Chapter 6
Carotenoids as Coloring Agents

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6.1 Introduction

Pigments are the chemical compounds responsible for producing color on an organism and are present in photosynthetic plants and microbes including colored fruits, vegetables, leaves, flowers, bacterial colony, skin, eye and many more. Color is one of the essential features of foods, influencing its demand among consumers. The proper color increases its appeal, whereas inappropriate coloration leads to an impression that the food is ineligible of consumption [1]. Food colors can be of various categories: (i) *natural colors* are pigments raised by living organisms, (ii) industrially produced natural pigments are known as *nature identical colors*, (iii) s*ynthetic colors* are laboratory produced colors that are not found in nature, (iv) *inorganic colors* are obtained from inorganic salts and metallic compounds. The colorant is added to the food for various reasons like (i) enhancing or replacing the color lost during processing or storage, (ii) maintaining color uniformity due to seasonal (batch to batch) variation, (iii) coloring the uncolored food products, (iv) increasing the acceptability of food items to the consumers, etc. [2].

From ancient times, colorants are used for marketing purpose in better satisfactoriness of food products as well as cosmetics, textile and other kinds of stuff [3]. Although colored garments were found in the remnants of Mohenjodaro and Harappa civilization (3500 BC); the oldest written record of usage of natural dye was found in China dated 2600 BC. Subsequently uses of dyes were observed in other parts of the world including the Indian subcontinent, Egypt, Europe and Brazil [2]. The first synthetic organic dye was mauveine, discovered by Sir William Henry Perkin at 1856 [4]. Although the synthetic colorants gained huge popularity at that time, gradually the demand of natural colorants increased due to perspectives of toxicity, hygiene and environmental consciousness. Color additives used in food,

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drug and cosmetics must be on a positive note as directed by different organizations like Food and Agricultural Organization (FAO), World Health Organization (WHO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), etc. [5].

Based on the chemical structure, pigments are divided as tetraterpenoids (carotenoids), anthraquinones (carmine), flavonoids (anthocyanins) and tetrapyrroles (chlorophyll) [6]. Carotenoids are natural pigment synthesized by plants and microbes. These pigments may be red, yellow or orange depending on its necessity of fulfilling its physiological function [7]. Carotenoids are heavily used as a biocolorant. Large scale production of nature identical carotenoids has been flourished due to its demand.

6.2 Mechanism of Coloration by Carotenoids

Carotenoids are broadly characterized based on their molecular composition. Carotenoids made up of solely hydrogen and carbon is known as carotene (α-carotene, β-carotene, lycopene etc.) whereas molecules bearing oxygen atoms are known as xanthophylls (lutein, zeaxanthin, astaxanthin etc.) (Figs. 6.1 and 6.2) [7]. These molecules are tetraterpene derivatives, i.e., they are generated from 8 isoprene molecules and bear 40 carbon atoms. Usually, carotenoids absorb light in

