Chapter

Carotenoids as Natural Colorful Additives for the Food Industry

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Abstract

The application of natural colorants is increasing in the food industry because they are considered safer and healthier than some synthetic pigments. Natural colorants can improve the organoleptic properties of foodstuffs, provide additional benefits such as enhance their nutritional value and/or extend shelf-life. Plants, fungi, bacteria or algae naturally produce different natural colorants, including carotenoids. These compounds are classified into two main groups: pure hydrocarbon carotenes (α- and β-carotenes, lycopene) and oxygenated derivatives of xanthophylls (lutein, zeaxanthin, astaxanthin, fucoxanthin, cryptoxanthin, etc.). Carotenoids have been related with beneficial properties like antioxidant, antidiabetic, antitumor or antimicrobial, so they are a natural and healthy alternative to the use of synthetic colorants. Thus, it is critical to optimize their extraction, by utilizing novel and green techniques, and their stability through encapsulation processes. This chapter aims to review natural sources of carotenoids, strategies to efficiently extract and produce them and their potential application as food colorants.

Keywords: carotenoids, natural colorants, natural pigments, natural additives, antioxidant, green carotenoid extraction

1. Introduction

1.1 Carotenoids: natural pigments for coloring in food industry

Carotenoids are a class of natural pigments broadly distributed in nature and synthesized by plants, certain bacteria, fungi and algae. These molecules are classified in two main groups: carotenes, which are pure hydrocarbons (α−/β-carotenes and lycopene), and xanthophylls, which represent the oxygenated derivatives (lutein, zeaxanthin, astaxanthin, fucoxanthin and cryptoxanthin) [1]. These hydrocarbons are formed by eight five-carbon isoprenoid units with conjugated double bonds, responsible of multiple geometrical isomers (cis/trans), although carotenoids are mainly found in the most stable configuration, the all-trans one [2, 3]. These double bonds act as chromophores and are responsible for light absorption in the visual range of the spectrum [4], providing yellow, orange and red coloration [5]. Among the main biological properties described for carotenoids, they stand out for their antioxidant capacity and ability to quench singlet oxygen species [6]. Carotenoids have also been described to wield anti-inflammatory, antimicrobial and anti-hyperglycemic activities, to prevent cardiovascular and/or neurodegenerative diseases and to stimulate the immune system [7, 8]. These beneficial properties made them emerge as a promising alternative to synthetic additives, which have been related with negative side-effects. Besides, these pigments improve the nutritional value of foodstuff and can be used for food coloring. These reasons have boosted carotenoids' market size, which is expected to reach $300 billion by 2024, due to the interest shown by food, animal feeding, pharmaceutical, nutraceutical and cosmetic industries [9].

1.2 Carotenes

1.2.1 Alpha and beta-carotene

Found primarily in microalgae species such as *Dunaliella sp.* or *Arthrospira sp.* and vegetables like carrots and pumpkins, β-carotene is an isomer form of α-carotene (**Figure 1**). The latter compound is also found in these vegetables and in cereals like corn and fruits like peaches and apples [10]. Since β-carotene bioconversion efficiencies surpass those of α-carotene, it is more abundantly distributed among the vegetal kingdom [11]. Carotenes have been approved as Group II food additives by the European Commission under the E160 number [12], being used as an orange-red pigment on non-alcoholic beverages, cheese, pastry and ice cream [13]. Moreover, both pigments are known for being vitamin A (retinal, retinol and retinoic acid) precursors [5]. Vitamin A intake has proven to prevent the development of ocular diseases associated with its deficiency [11, 14] and systemic affections involving an increase of the oxidative status as in immunological diseases or cancers [5]. Thus, their versatility and multiple benefits have made them emerge as high-valued food additives with large economic significance, catching the attention of food industry.

1.2.2 Lycopene

Lycopene can be found in fruits and vegetables, especially in tomatoes (**Figure 2**), being the carotenoid with the highest antioxidant capacity. It has been seen that this pigment is involved in modulating many anti-inflammatory processes, and some authors have linked it with the prevention of bone diseases, such as osteoporosis [15]. Furthermore, lycopene also shown anticancer effects against several tumoral and normal cell lines, particularly prostate cancer cell lines (PrEC and PC-3), in *in vitro* and *in vivo* studies [16, 17]. For all these health benefits and for being easily obtainable, lycopene is widely used by the food industry as a colorant, being applied into many foodstuffs like cheese, sausages

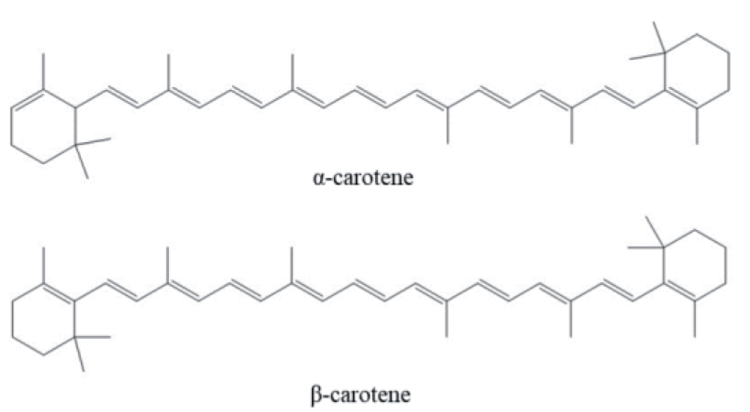

Figure 1.
Chemical structure of α-carotene and β-carotene.

