Chapter 8

Biotechnological Production of Carotenoids and Their Applications in Food and Pharmaceutical Products

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Abstract

Pigments can be divided into four categories: natural, nature-identical, synthetic, and inorganic colors. Artificial colorants are the most used in food and pharmaceutical industries because of their advantages related to color range, price, resistance to oxygen degradation, and solubility. However, many natural pigments present health-promoting activities that make them an interesting option for human use and consumption. Natural colorants are derived from sources such as plants, insects, and microorganisms. Carotenoids are natural pigments with important biological activities, such as antioxidant and pro-vitamin A activity, that can be either extracted from plants and algae or synthesized by various microorganisms, including bacteria, yeasts, filamentous fungi, and microalgae. Advantages of microbial production include the ability of microorganisms to use a wide variety of low cost substrates, the better control of cultivation, and the minimized production time. After fermentation, carotenoids are usually recovered by cell disruption, solvent extraction, and concentration. Subsequent purification steps are followed depending on the application. The most prominent industrial applications of carotenoids, considering their health benefits, are in the food, feed, and pharmaceutical industries.

Keywords: biotechnology, natural pigments, microbial carotenoids, downstream, industrial applications

1. Introduction

Color has a great influence on the appearance, processing, and acceptance of food products, textiles and pharmaceutical products. The first quality impact by which consumers make the decision to purchase a product is its visual appearance.

Food colorants can be divided into four categories: natural, nature-identical, synthetic, and inorganic colors [1]. The production of synthetic coloring agents and other chemicals used as food additives is under increasing pressure due to a renewed interest in the use of natural products and the strong interest in minimizing the use of chemical processes [2]. Since the number of permitted synthetic colorants has decreased because of undesirable toxic effects including mutagenicity and potential carcinogenicity, interest focuses on the development of food grade pigments from natural sources [3–5].

Natural pigments are derived from sources such as plants, insects, and microorganisms. Algae and microalgae, bacteria, fungi, and yeasts are organisms commonly found in nature that can produce natural pigments in different color spectra, such as violacein, phycocyanin, monascins, flavins, quinones, and carotenoids.

Carotenoids represent one of the most important groups of natural pigments, they are responsible for the yellow, orange, red, and purple colors in a wide variety of plants, animals, and microorganisms [6]. They are lipid-soluble, commercially, and biotechnologically significant pigments produced from various organisms such as plants [7], algae and microalgae [8–12], bacteria [13–15], fungi [16–20], and yeasts [4, 21–25].

Pigments from natural sources have been obtained since long time ago, and their attractiveness has increased due to the toxicity problems caused by the synthetic pigments [26–28]. Carotenoids are obtained industrially by chemical synthesis or extraction from plants or algae; however, there has been an increasing interest in biotechnological processes for carotenoids production [29]. The pigments from microbial sources are a good alternative to obtain natural colorants for industrial uses.

The biotechnological production of carotenoids has advantages related to the diversity of microorganisms in nature, versatility in the use of substrates and agro-industrial wastes and the possibility to control operating conditions such as pH, temperature, dissolved oxygen, and light intensity; also, biomass from other bioprocesses can be submitted to the extraction of carotenoids. The production of microbial carotenoids has become a potential alternative for the replacement of artificial pigments, even with technological, economic, and legislation limitations.

Studies have demonstrated that carotenoids play an essential role for the maintenance of living bodies. In plants, carotenoids play an important role in photosynthesis, acting as light-harvesting pigments and protectors against photo-oxidation. In foods, carotenoids confer yellow, orange, or red color, serve as precursors of aroma compounds, and, as natural antioxidants, may help to extend the shelf-life [30, 31]. In humans, carotenoids have been associated with the reduction of the risk of developing chronic diseases such as cancer, cardiovascular diseases, high levels of cholesterol, cataract, and macular degeneration, aside from the pro-vitamin A activity of some of these compounds [31–33]. This is important because in the developed world,

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as life expectancy increases and the birth rate declines, the demand for solutions focusing on longevity and life quality increases too. The number of people aged >60 years is expected to account for approximately one-fifth of the world's population by 2050 [34].