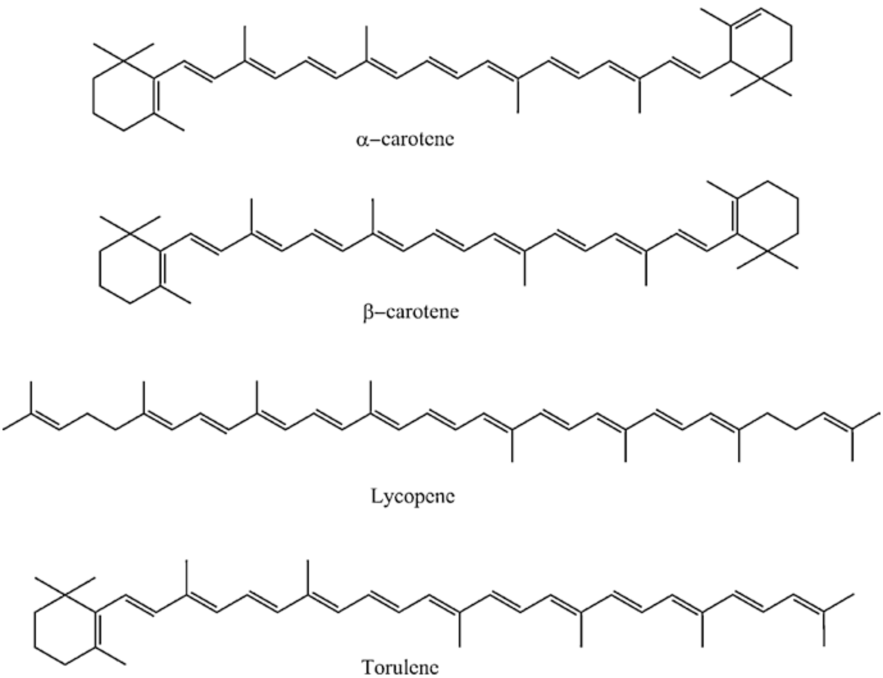

Fig. 6.1 Different types of carotenes

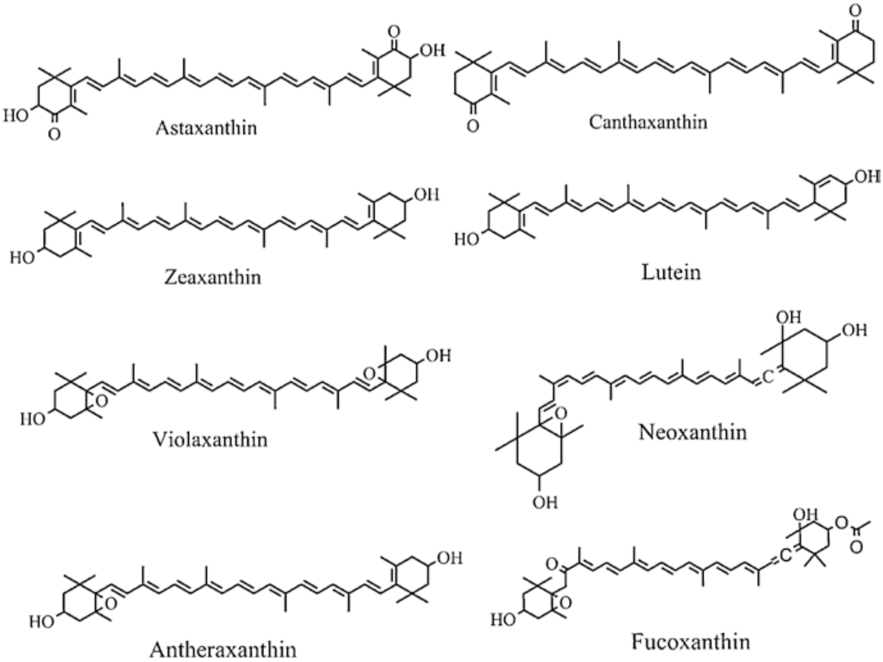

Fig. 6.2 Different types of xanthophylls

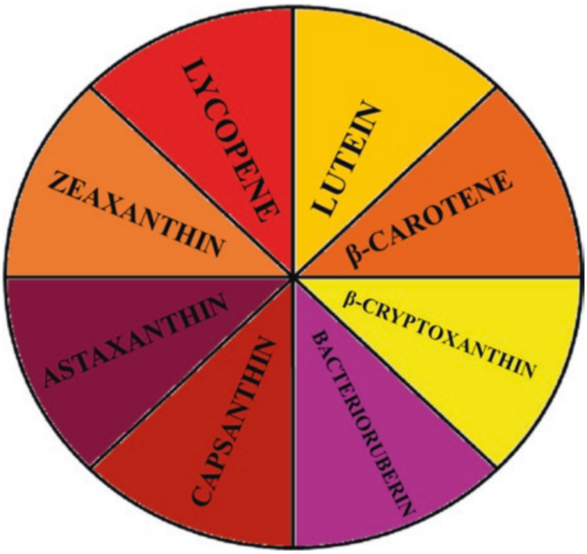

Fig. 6.3 Color of various carotenoids: Carotenoids can have different colors based on the number of double bonds present in its polyene tail

wavelengths ranging between 400–550 nm (i.e., violet to green light) causing the compounds to be yellow, red, or orange (Fig. 6.3). Their color is directly associated with their structure. The carbon-carbon (C=C) double bonds in their structure interact with each other. This process of conjugation permits electrons in the mole-