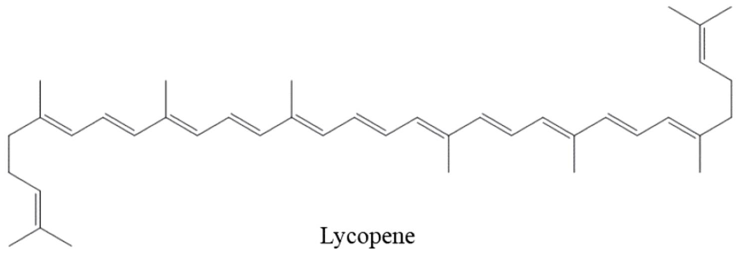

Figure 2.
Chemical structure of lycopene.

or dairy drinks, among many others [10]. However, the major drawback of lycopene is its bioavailability, which depends on several factors, including the food source, people's metabolism and even the interaction with other food [18].

1.3 Xanthophylls

Xanthophylls comprise oxidized derivatives of carotenoids, being broadly available in nature. These pigments are characterized for having yellow, orange or red coloration. Some of the most common xanthophylls present in nature include lutein, zeaxanthin, astaxanthin, β-cryptoxanthin and fucoxanthin (**Figure 3**) [6]. These compounds are polar molecules and, unlike non-polar carotenes, they get accumulated, contributing to skin pigmentation [1]. Antioxidant, neuroprotective, antiplasmodial or anticancer are some of the biological activities that pointed xanthophylls as a promising nutraceutical. These beneficial bioactivities may have preventive effects in an extensive variety of diseases such as oral, allergic, neurologic, ophthalmologic and immune affections [6]. Moreover, beneficial properties may be transferred to food. Hence, these characteristics have prompted the incorporation of xanthophylls as natural additives to obtain products with a better appearance according to the consumers' standards [1, 19].

1.3.1 Lutein

Lutein is a dihydroxy derivative of β-carotene with hydroxyl groups at both sides of the molecule (**Figure 3a**), converting it in a dipolar xanthophyll. This chemical configuration confers hydrophilic characteristics and improves its capacity to scavenge free radicals [6, 20]. The most common chemical configuration of lutein

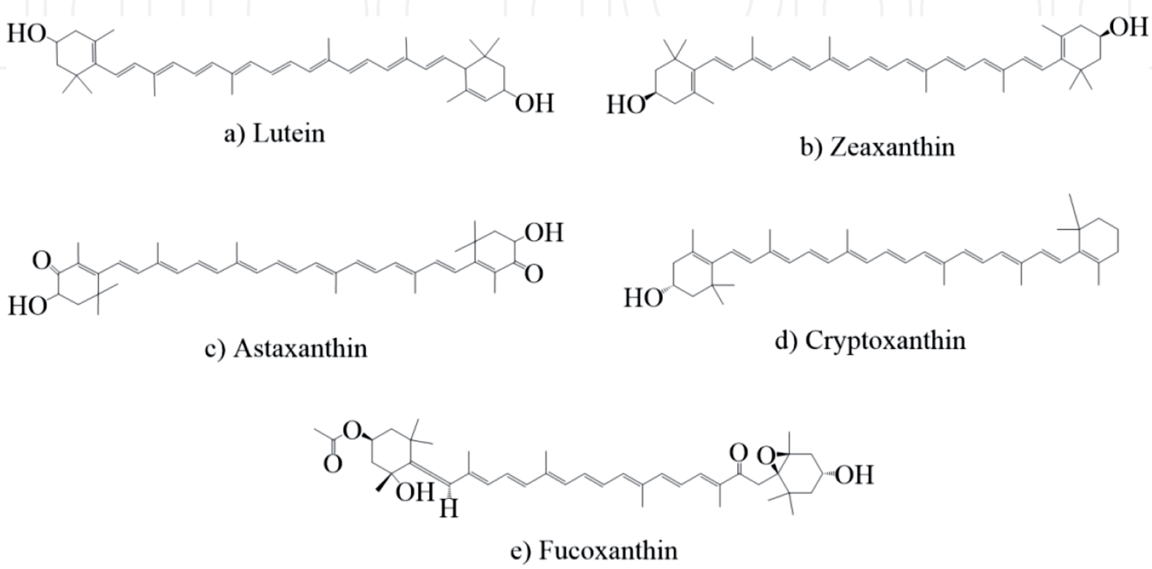

Figure 3.
Chemical structure of a) lutein, b) zeaxanthin, c) astaxanthin, d) cryptoxanthin and e) fucoxanthin.

is acylated with different fatty acids [1], such as lauric (C12:0) or palmitic acid (C16:0), becoming mono- and diacylated derivatives [21]. Leafy vegetables and plants, flower petals and yellow and orange fruits are the most important sources of lutein. Its extraction is mainly carried out with organic solvents from flower petals that have been previously fermented and/or dried [1].