2. Biotechnological production of carotenoids

2.1. Carotenoids diversity

Carotenoids are lipid-soluble pigments, colored from yellow to red, with a basic structure consisting in a tetraterpene with a series of conjugated double bonds. They can have only carbon and hydrogen in their structures or have one or more oxygen atoms, being classified as xanthophylls. The majority of carotenoids are C_{40} terpenoids, which act as membrane-protective antioxidants scavenging O_2 and peroxyl radicals [35].

There are more than 700 types of carotenoids described and only about 50 are precursors of vitamin A. Carotenoids can reduce risks for degenerative diseases such as cancer, cardiovascular diseases, macular degeneration, and cataract. The biological activities, specially the antioxidant properties, depend on their chemical structure: number of conjugated double bonds, structural end-groups, and oxygen-containing substituents [36].

Carotenoids occur in photosynthetic systems of higher plants, algae, and phototrophic bacteria. In plants, carotenoids are embedded in the membranes of chloroplasts and chromoplasts. The colors of these pigments are masked by chlorophyll, but they contribute to the bright colors of many flowers and fruits [37].

Nonphotosynthetic organisms, as some bacteria and fungi, present carotenoids as protectors against photo-oxidative damage, a way of protection in growth conditions with light and abundant air. The main carotenoids produced by fungi are β-carotene, torulene, torularhodin, and astaxanthin [38]. Bacteria have been reported as producers of cantaxanthin mainly. The microalgae are producers of lutein, β-carotene, and astaxanthin [35].

Animals usually present carotenoids provenient from their diet. Marine animals that feed on algae or on products rich in carotenoids may exhibit the coloration of these pigments, as the salmon fish. The color of the feathers of some birds also comes from a diet rich in carotenoids, as flamingos [39].

The industrial production of carotenoids by plants is dependent on the season and geographic variability, and these cannot always be controlled. The chemical synthesis of carotenoids generates wastes that can cause damage to the environment and resistance by the consumers. Because of this, the biotechnological resources are becoming more interesting. The microbial production of carotenoids can be performed using low-cost substrates or substrates that are residues from industrial processes, like molasses, resulting in lower costs of production [40]. All conditions of this kind of production can be controlled and optimized, especially knowing the metabolic route of each microorganism utilized.

Carotenoids are intracellular products, and a process to increase their accessibility at the downstream stage is necessary. The techniques most used combine physical and chemical methods like maceration and contact with organic solvents [4].

2.2. Main carotenoid biosynthesis pathways

Carotenoids are usually produced from the building blocks geranyl geranyl diphosphate (GGPP) and farnesyl diphosphate (FPP), like other secondary metabolites such as sesquiterpenoids and steroids. The most common pathway is the condensation of 2 GGPP units into prephytoene diphosphate and then to phytoene, a 40-carbon polyunsaturated precursor which is colorless. This precursor is converted into lycopene and then into several derived carotenoids such as β-carotene and oxidized derivatives such as lutein. The condensation of two units of FPP leads to 30-carbon precursors that are converted to steroids or apocarotenoids such as staphyloxanthin [41, 42]. Apocarotenoids can also be produced by oxidative cleavage of carotenoids. **Figure 1** presents a simplified carotenoid biosynthesis pathway.

Most carotenoids present maximal absorption in the violet to green region of the visible spectrum, so these substances appear as red to yellow pigments. **Table 1** shows the carotenoids with permitted food use according to the Food and Drug Administration (FDA) and the Food and Agriculture Organization (FAO).

