporządzenie Ministra Zdrowia... 2010].

Among the carotenoid preparations used in food colouring which are received by extraction from plant, one can single out the mixture of carotenes, the lycopene, the saffron, the annatto, the oleoresins from sweet peppers and the lutein. In Table 3 the examples of the natural carotenoid preparations obtained from plant material, as well as the types of the extraction solvents and their colouring, are presented.

Beyer and his co-workers introduced the genes responsible for synthesis of β , β -carotene derived from the bacterium *Erwinia uredovora* and daffodil (*Narcissus pseudonarcissus*) into the rice endosperm (*Oryza sativa*). As a result the heterogenic expression of enzymes responsible for the synthesis of phytoene and β -carotene was obtained, which resulted in an over twenty-fold increase in the total quantity of carotenoids content from 2 to 37 µg/g_{db} [Beyer et al. 2002].

Another strategy to increase the biosynthesis of provitamin A is a modification of the carotenogenesis pathway in tomatoes (*Solanum lycopersicum*) by the phytoene desaturase expression from *Erwinia uredovora*, the main factor affecting the accumulation of lycopene, under the control of a 35S promoter from the tobacco mosaic virus (CaMV). These studies have led to a reduction in the overall number of carotenoids by 50% but the contents of the β -carotene have been almost doubled from 270 to 520 µg/g_{db} [Romer et al. 2000].

There have also been examples of changes in the expression of phytoene synthase from *Erwinia uredovora* in rapeseed (*Brassica napus*). Shewmaker achieved a fifty-fold increase in the total carotenoids content, of which the highest concentration was reached by β -carotene in the amount of 400 µg/g_{db} [Shewmaker et al. 1999].

The crucial limitation of the production of carotenoids from plants is the high cost, hence the constant search for other methods of obtaining them, which provide high efficiency, low cost and simple technological process.

The application of carotenoids is inextricably linked with their durability. The storage of these colorants is of great importance for the maintenance of their property. Carotenoids are easily oxidized and therefore should be kept in an atmosphere of inert gas. Among the factors affecting the durability of carotenoids are: the presence of oxygen and radicals, light, temperature and water content in the preparation. In the selection of the colours used in food production one should take into account not only the products but also the type of product, its preservation requirements, packaging and storage. Stability studies of carotenoids also are related to their storage on a variety of carriers such as microcrystalline cellulose, fibre, wheat fibre and potato starch [Shewmaker et al. 1999; Jintasataporn, Yuangsoi 2012; Boon et al. 2010; Szterk, Lewicki 2007; Dłużewska, Bednarek 2005].

The biological properties of carotenoids and the growing consumer awareness make the natural origin carotenoid preparations increasingly used in health protection and balanced nutrition. Research carried out in this area is intended to develop such technology which would ensure the high efficiency industrial production of carotenoids.

5. Vegetable carotenoids market

The size of the worldwide market for carotenoids measured in U.S. dollars is estimated at 1.24 billion USD in 2016, and its value predicted for the year 2021 is 1.53 billion USD, while CAGR (compound annual growth rate) up to 3.78% from 2016 to 2021 [Carotenoids Market by Type 2016]. The market of carotenoids is divided into segments corresponding to products (Figure 2).

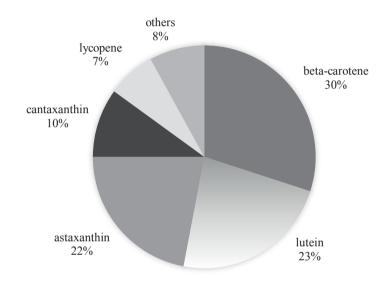


Fig. 2. Market segmentation depending on the type of compounds

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Rys. 2. Podział rynku karotenoidów
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Source: own study based on [*Carotenoids Market by Type...* 2016]. Źródło: opracowanie własne na podstawie [*Carotenoids Market by Type...* 2016].

The dramatic increase of interest on carotenoids translated into the increase in demand for these compounds is especially noticeable when compared to the previous decade, in which the world market of carotenoids was worth 766 million dollars in 2007 and was expecting growth to the level of 919 million dollars in 2015, with a CAGR rate of 2.3%.

The growing trend of the market of natural plant carotenoids is observed which results from healthy consumer behaviour and, at the same time, the increased demand for better quality food with attractive design [The World Beta-Carotene Ingredient Market].

According to Global Industry Analysts (GIA), β -carotene represents the biggest segment of the market of carotenoids estimated in 2010 at 392 million dollars. The dominance of β -carotene is associated with the certified health benefits resulting from consuming it.

Along with more and more media reports on scientific research showing the harmful or carcinogenic effects of the synthetic β -carotene on the health of humans and animals, there is a clearly noticeable return towards natural products which are a source of β -carotene such as carrot juice [Prieto et al. 2012]. This phenomenon is observed particularly in the European market. This is due to increased consumer awareness and the constantly widening knowledge of the consequences of the quality of consumed food [www.nutraceuticalsworld.com].

The food sector, however, is not the biggest recipient of carotenoid compounds. Carotenoid preparations are most commonly used as an additive in feed, since they provide the desired colour to meat. According to the GIA report, manufacturers expect the astaxanthin, canthaxanthin and lutein use in food and feed increases due to the proven antioxidant properties. The target market is the pharmaceutical industry and especially the market of dietary supplements and non-prescription drugs. This trend is associated with the deepening of knowledge in the area of the influence of oxidative stress on the welfare of humans and animals. It is noteworthy that the cosmetics market has been selected as a benchmark, because it is characterized by the greatest impact on the market of nutraceuticals. Countries such as Japan, China and Germany are on the list of the most active providers of supplements that affect the external appearance, in the U.S. has also been observed a significant activation of this market. Currently the sales volume in China is matching the level in Japan. This situation relates the increase in wealth of the middle class of Chinese society which affects consumer behaviour regarding the increase in the expenditure related to the appearance.

In Western Europe the increasing trend of cosmetic supplements segment is observed, with the increase in their availability in a variety of retail outlets such as health food shops in Germany; also the sale of cosmetic supplements in the United States is increasing at a faster rate compared to other sectors because consumers are looking for nutraceuticals as an alternative to plastic surgery in order to maintain a youthful appearance. The result of this trend is an increase in the number of components of cosmeceuticals offered by distributors in the food and pharmaceutical industry [www.nutraceuticalsworld.com].

6. Conclusion

The growing understanding of the impact of carotenoids on health has forced a retreat from the synthetic methods of obtaining them. The active forms of carotenoid compounds available from natural sources far outweigh the effectiveness of compounds of a synthetic origin. In addition, they do not show toxic or carcinogenic effects. This is the main cause of the increased demand for these compounds produced naturally, whose primary sources are fruit and vegetables. However, the method for obtaining carotenoids from vegetable raw materials is economically unprofitable and controversial for the sustainable development of the economy. This leads to the increased interest in biotech methods of recovering them from yeast, algae and microalgae. Colouring properties and the biological activity associated with them hac meant that the range of applications of carotenoids is still expanding. This will not surely not remain without having an effect on the size of the market and the sales of these substances.

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