1.3.2 Zeaxanthin

Zeaxanthin (**Figure 3b**) is a structural isomer of lutein with a darker yellow tone, closer to orange [20]. It is naturally found in leaves of green vegetables, flower petals, in some yellow and orange fruits, corn and even in microbial *Flavobacterium* sp. Also, it can be transferred into animal products such as egg yolks [6, 20, 22]. Zeaxanthin's poor stability in presence of oxygen and light and its lipophilic nature limit its applications in food industry. Nanoencapsulation of the molecule seems to be a promising strategy to improve zeaxanthin stability in the final product [22]. Currently, in the food industry, zeaxanthin is used as colorant and feed additive in birds to color skin and egg's yolk, and in swine and fish for skin pigmentation [20].

1.3.3 Astaxanthin

Astaxanthin (**Figure 3c**) is a lipophilic carotenoid with a reddish-orange color [23]. This pigment is found in high concentrations in microalgae like *Haematococcus pluvialis*, and *Chlorella zofingiensis*. Furthermore, it is also encountered in red yeast like *Xanthophyllomyces dendrorhous* and some bacteria like *Agrobacterium aurantiacum* [24]. Ascending in the food chain, astaxanthin gets accumulated in crustaceans like krill, shrimps, lobsters or crabs, and fish flesh like in salmon [20, 25]. This xanthophyll is mainly used as a food additive in aquaculture for animal feeding, as well as in poultry, to provide the characteristic pigmentation [24]. However, astaxanthin presents several disadvantages such as undesirable sensory attributes, low solubility in water and is easily oxidated, problems that can be overcome by microencapsulation [26]. Moreover, this molecule exerts a powerful quenching of singlet oxygen activity and scavenging oxygen free radicals, translated into a high antioxidant activity. These qualities convert astaxanthin into a promising supplement with antioxidant and anti-inflammatory properties [24, 27].

1.3.4 Cryptoxanthin

β-cryptoxanthin is a naturally occurring pigment mainly found in tropical fruit like papaya, highlighting its accumulation in citrus fruit such as oranges and tangerines [28]. This xanthophyll is closely related to β-carotene since, aside from being a vitamin A precursor, their structures are very similar, varying by just the addition of a hydroxyl group in one of the β-ionone rings in β-cryptoxanthin's structure (**Figure 3d**), resulting in a bipolar conformation. These conformation makes its bioaccumulation easier, facilitating food coloring as well as being more nutritionally valuable, contributing to vitamin A production [29]. Moreover, β-cryptoxanthin intake has been associated with a reduced risk of inflammatory diseases, like polyarthritis or rheumatoid arthritis, by suppressing bone resorption and stimulated bone formation [30].

1.3.5 Fucoxanthin

Fucoxanthin (**Figure 3e**) is mostly known for giving the characteristic brownish/olive-green color to brown algae (Phaeophyceae), as in species belonging to the genus *Undaria*, *Sargassum* and *Laminaria*, although some microalgae, mainly diatoms and

Chrysophyta, can accumulate higher concentrations [31, 32]. This pigment has been related with antioxidant, anticancer, antihypertensive, anti-inflammatory, anti-diabetic, anti-obesity, neuroprotective, anti-angiogenic and photoprotective bioactivities [33], being considered as a non-toxic and safe bioactive ingredient for coloring and food supplementation purposes [34]. However, some limitations arise due to its low water-solubility, reduced bioavailability, and sensitivity to temperature, light and oxygen [35]. In addition, its synthesis is complex and expensive and extraction procedures from algae have not been standardized yet [36, 37]. Despite these drawbacks, some studies reported great fucoxanthin stability after encapsulation [38, 39].

2. Natural sources of carotenoids

Generally, natural sources of carotenoids are divided into: i) fruits, vegetables and flowers; ii) microorganisms (microalgae, cyanobacteria, fungi, bacterial and yeasts); and iii) by-products (peels, seeds and skin).

2.1 Fruits, vegetables and flowers

There is a wide variety of fruits and vegetables recognized as natural sources of carotenoids in human diet. Besides, flowers, recently introduced in gastronomy, appeared to be a suitable source of carotenoids (**Table 1**). In general terms, the most relevant carotenoid found among these groups is β-carotene, although lutein, β-cryptoxanthin, lycopene and zeaxanthin are also highlighted as major carotenoids. Reviewed literature showed very different ranges of carotenoid concentrations depending on the analyzed tissue, variety, ripening stage, geographical origin, etc. [46, 47]. Nevertheless, **Table 1** points to fruit as the most relevant source of carotenoids.