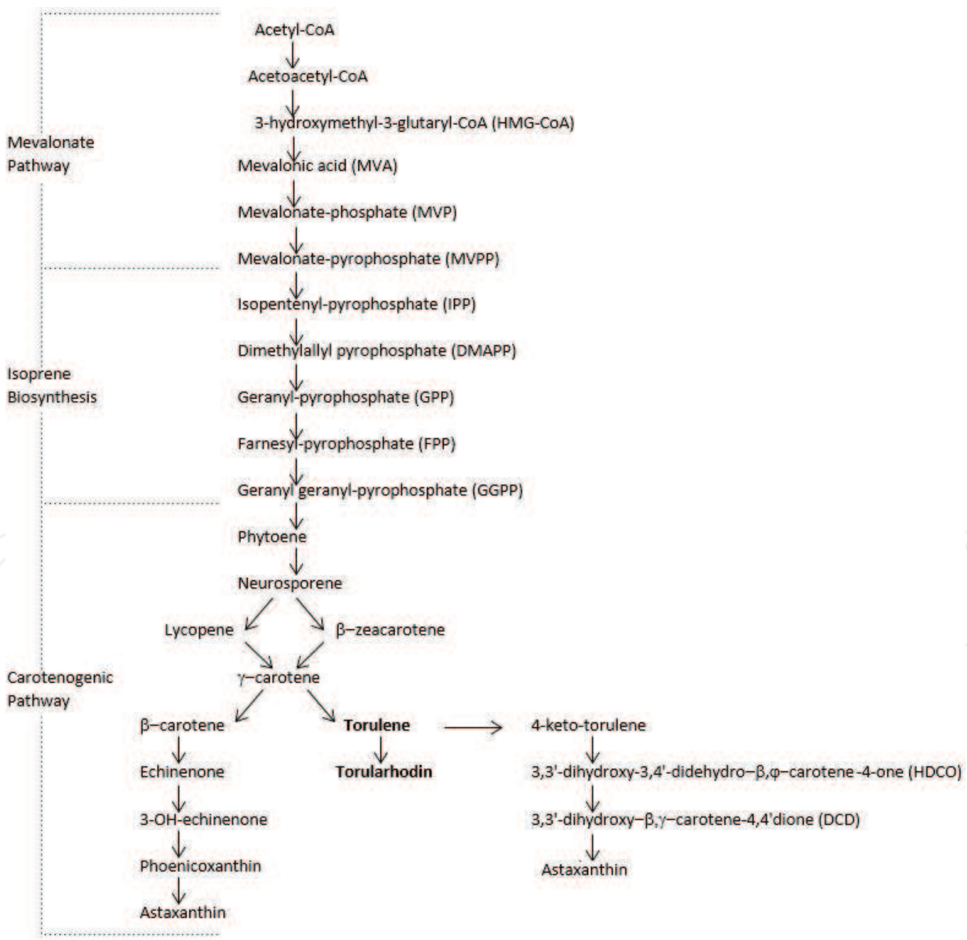

Figure 1. Biosynthesis pathways of common carotenoids. Source: Adapted from Ref. [38] with permission.

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Additive/source	Color	Main component	International Numbering System
Algae meal, dried	Green to red	Mixture of carotenoids, xanthophyll, and chlorophylls	
Astaxanthin and astaxanthin dimethyldisuccinate (several microorganisms)	Orange-red	Astaxanthin	
β-apo-8′-carotenal (from carrot oil)	Reddish orange	All-trans-β-apo-8′-carotenal	160e, 160f
β-carotene, synthetic and natural: from vegetables, *Blakeslea trispora* and *Dunaliella salina*	Orange	β-carotene	160a(i), 160a(ii)
Canthaxanthin; most of the pigment used in feeds is synthetic	Orange pink	β-carotene-4,4′-dione (canthaxanthin)	161g
Carrot oil	Orange to yellow		160a(ii)
Gardenia red and yellow	Red, yellow	Crocin, crocetin	
Haematococcus algae meal	Orange-red	Astaxanthin	
Lycopene, tomato extract (i) or concentrate (ii) or from *Blakeslea trispora* (iii)	Red	Lycopene	160d
Lutein (bras), from marigold oleoresin		Lutein	161b
Marigold color	Yellow	Lutein	
Orchil dyes	Red	Orcein	
Paprika and paprika oleoresin	Red	Capsanthin, capsorubin	160c
Paracoccus pigment	Red	Astaxanthin	
Phaffia yeast	Orange-red	Astaxanthin esters	
Saffron	Yellow to orange	α-crocin	164
Tagetes (Aztec marigold) meal and extract	Yellow to orange	Lutein	161b

Sources: Compiled from the FDA Color Additive Status List [http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm106626.htm] and from the Combined Compendium of Food Additive Specifications [ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e00a.pdf].