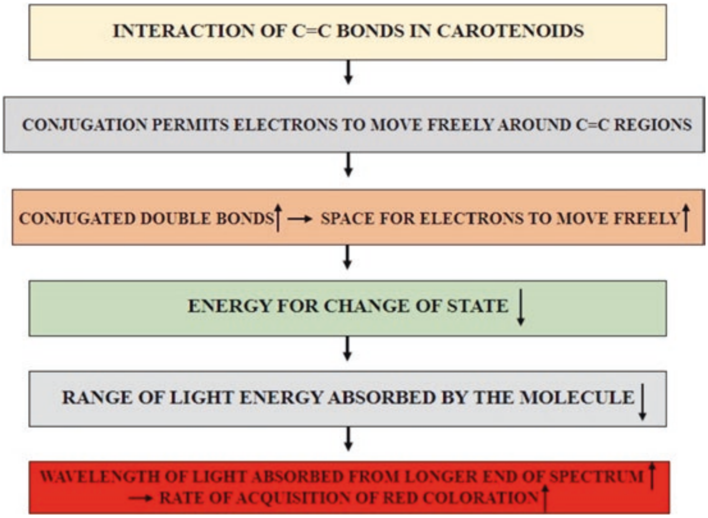

Fig. 6.4 Mechanism of color formation

cule to move freely around the C=C regions [8]. With the increase in the number of conjugated double bonds, electrons associated with the conjugation system experience more space to move freely and the energy requirement for the change of state decreases. With the resultant decrease in the range of light energy absorbed by the molecule, higher the wavelength of light absorbed from the longer end of the visible spectrum, higher is the rate of acquisition of red appearance by the compounds. On the other hand, the length of the carotenoids plays an essential role in the coloration of plants. The length of the polyene tail defines the chromophore, thus determining the wavelengths of light to be absorbed by the plant. Wavelengths, not absorbed, are reflected as the plant's color [9]. Therefore, different species of plants containing carotenoids of varying tail lengths allowing the absorption and reflection of different colors. Carotenoid based coloration is more complicated in animals (Fig. 6.4). For example, the bright feather colorations of birds can be due to pigments or by well-organized tissue. Reports reveal that yellow plumage color of American goldfinches is generated by both reflection of light from the white structural tissue and absorption of light by the carotenoids. Thus, structural components of feathers are linked with the carotenoid display of yellow color [10]. Several prominent carotenoids found on different classes of organisms are listed on Table 6.1.

6.3 Carotenoid Coloration in Microbes

Microorganisms are massively used in industries to produce various enzymes [11], organic acids [12], insecticides [13], antibiotics, recombinant proteins [14], bread and other beverages [15] along with pigments and natural colors. The large-scale

Table 6.1 **Major carotenoids** found in different classes of organism

Organism	Possible carotenoids found
Green algae	β-Carotene, lutein, astaxanthin, canthaxanthin, zeaxanthin
Micro algae	Fucoxanthin
Paracoccus	Astaxanthin, zeaxanthin
Fungi	β-Carotene, astaxanthin, neurosporaxanthin, etc.
Yeast	β-Carotene, torulene, torularhodin etc.
Plants	β-Carotene, xanthophyll, lycopene, capsanthin, lutein, violaxanthin, etc.
Sea sponge	Bastaxanthin
Corals	Diadinoxanthin, pyrrhoxanthin, peridinin, etc.
Sea anemones	Actinioerythrin, 2-nor astaxanthin
Arthropods	Canthaxanthin, astaxanthin, crustacyanin, 3-hydroxy echinenone, etc.
Molluscs	β-Carotene, astaxanthin, alloxanthin, fucoxanthin, pectenolone, 7,8-didehydro astaxanthin, etc.
Echinoderms	β-Carotene, 4-keto depoxy neoxanthin, etc.
Tunicates	Fucoxanthin, diatoxanthin
Fishes	Tunaxanthin, taraxanthin, eichinenone, β-carotene, lutein
Birds	β-Carotene, xanthophyll, etc.
Mammals	β-Carotene, lutein, astaxanthin, etc.

production of pigments from microorganisms is developing very fast due to easy fermentation, low cost production from agro waste products and relatively easy isolation process. Several critical factors are considered during pigment formation like optimum temperature, pH, carbon source, nitrogen source, fermentation time, etc [16].

6.3.1 Algae

Characteristically, algal carotenoid is present in a complex form inside chloroplasts. The accumulation of pigments is observed under different stress conditions like high light intensity, high temperature, nutrient starvation, etc. [17]. Among the chlorophyte algae (green algae), *Dunaliella bardawil* is the most notable green algae, acting as a source of a massive amount of β-carotene [18]. *Dunaliella salina* is rich in 9-cis-β-carotene and lutein [19]. Another green algae, *Haematococcus pluvialis,* is rich in astaxanthin [20], canthaxanthin and lutein [21]. *Scenedesmus* sp. contains lutein and β-carotene [22]. *Chlamydomonas reinhardti,* another type of green algae, produce zeaxanthin under stress conditions [23]. Other microalgae like *Isochrysis galbana (*haptophyte)*, Mallomonas* sp., *Phaeodactylum tricornutum* (o*chrophyta) and Odontella aurita* (bacillariophyte) *contain*s fucoxanthin and diatoxanthin [24]. Rhodophytes or red algae contain α- and β-carotene derivatives [25].