Carotenoids extracted from fruits, vegetables and flowers become too expensive due to high production costs associated with large production areas required. Besides, the supply of carotenoids extracted from plants becomes unstable, since it is dependent on unpredictable climatologic conditions [66]. Therefore, more sustainable and green approaches have been explored for a more efficient carotenoids' collection, including the use of microorganism or the reutilization of agricultural by-products

2.2 Microorganisms

Nowadays, the interest on microbial carotenoids has increased because of their low production area requirements when compared to plants. Besides, microbial cultures are nearly independent of climatic conditions, seasonality and soil composition. Current technological advances permit a tight control of culturing conditions, which improves the efficiency of microbial carotenoid production and reduces costs. Examples of efficient production of carotenoids using microalgae, bacteria, yeasts or fungi are displayed in **Table 2**, that demonstrates the huge variability of microorganisms capable of producing specific types of carotenoids being the most relevant β-carotene, lutein, astaxanthin, canthaxanthin and torulene (**Table 2**).

2.3 By-products

Food waste has been increased in the last years driven by an increasing population, expected to reach 10 billion people by 2050, and inefficient and unsustainable

Source		Main Carotenoids	Carotenoid Content (mg/g)	Ref.
Fruits				
Apricot	*Prunus armeniaca*	β-car, β-crypt, Lut, Zea	0.07–0.08 (DW)	[40]
'Gac' oil	*Momordica cochinchinensis*	α–/β-Car, Lyc	1.8–11 (FW)	[41, 42]
Goji	*Lycium barbarum*	β-car, β-crypt, Zea	0.04–0.51 (FW)	[43]
Kaki	*Diospyros kaki*	β-car, β-crypt, Lut, Zea	0.03–0.07 (DW)	[40]
Banana and plantain	*Musa* sp.	α–/β-Car, Lut	0.01–0.04 (DW)	[44, 45]
Mandarin juice	*Citrus reticulata*	ζ–/ β-car, β-crypt	0.01 (DW)	[46]
Mango	*Mangifera indica*	α–/β-Car, β-crypt, Lut, Zea	3–129 (FW)	[47]
Orange	*Citrus sinensis*	α–/ β–/ζ-car, β-crypt, Lut, Zea	0.01–0.03 0.01–0.02 (DW)	[46, 48]
Papaya	*Carica papaya*	β–/ζ-car, β-crypt, Lyc, Vio, Zea	0.14–4.13 (FW)	[49, 50]
Peach	*Prunus persica*	β-car, β-crypt, Lut, Zea	0.04–0.09 (DW)	[40]
Vegetables and cereals				
Broccoli	*Brassica oleracea* var. *italiaca*	Lut, Neo	8.5–11.6 (DW)	[51]
Carrot	*Daucus carota*	α–/β-car, Lut, Lyc	0.01–0.8 (DW) (TC's)	[52, 53]
Kale	*B. oleracea var. sabellica*	Zea	1.6–2.5 (DW)	[54]
		β-car	0.10 (DW)	
		Neo	0.12 (DW)	
Lettuce	*Lactuca sativa*	Lut	0.1–0.13 (DW)	[55]
		β-car	2.2–2.9 (DW)	
Pea	*Pisum sativum*	Lut	0.01–0.02 (DW)	[56]
		β-car	0.01–0.02 (DW)	
Pepper	*Capsicum annuum*	β-car	0.39–0.71 (DW)	[57]
		Zea	0.31–0.73 (DW)	
Spinach	*Spinacia oleracea*	Neo	0.1–0.2 (FW)	[58]
		Lut	0.34–0.53 (FW)	
		β-car	0.2–0.32 (FW)	
Sweet corn	*Zea mays*	Lut	0.02 (DW)	[59]
		β-car	0.01 (DW)	
Tomato	*Lycopersicon esculentum*	β-car	0.01 (FW)	[60]
		Lyc	0.05–0.08 (FW)	
		TCs	0.04–0.2 (FW)	
Flowers				
Blue centaurea	*Centaurea cyanus*	β-car, Lut	0.06 (DW)	[61]
Blue borage	*Borago officinalis*	β-car, Lut	1.8 (DW)	
Camelia	*Camelia japonica*	β-car, Lut	0.2 (DW)	
Carnation	*Dianthus caryophyllus*	Xanthophylls	0.001–0.003 (P) (DW) 0.04–0.07 (L) (DW)	[62]
Marigold	*Tagetes* sp.	Lut	0.0002–0.006 (DW)	[63]

Source		Main Carotenoids	Carotenoid Content (mg/g)	Ref.
Nasturtium	*Tropaeolum majus*	Lut	0.4–1.2 (DW)	[64]
Pansies	*Viola x wittrockiana*	β-car, Lut, Zea	0.2–1.1 (DW)	[61, 65]
Snapdragon	*Antirrhinum majus*	β-car, Lut, Zea	0.03 (DW)	[65]

Abbreviations: DW: dry weight, FW: fresh weight, L: leaves, P: petals. Carotenoids: α−/β−/γ−/ζ-car: α−/β−/γ−/ζ-carotene, β-crypto: β-cryptoxanthin, lyc: lycopene, lut: lutein, neo: neoxanthin, TCs: total carotenoids content, vio: violaxanthin, zea: zeaxanthin.