Table 1. Carotenoids and carotenoid-rich products used as food color additives.

2.3. Carotenoid sources

The most common sources for natural carotenoids for food and cosmetic use are plants, although microorganism biomass is becoming more common as a source for these substances. **Table 2** illustrates some commercial sources for microorganism-based carotenoids.

Microorganism	Molecule	Culture medium*	X_{max} (g/L)	P_{max} (mg/L)	Conc. (mg/g)**	μ_x (h^{-1})	References
Blakeslea trispora (fungus)	β-carotene	Corn steep liquor	20	800	40	0.022	[43]
Blakeslea trispora	β-carotene	Whey	8	1360	170	0.023	[44]
Sporobolomyces roseus (yeast)	β-carotene	Reconstituted whey	4.71	2.58	0.55	–	[40]
Rhodotorula glutinis (yeast)	β-carotene	Potato extract	5.70	1.08	0.19	–	[40]
Dietzia natronolimnaea (bacterium)	Canthaxanthin	Whey	3.29	2.87	0.87	0.020	[45]
Phaffia rhodozyma (yeast)	Astaxanthin	Cassava residues	8.6	2.98	0.35	0.060	[46]
Sporobolomyces ruberrimus (yeast)	Torularhodine	Technical glycerol	30	3.7	0.12	0.040	[47]
Chlorella zofingiensis (microalga)	Astaxanthin	BBM with glucose	10.2	–	1	0.031	[48]
Coelastrella striolata (microalga)	Canthaxanthin Astaxanthin β-carotene	BBM	2.7	–	47.5 1.5 7	0.30	[49]
Coccomyxa onubensis (microalga)	β-carotene Lutein	K9	1.6	–	2.88 6.48	0.50	[50]
Haematococcus pluvialis (microalga)	Astaxanthin	BBM	2.2	–	13.5	–	[51]
Chlorella zofingiensis	Astaxanthin	Bristol, modified	10	–	1.25	0.043	[52]
Dunaliella salina (microalga)	β-carotene	f2	–	–	14***	0.55	[53]
Haematococcus pluvialis	Astaxanthin	Standard	3	–	12–15	0.56	[54]
Muriellopsis sp. (microalga)	Lutein	Arnon, modified	5.37	–	6.51	0.17–0.23	[55]
Haematococcus pluvialis (wild-type) *Haematococcus pluvialis* (mutant)	Astaxanthin	NIES medium	1.6 2.25	– –	47.62 54.78	0.07 0.08	[56]
Paracoccus carotinifaciens (bacterium)	Astaxanthin Canthaxanthin	Glucose and peptone based	–	–	25–40	–	[57]

*Except where specified, these are mineral-based media. Recipes may be found at UTEX, SAG, or CCMP collections web sites.

**Milligrams of carotenoids per gram of biomass.

***Estimated. The original reference reports 28.1 mg/L carotenoids.

X_{max}—maximum biomass concentration; P_{max}—maximum carotenoids concentration; μ_X—biomass production rate.

Source: Adapted from Ref. [58].

Table 2. Main sources for concentrated carotenoids.

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2.4. General downstream operations for carotenoid production

Carotenoids are nonpolar molecules that accumulate intracellularly in plant tissues and microorganisms. Therefore, the production usually consists in a biomass pretreatment that may accelerate the dissolution of these substances, followed by a solid-liquid extraction (leaching) with a suitable, low-polarity solvent. The resulting solution can be a final product, can be desolventized, and can be further purified, depending on the use intended for the extract. **Figure 2** illustrates the main steps in the production of carotenoids.

The first step in carotenoid production is the pretreatment of the raw biomass, usually by drying and milling. Drying is convenient because it reduces the weight of the material to be processed, facilitates the access for solvents to the biomass, and reduces contaminants that could be extracted in water micelles with the solvent. The milling step is also important because it increases the surface area of the biomass matrix, facilitating contact with the solvent. In the case of tough-walled organisms, chemical or mechanical cell disruption may be done prior to drying. Fine milling of the dry biomass is less common.