6.3.2 Bacteria

Pigments in bacteria are mainly found as secondary metabolites [26]. Different species of *Brevundimonas* [27] and *Paracoccus* [28] are rich in astaxanthin. Zeaxanthin can be found on *Paracoccus zeaxanthinifaciens* [29], *Erwinia herbicola* [30] and *Synechocystis* sp. [31]. Another carotenoid Canthaxanthin is mostly found on *Bradyrhizobium* sp. which is used in salmon and poultry feed as a colorant [32]. Apart from the native carotenoid producing bacterial strains, several other E. coli strains are present with modified metabolic pathways to produce a more considerable amount of β-carotene and other carotenoids [33].

6.3.3 Fungus

The main three carotenoids, namely β-carotene, astaxanthin and neurosporaxanthin are abundant among fungal families. β-carotene can be found in numerous species of the order Mucorales like *Blakesleatri spora* [34] and *Phycomyces blakesleeanus* [35]. Different types of basidiomycetes like *Rhodosporidium* sp., *Sporidiobolus pararoseus, Sclerotinia sclerotiorum*, *Ustilagomaydis* sp. were found to exhibit β-carotene biosynthetic pathways [36]. Several ascomycetes like *Penicillium* sp., *Aschersonia aleyroides* and *Aspergillus giganteus* also contain β-carotene [36]. Astaxanthin can be found on *X. dendrorhous* which is used in the industrial purpose for large scale pigment production. Neurosporaxanthin was first discovered in *Neurospora crassa* but can also be found on *Fusarium fujikuroi* [36] and *Verticillium agaricinum* [37]. Different types of yeast species are of industrial importance for carotenoid production. The yeast *Phaffia rhodozyma* sp. is used as an industrial source of astaxanthin [38]. Carotenoid profiling of *Rhodotorula* sp. has shown the presence of β-carotene, torulene and torularhodin. The latter two carotenoids are also synthesized by *Sporidiobolus*, *Sporobolomyces* and *Rhodosporidium* sp. [39].

6.4 Carotenoid Coloration in Plants

In plants, the carotenoids are synthesized in both chromoplasts and chloroplasts. They impart color to photosynthetic tissues and also to fruits, flowers, storage organs, etc. [40]. Carotenoids found in mature chloroplasts mainly take part in photosynthesis [41, 42] whereas carotenoids found in chromoplasts primarily function as a coloring agent and lead to the attraction of some pollinators, some seed-distributing herbivores, etc. [43]. Chromoplast derived carotenoids are a rich source of antioxidants and pigments.

6.4.1 Leaves

Carotenoids are found in leaves and their occurrence varies according to the change of seasons. Tree leaves undergo senescence in autumn, which causes their color change from green to yellow.

During spring, some xanthophylls are found to be absent in tree leaves, whereas they come back during autumn. Earlier it was thought that some oxidation process in the plastid caused carotenoids formation, resulting in the phenomena. Later, detailed studies reported that the xanthophyll-carotene ratio increases during autumn, which causes a color change in leaves [44, 45].

6.4.2 Flowers

The flower coloration is solely dependent on the synthesis of carotenoids in chromoplast. Thus, carotenoids have a substantial impact on the flower industry mainly for aesthetic reasons as well as in the areas of traditional medicine because some carotenoids extracted from dried flowers are used as a food colorant and flavoring agents. The diversity and quantity of carotenoids differ widely in plants even within the same species. Flowers with white petals contain very few carotenoid molecules. In contrast, flowers with bright and dark colored petals consist up to 20-fold of the carotenoid content of leaves [46].

Marigold flowers are the major source of lutein, which is used commercially in flower and food industries. Lutein is responsible for the orange to yellow hues of the marigold petals [47]. Bright yellow colored flowers of Gentian *(Gentiana lutea)* are abundant in β-carotene and xanthophylls [48]. Development of such flowers shows upregulation of carotenoid synthesis as well as a shift in carotenoid profile from lutein to neoxanthin, zeaxanthin, and antheraxanthin [48, 49]. Another spectacular flower called Morning glory of genus *Ipomoea* is renowned for its diverse flower colors. Many species are rich in carotenoid due to their bright orange and yellow petals.