Table 1.
Quantitative and qualitative analysis of carotenoids content in different species of fruits, vegetables, and flowers.

Species		Most abundant Carotenoids	Content (mg/g)	Ref.
Microalgae				
Dunaliella tertiolecta		β-car	0.001–0.0045 (DW)	[67]
Haematococcus pluvialis		Ast	2–20 (DW)	[68]
Haematococcus alpinus		Ast	6–19 (DW)	[69]
Nostoc commune		Canthaxanthin	N.D	[70]
Scenedesmus almeriensis		Lut	0.01 (DW)	[71]
		β-car	1.50 (DW)	
Bacteria				
Arthrobacter sp. P40		Decaprenoxanthin and derivatives mono−/diglucosides; Lyc	0.3–0.4 (DW)	[72]
Corynebacterium glutamicum		β-Car	0.01–3.1 (CDW)	[73]
		Zea	0.01–0.9 (CDW)	
Cryobacterium sp. P19		Carotenoids, glucoside derivatives	0.4–0.5 (DW)	[72]
Chryseobacterium sp. P36		Zea; β-Crypto; β-Car; β-Zeacarotene	0.5–0.6 (DW)	
Flavobacterium sp.P33		Zea; β-Crypto; β-Car; β-Zeacarotene	0.7–0.8 (DW)	
Planococcus sp. 48		Carotenoids and glucoside derivatives	0.7 (DW)	[72]
Salinibacterium sp. P15		Carotenoids and glucoside derivatives	0.5 (DW)	[72]
Yeasts and filamentous fungi				
Blakeslea trispora	Fungi	β-car	30 (DW)	[68]
		Lyc	>900 (DW)	
Mucor circinelloides		β-car	0.275–0.698 (DW)	[74]
Phycomyces blakesleeanus		β-car	0.05–10 (DM)	[68]
Phaffia rhodozyma	Yeasts	Ast	0.000725–0.007642 (DW)	[75, 76]
Rhodotorula minuta		β-car	0.0172 (DW)	[77]
Rhodotorula glutinis		Torulene	5–14 (DW)	[78, 79]
		Torularhodin	32.2 (DW)	
Rhodotorula graminis		Torulene	18.2 (DW)	[79, 80]
		Torularhodin	9.3 (DW)	
Sporobolomyces sp.		Torulene	0.0001 (DW)	[79]
		Torularhodin	0.00001 (DW)	
Xanthophyllomyces dendrorhous		Ast	0.0026–0.001 (DW)	[81]

Abbreviations: DW: dry weight; CDW: cold-water-dispersible; N.D: not determined. Carotenoids: ast: astaxanthin, β-car: β-carotene, β-crypto: β-cryptoxanthin, lut: lutein, lyc: lycopene, TCs: total carotenoids content; zea: zeaxanthin.

Table 2.
Quantitative and qualitative analysis of carotenoids content in different species microorganisms such as microalgae, bacteria, yeasts, filamentous fungi and cyanobacteria.

By-product	Most abundant Carotenoids	Content (μg/g)	Ref.
Tucumã peels	β-car	68–88 (FW)	[84]
Peach palm peel		71–75 (FW)	
Mandarin epicarp	β-car	1397–1417 (DW)	[85]
Melon peels	β-car	67–915 (DW)	[86]
	β-crypto	3–49 (DW)	
Atlantic shrimp cooked shell	Ast	57.3–284.5 (DW)	[87]
Grape canes	Lut; β-car	0.3–2.4 (DW)	[88]
Peels and pulp of persimmon	β-crypto	6500–167,000 (DW)	[89]
	β-car	6900–45,000 (DW)	
Pressed palm fibers	α-car	142–305 (DW)	[90]
	β-car	317–713 (DW)	
Mango peel	α–/β-car; crypto	5600 (β-car) (DW)	[91]
Skin and seeds of tomatoes	Lyc	3.8–166.4 (DW)	[92]
	β-car	0.6–26.4 (DW)	
	Lut	0.8–10.8 (DW)	
Carrot by-products	β-car	230 (FW)	[93]
Carrot juice processing waste	β-car	240 (DW)	[94]
Tomato peels and seeds	Lyc	410 (P);28 (S) (FW)	[95]
	β-car	31 (P); 5.2 (S) (FW)	

Abbreviations: P: peel; S: seeds. Carotenoids: ast: astaxanthin, α–/β-car: α–/β-carotene, β-crypto: β-cryptoxanthin, lut: lutein, lyc: lycopene. FW – Fresh weight, DW – Dry weight.