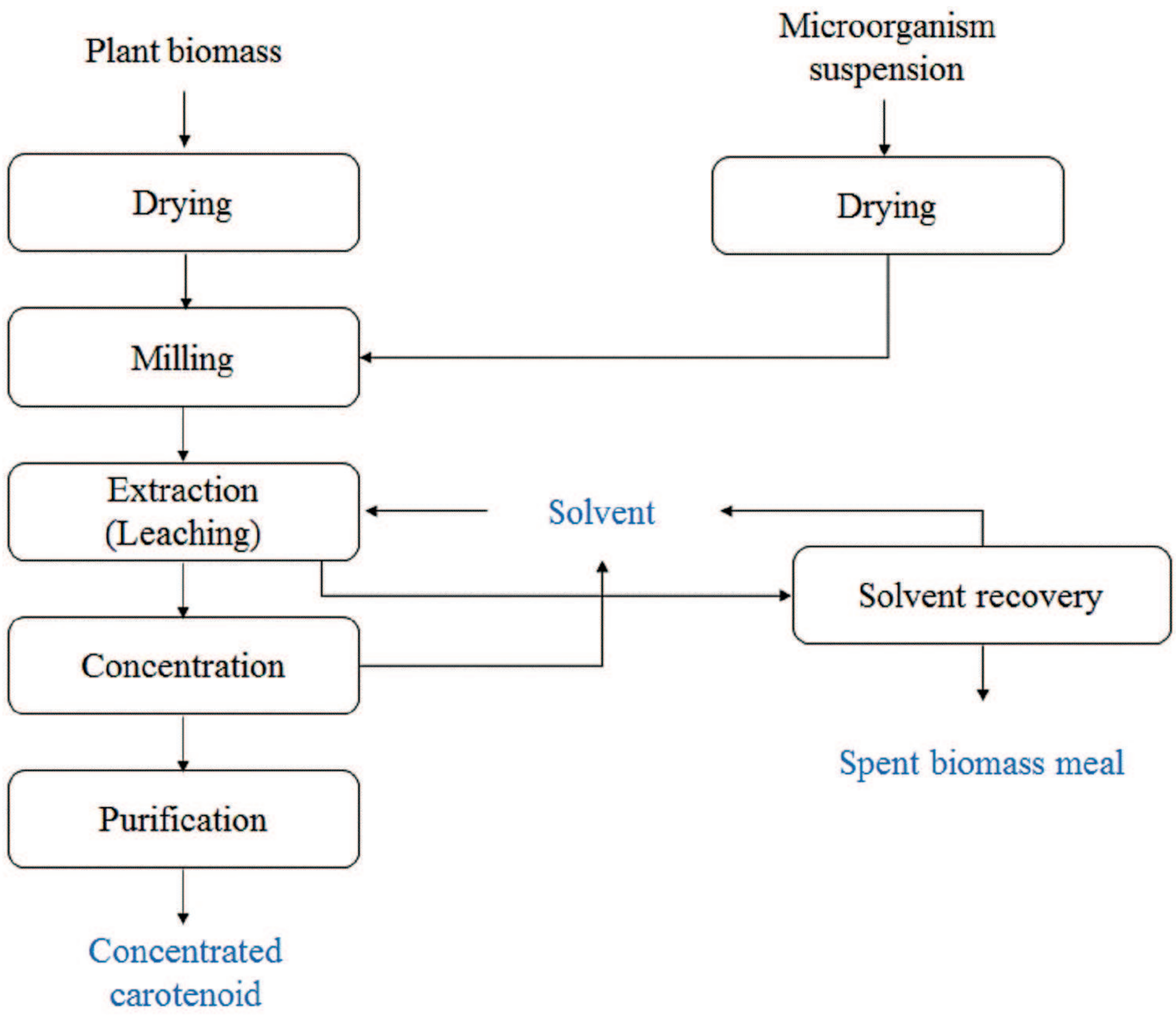

Figure 2. Main steps in carotenoids production.

The dry biomass is then extracted using a nonpolar solvent such as hexane or a vegetable oil, for the dissolution of carotenoids. A higher polarity solvent such as acetone can be used for the extraction of xanthophylls. In both cases, lipids are extracted in the mix. This extraction is an equilibrium operation; therefore, the final concentration in the solvent affects the extraction efficiency. Following extraction, the solution containing carotenoids must be concentrated and desolventized. This is why low boiling point solvents, which are easy to evaporate, are more common extractants than oils.

The carotenoids in the concentrated extract may be purified or not, depending on the intended use. For example, β-carotene that will be used as a vitamin A precursor must be purified, while paprika oleoresin is a mixture of carotenoids used mainly as a color and flavor additive and needs no further purification. In general, for carotenoids used as color additives, it is enough to concentrate the extract because (1) the tinctorial strength of the molecules is large—therefore, the additive is added at a low concentration to the formulated product and (2) the sources used are generally regarded as safe (GRAS), and the molecules extracted with the carotenoid are harmless in the concentrations used.

In the case of purified carotenoids, the operations to be used—adsorption, chromatography, crystallization, etc.—depend largely on the properties of the target molecule and the contaminants in the mixture, such as melting point, polarity, solubility, etc. All sorts of nonpolar compounds are extracted with the solvent, such as neutral and slightly polar lipids, steroids, and waxes. The differences in the properties of the carotenoid and the contaminants will be explored in the purification strategy.

Following extraction and purification, the carotenoid must be formulated for further application. This formulation will also depend on the intended use. The formulation may be as simple as adding an antioxidant such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) to the extract or may be more complex, such as emulsifying the carotenoid as an oil-in-water product for use in polar matrixes such as juices.

3. Industrial application of carotenoids as additives in food, feed and pharmaceutical products

Because of the rising of health concerns by consumers, the demand for carotenoids as natural coloring products is growing. Beta-carotene, astaxanthin, canthaxanthin, lycopen, and lutein are the most required and valuable carotenoids, and they are currently used by the food, feed, and cosmetic industries (**Table 3**). The use of carotenoids is regulated by the legislation of each country that specifies the source, purity, product, and quantities of the colorant that can be used [59].

According to BBC Research [65], the carotenoid global market in 2014 was of US$ 1.5 billion, this value is increasing year by year and is expected to reach US$ 1.8 billion in 2019, with an annual growth rate of 3.9%. Beta-carotene, the carotenoid of highest value, had a global market of US$ 233 million in 2010, which is expected to reach US$ 309 million by 2018. Astaxanthin, due to its powerful antioxidant activity, is the third carotenoid in terms of high added value, with a global market size of US$ 225 million, estimated to increase to US$ 253 million by 2018.

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Carotenoid	Color	Application	Activities	References
Lutein	Yellow	Poultry feed; functional nutrient	Antioxidant	[60]
Canthaxanthin	Orange	Poultry feed; fish feed; cosmetic	Antioxidant, anticancer	[61]
Lycopene	Red	Supplement in functional foods; additive in cosmetics	Antioxidant, anticancer	[62]
β-carotene	Orange-red	Nutraceutical; cosmetic; animal feed industries	Antioxidant, anticancer, precursor of vitamin A	[63]
Astaxanthin	Pink-red	Fish feed; cosmetic industry	Antioxidant, photoprotectant, anticancer, anti-inflammatory	[64]

Table 3. Carotenoids' colors, applications and biological activities.

3.1. Importance and use of carotenoids in food products

Commercial food products using carotenoids are expanding, and the greatest demand is in the Asian continent. The pigment is extracted from microalgae such as *Chlorella*, *Dunaliella*, *Haematococcus* [66, 67], from the cyanobacterium *Spirulina* [68], and from the fungus *Monascus* [69].