Table 3.
Quantitative and qualitative analysis of carotenoids content in different by-products derived from agricultural and food industries.

production systems [82]. These factors boosted waste production, which is usually composted or burnt, emitting high amounts of CO_2 to the atmosphere. To counteract this situation, multiple strategies have been explored in the last decades, such as the revalorization of wastes as source of biomolecules. In fact, peels, seeds, husks, pomace or pulp are recognized as alternative sources of compounds with diverse biological properties [83].

Table 3 collects information about potential agricultural and food by-products as sustainable sources of carotenoids.

2.4 Macroalgae

In the last decades, macroalgae have been pointed out as a promising source of carotenoids. These photosynthetic organisms contain high amounts of pigments involved in light absorption for nourishment. However, they also have a secondary role related with damage protection from UV exposition. The main xanthophylls found in macroalgae include fucoxanthin, lutein, or zeaxanthin, being fucoxanthin the most abundant one, while β-carotene stands out from carotenes (**Table 4**). The main advantage of using macroalgae, is that invasive species can be used as an alternative source of carotenoids.

Species	Most abundant Carotenoids	Content (mg/g)	Ref.
Cystoseira sp.	Fuco	2.0–3.5 (DW)	[96]
Dictyota sp.	Fuco	0.4–6.4 (DW)	[97]
Eisenia bicyclis	Fuco	0.42 (DW)	[98]
Fucus serratus	Fuco, Lut	5.2 (DW) 0.3 (DW)	[99]
Laminaria digitata		1.4 (DW) 0.1 (DW)	
Himanthalia elongata	Fuco	18.6 (DW)	[100]
Hypnea musciformis	β-car, lut, zea	0.0029 (TCs, FW)	[101]
Monostroma nitidum	Lut	0.3 (FW)	[102]
Sargassum muticum	Fuco	0.0084 (TCs, DW)	[103]

Abbreviations*: DW: dry weight, FW: fresh weight,* β*-car:* β*-carotene, fuco: fucoxanthin lut: lutein, TCs: total carotenoids content, zea: zeaxanthin.*

Table 4.
Quantitative and qualitative analysis of carotenoids content in different macroalgae species.

3. Extraction and production techniques for carotenoids recovery

3.1 Conventional extraction

In the last century, pigment extraction has been performed using solid–liquid extraction with different organic solvents. Extracts were later purified via semi-preparative high-performance liquid chromatography (HPLC) [104] or clean up and separation columns using organic solvents such as hexane or dichloromethane [105]. The use of non-polar solvents for carotenoid extraction like petroleum ether or hexane has been linked with toxicity, having a negative impact in the environment in the long term. In addition, in the current legislation regarding the use of these solvents for the production of food ingredients is not allowed. For this reason, in the latest years, novel "greener" extraction processes have been developed for pigment recovery, including supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE) (**Table 5**). Implementing these techniques improved, among other things, extraction times, yields and solvent usage [114].

3.2 Novel techniques

Supercritical fluid extraction (SFE) emerged in 80s decade as a promising alternative to conventional organic extractions [115]. This is a process where a compound is separated from its matrix making use of the unique properties of supercritical fluids as solvents, being CO_2 the most commonly used. Supercritical fluid technology applies pressures and temperatures above the critical point of the extracting solvent, leading to a balanced state between liquid and gas phases. This balance confers low viscosity, high diffusivity, enhanced solubility and no surface tension, facilitating mass transfer [116]. However, this process involves a high cost, due to high temperatures and pressures requirements. Moreover, CO_2 only dissolves non-polar molecules, although using a co solvent overcomes this issue, being ethanol the most employed [107, 108].

As well as in SFE, pressurized liquid extraction (PLE), also called accelerated solvent extraction (ASE), makes use of high temperature and pressure, although

Source	Carotenoids	Conditions	Recovery (μg/g)	Ref.
SFE				
Dunaliella salina	β-car	CO_2, 60 °C, 300 bar	15,000 (DW)	[106]
Hachiyakaki sp. (Pe)	α−/β-car, β-crypto, lyc, lut, zea	CO_2 + EtOH, 30 MPa	392 (TCs)	[107]
Rosmarinus officinalis (L)	Carotenoids	CO_2 + EtOH, 25°C, 20 min, 20 MPa	47,000–53,000	[108]
Scenedesmus almeriensis	Lut	CO_2, 65°C, 55 MPa	3000 (DW)	[109]
Scenedesmus sp.	Ast, β-car, lut, neo, zea	CO_2 + 10% EtO, 25°C, 20 min, 20 Mpa	73; 60, 436, 671, 90	[110]
Tomato, apricot, peach, pumpkin (Fl, Pe), pepper (Fl, wastes)	β-car, lut, lyc	CO_2/EtOH, 59°C, 30 min, 350 bar	88–100% β-car	[111]
PLE				
Carrot by-products	β-car	EtOH 99%, 60–180°C, 5 min, 50 bar, 1–5 cycles of 2 min	120–230 (FW) (soft soggy carrots) 80–190 (FW) (orange carrots)	[93]
Diospyros kaki, *P. armeniaca*	β-crypto, β-car, lut, zea	MeOH: THF 2:8 (v:v), 40 °C, 5 min, 103 bar	Kaki: β-crypto ≤29, lut ≤ 13, zea ≤ 18 Apricot: ≤48 (β-car)	[40]
Eisenia bicyclis	Fucoxanthin	90% EtOH, 110°C, 5 min, 1500 psi	420	[98]
Porphyridium cruentum	β-car, zea	125°C, 20 min, 10.5 MPa	Zea ≤14,000 β-car ≤8000	[112]
UAE				
Dark red tomato	Lyc	EtAc: tomato paste 8:1 (V/W), 86°C, 29 min	89,000	[113]
MAE				
Carrot juice wastes	β-car	Flaxseed oil: wastes 8:1 g/g, 165 W, 9.4 min	775,000	[94]