In Asia, the production red *koji* dates of hundreds of years and uses the fermentation of rice by *Monascus* to produce the typical reddish color. These red pigments are also used as food colorants for wine, red soy cheese, meat, and by-products of meat and fish [26]. The French cheese named *vieux-pan* contains the carotenoid produced by *Brevibacterium linens* due to its orange-red-brown color that improves the sensory quality of the product [70]. In Russia, infant formulas are enriched with natural pigments such as lutein, which is present in breast milk, in order to improve children's health [71].

Nutraceutical food products have also been applied in bakery products and pasta. In Japan, *Undaria pinnatifida* (wakame), an edible seaweed rich in fucoxanthin, is commercialized as an ingredient for pasta [72]. In India, a pasta containing fucoxanthin as an ingredient to improve its biofunctional and nutritional qualities was developed [73].

3.2. Importance and applications of carotenoids in the pharmaceutical industry

Besides the use of nutraceutical foods as a form of prevention and treatment of diseases, the administration of the bioactive compounds in their concentrated form is also a possibility for promoting health. The transport of carotenoids occurs from the intestinal mucosa to the blood vessels carried by lipoproteins [74]. Carotenoids functional properties are related to reactions such as oxidation, reduction, hydrogen abstraction, and addition in biological membranes, and their antioxidant power is fundamental for cell protection against free radicals and singlet oxygen formed in tissues [75].

Some carotenoids are precursors of vitamins, and they also present activities such as anti-inflammatory, antioxidant, immunomodulatory, anticancer, for cardiovascular therapy and neurodegenerative diseases [76], and anti-obesity [77]. The carotenoids included as pro-vitamin A are β-carotene, α-carotene, and cryptoxanthin. Vitamin A is an essential nutrient for operation and maintenance of biological functions including vision, reproduction, and immunity [78]. Beta-carotene is present in blood and tissues, which is associated with antioxidant activity and concomitantly with other carotenoids or antioxidants can enhance their activity against free radicals. However, it can bring health risk at high doses [79].

Carotenoids, acting as antioxidants eliminating free radicals, can modulate the risk of developing chronic diseases by inhibiting reactions mediated by reactive oxygen species (ROS). Reactive species are produced during cellular metabolism as a defense to infectious and chemical agents that may cause damage to DNA, proteins, and tissues, contributing to the development of chronic diseases such as diabetes, Parkinson's, Alzheimer's, cardiovascular diseases, and cancer [80].

In addition to the antioxidant properties, carotenoids exhibit anti-inflammatory activities owing to the protective effects of phytochemicals such as lutein and astaxanthin. Astaxanthin has been shown to inhibit the production of pro-inflammatory mediators such as nitric oxide (NO) in macrophages, to increase the level of inflammatory cytokines, and to reduce oxidative stress. Neuroprotective effect, reduced neuroinflammation, improvement of insulin signals, and reduction of lipid levels were also verified [81].

Inhibition of cell proliferation of colon cancer cells by the use of *Neochloris oleoabundans* carotenoids was observed, enabling its use as a functional food additive or nutraceutical with potential for the prevention of colon cancer [82]. Beta-carotene, astaxanthin, and capsanthin demonstrated antiproliferative effects on leukemic K562 cells [83]. Studies indicated that the simultaneous use of different carotenoids was efficient against liver cancer. Patients were administered with β-cryptoxanthin-enriched mandarin orange juice and capsules of a carotenoids mixture-containing lutein, β-cryptoxanthin, lycopene, zeaxanthin, and fucoxanthin. Analyses of DNA array and protein-antibody array showed that the carotenoids interferred in the induction of genes such as p16 and p73 [84].

4. Conclusion and final remarks

There are many advantages related to the use of carotenoids instead of artificial pigments in food products and for pharmaceutical applications. Their biological properties such as antioxidant, anti-inflammatory, antitumoral, and pro-vitamin A activities contribute to the quality of the product and to the consumer's health. Among the production strategies, microbial synthesis is considered advantageous, and the downstream techniques usually involve cell disruption, solvent extraction, concentration, and purification, when necessary. Several researches have proved the beneficial effects of carotenoids on health, so they can meet the demand for solutions focusing on longevity and life quality.

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