Abbreviations: DW: dry weight, EtOH: ethanol, EtAc: ethyl acetate, Fl: flesh, FW: fresh weight, L: leaves, MeOH: methanol, Pe: peels, THF: tetrahydrofuran. Carotenoids: ast: astaxanthin, α−/β-car: β-carotene, β-crypto: β-cryptoxanthin, lyc: lycopene, lut: lutein, neo: neoxanthin, TCs: total carotenoids, zea: zeaxanthin.

Table 5.
Novel extraction techniques to efficiently recover carotenoids from natural sources.

along with a liquid solvent to accelerate the extraction of specific analytes from solid matrices. In this system, pressure is high enough to keep the solvent liquid without hampering extraction performance. However, extraction time, temperature, solvent type and volume have influence on extraction performance, especially temperature and solvent type. Temperature range is mostly comprised from 40 to 180°C and it has been seen that the use of *Generally Recognized as Safe* (GRAS) solvents, like water, ethanol or its mixtures enhance extraction efficiencies [117]. Nevertheless, other more non-polar solvent mixtures like methanol: tetrahydrofuran can also be used [40].

Ultrasonic assisted extraction (UAE) also emerged as a novel technique, which employs using ultrasonic waves that propagate causing the implosion of bubbles, phenomenon known as cavitation. This perturbation leads to a diffusion of the solute from the porous matrix to the solvent. Nowadays, UAE is used for extracting various compounds including carotenoids from a wide diversity of matrices, such as macroalgae, microalgae and plants. This technique is environment-friendly, simple, cheap and efficient, reporting high yields when compared to conventional techniques, although the reproducibility of the samples is jeopardized by equipment's aging [118].

Microwave assisted extraction (MAE) is a relatively new extraction technique that combines microwave and traditional solvent extraction. Since the late 1980s, MAE has become one of the most popular and cost-effective extraction methods [81]. This technique is based on the application of microwaves for heating both solvents and matrices, increasing the kinetic of the extraction. Compared to conventional and novel (SFE and PLE) techniques, MAE reduced extraction time and solvent usage, leading to higher extraction rates and reduced costs [94].

4. Carotenoids' incorporation into food: reported and future applications

Color is an important sensory attribute associated with safety and nutritional values of food, reason why, in the last years, consumer awareness regarding the use of synthetic food coloring has been increased. In order to develop a more natural food industry, natural pigment demand has raised, as is the case for carotenoids. These pigments are used for their coloring properties as well as for their antioxidant potential and biological functions. Carotenoids can be either applied directly into food matrices like beverages or pasta, among others [119, 120], or indirectly, into animal feeding to improve pigmentation of final products as in eggs or fish flesh [1, 29, 121]. Moreover, carotenoids have pointed out as promising ingredients in active packaging films. Their inclusion in protective films can improve the storing properties of the package, extending the shelf-life of the product, as well as transferring carotenoids' nutritional values [122, 123]. Several applications of carotenoids in the food industry have been collected in **Table 6**.

Since the late 1980s, carotenoids implementation into food has significantly increased. Among all, β-carotene is the most applied one, being used for coloring oils and butters, providing a yellowish color. In addition, it has been also used to fortify different food matrices for its provitamin A activity [127]. Apart from β-carotene, other carotenoids have been incorporated as free molecules into food matrices (**Table 6**). However, the direct application of these natural pigments is limited by their low stability, so micro- and nanoencapsulation technologies have been applied. Multiple encapsulation technologies including spray or freeze drying, emulsion, spray chilling, extrusion coating, liposome entrapment, coacervation and ionic gelation [128] have been applied to improve solubility, chemical stability and bioavailability of pigments, as well as for masking unpleasant organoleptic properties [129]. Most of these technologies have been applied to encapsulate carotenoids, generally on a nanometric scale (≤100 nm). The type of encapsulation materials used for food applications have to be food-graded biopolymers such carbohydrates or gums (Persian gum), proteins (gelatin or whey), and animal or vegetal lipids [22, 26, 130]. Emulsion is also a prominent encapsulation processes, which results in an improved bio accessibility and

Carotenoid	Origin	Application	Properties	Ref.
β-Carotene	Fruits & vegetables	Free and encapsulated	Yellow colorant and antioxidant	[120]
		Incorporated into polymer materials	Antioxidant, O_2 and light barrier	[123]
α-Carotene		Free and encapsulated	Yellow colorant, anti-carcinogenic and antioxidant	[120, 124]
α-Tocopherol	High fat vegetables	Incorporated into polymer materials	Antioxidant	[123]
Astaxanthin	Marigold flower	Incorporated into packaging material	Fish feeding for antioxidation and flesh coloring	[125]
Bixin	Annatto seeds	Incorporated into polymer materials	Antioxidant, O_2 and light barrier	[123]
Canthaxanthin	Mushrooms	Alginate-pectin microencapsulation	Red colorant and antioxidant	[126]
Cryptoxanthin	Mandarin, papaya, orange	Free and encapsulated	Orange colorant and antioxidant	[120]
Lutein	Green vegetables	Encapsulation in food	Eye protection against AMD development or cataracts. Anticancer	[124]
Lycopene	Tomato, watermelon, pink grapefruit, guava	Free and encapsulated	Red colorant, eye UV-protection, antioxidant	[120, 123, 124]
		Incorporated into polymer materials	Antioxidant, O_2 and light barrier	
Zeaxanthin	Mandarin, papaya, orange	Orange colorant	Eye protection against macular degeneration and cataracts	[120, 124]

Table 6.
Carotenoids applications in food industry.

bioavailability [131]. Lutein emulsions, β-carotene oil-in-water emulsions or microcapsules containing lycopene are just some examples of microencapsulation found in the food industry to improve the stability, bio accessibility and bioavailability of these pigments [129, 132, 133]. Similarly, multiple studies support carotenoids' nanoencapsulation [134, 135].

A different way to incorporate natural pigments in human food is through animal feeding. By doing so, pigments get incorporated in foodstuff such as in fish flesh or eggs, giving a characteristic pigmentation and an increased nutritional value that will be further transferred to humans or animals [121, 136]. One of the main industries where carotenoids have been implemented is aquaculture. Fish factories have been making use of pigments such as β-carotene as an important source of provitamin A, which has been shown to improve the antioxidant capacity and immune system of fish, enhancing growth and preventing lipid peroxidation [137]. In fact, in aquaculture, different biological sources of xanthophylls such as green microalgae, yeast, krill, or crab waste have been utilized as feeding supplements. This complementary pigmentation enhances the nutritional value of fish products by providing strong antioxidant activity and higher amounts of provitamin A [1]. Other industry where pigments are widely use is poultry. EU approved egg yolk and poultry tissues pigmentations with yellow and red carotenoids, including lutein, zeaxanthin, β-cryptoxanthin, violaxanthin and capsanthin [136].

Natural pigments can also be incorporated into packaging materials to improve food preservation. Carotenoids such as lycopene or β-carotene prevent color alterations due to oxidation processes and UV-induced damage, providing stability to packaging polymers [123]. Besides, pigment migration from active packaging into food matrices has been reported, transferring the beneficial properties. As could be seen in **Table 6**, several carotenoids such have been included in active packaging, achieving promising results.

5. Conclusions

Synthetic pigments have been frequently used as food additives to improve food appearance since colorful products have been associated with healthy and high-quality properties by consumers. However, tendency has slowly shifted towards a stronger presence of natural ingredients due to a raising concern about the negative side effects associated with synthetic molecules. In this context, carotenoids have come up as an attractive replacement of synthetic pigments, being found in multiple sources, like plants, algae, fungi, microorganisms and by-products. Moreover, carotenoids have been linked with diverse beneficial properties, such as antioxidant, prevention of degenerative diseases, cancer and stimulation of the immune system. For all these reasons, carotenoids have caught the attention of many industries, including food, nutraceutical and cosmetic industries.

In order to extract these pigments, novel technologies emerged to improve the extraction rates of traditional techniques, mostly based on maceration. Among these new strategies, SFE and PLE highlight. Equipment may result into an initial economic expense, but they offer satisfactory extraction rates while minimizing solvent usage and experimental times.

Regarding food industry, carotenoids have been widely used for their application into food matrices or as part of packaging materials. Their inclusion as food additives or feed supplements for animals is the most extended and explored application, improving the organoleptic properties and nutritional values, aiming for a higher commercial acceptance. Besides, carotenoids have also been used as ingredients for active packaging films to extend products' shelf-life. Regardless the matrix of inclusion, natural carotenoids have been incorporated as free molecules or encapsulated. This last strategy prolongs the stability and bio accessibility of carotenoids, protecting core ingredients from chemical degradation.

Furthermore, due to their extensive bioactivities, carotenoids are very useful to formulate new cosmetic ingredients. Besides, its antioxidant properties that can benefit the skin and promote skin regeneration and healthy aging, carotenoids also mitigate the harmful effects of UV radiation, which makes them excellent candidates for their application in cosmetic formulations as preservatives with photoprotective, antioxidant and anti-aging properties.

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Conflict of interest

The authors declare no conflict of interest.

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