Interestingly, the Japanese morning glory (*Ipomoea nil*) lacks carotenoid accumulation since its petals are white. In a particular study, an attempt was made to determine the accumulation of carotenoids by comparing Japanese morning glory to two yellow-flowered species of Morning glory [50]. It was found that during early development of the flowers, all the species accumulated β-carotene, lutein and violaxanthin in the petals, the same carotenoids present in the leaves. During later development, however, the yellow flowers switched to chromoplast-derived carotenoid accumulation including zeaxanthin, β-cryptoxanthin and β-carotene [46, 51]. The white flowers did not accumulate any chromoplast derived carotenoid even in later developmental stages [50].

The Asiatic hybrid lily (*Lilium* sp.) is another commercially valuable ornamental plant which has flowers ranging from red, yellow to pink. Carotenoids impart the red, orange and yellow colorations whereas the pink color is due to anthocyanin. Most carotenoids found in yellow petals are violaxanthin, antheraxanthin, cis-lutein, etc. An unusual accumulation of capsanthin is also found in red petals [52, 53].

6.4.3 Fruits and Vegetables

There are mainly three types of carotenoids found in fruits and vegetables: β-carotene, xanthophyll and lycopene. They are responsible for the orange-yellow and red pigmentation in various vegetables and fruits [54]. Orange-yellow carotenoids include carotene and xanthophyll occurring in fruits and vegetables. β-carotene is orange in color while α-carotene is yellow [55], and the former being more abundant in fruits and vegetables than the latter. β-carotene is found less in yellow colored fruits and vegetables and more in quantity in orange colored ones. Xanthophylls like lutein, which appear yellow and zeaxanthin which appears orange-yellow, are mainly found in many fruits and vegetables with yellow-orange color [56].

Vegetables like carrot, tomato and sweet potato are good sources of β-carotene. Along with that, many green vegetables are also reported to have a decent amount of β-carotene [57]. Tomato *(Solanum lycopersicum)* is the widely researched plant model system for the study of carotenoid accumulation [58]. Green leafy vegetables from the genera of *Moringa, Brassica, Coriandrum, Solanum*, etc. are reported to contain lutein and zeaxanthin [59, 60].

Lycopene is abundant in fruits like pink grapefruit, watermelon, papaya, etc. [61]. The red color of ripe fruits shows the accumulation of lycopene. The red coloration is believed to be originated from lycopene, though contributions from anthocyanins [62, 63] and xanthophylls [64–66] are also reported. Similarly, orange colored fruits like mango, papaya, pink guava, watermelon, etc. are good sources of β-carotene. Commercially important kiwi fruit of genus *Actinidia* is an important model for studying carotenoid accumulation in fruits. This fruit comes in a range of colors including green, red, purple, orange and yellow. In ripe kiwi fruit, *A. deliciosa*, green flesh is a distinguishing feature and it is due to the retention of chlorophyll during ripening. The carotenoids occurring in this species are those related to the chlorophyll containing tissues [67] including 9′-cis-neoxanthin, lutein, violaxanthin and β-carotene. *A. chinensis* Cv. Hort16A is a recently commercialized selection ZESPRI Gold Kiwi fruit. It has bright yellow flesh and also the presence of green color with varying levels of brightness is seen. It has been found that its yellow color is due to the presence of lutein and violaxanthin, but there is an absence of chlorophyll-b which is present in *A. deliciosa*. Also, the bright orange color of *A. macrosperma* is due to the presence of high level of lutein and β-carotene. Concentrations of β-carotene are high at about ~20 mg/100 g of fresh fruit weight that provides a total carotenoid concentration higher than that of most other yellow colored fruits [68]. Another fruit of this genus, *A. polygama,* is light orange to yellow in color. Here, the concentration of xanthophylls was found to be higher than in other species. Moreover, it showed a high degree of β-carotene and zeaxanthin concentration [69].

6.5 Carotenoid Coloration in Animals

Carotenoids in animals are not synthesized *de novo*; instead, they are taken from food or modified metabolically [70, 71]. Various kinds of carotenoids with structural diversity are found in marine animals, birds, etc.

6.5.1 Poriferans

Phylum Porifera consists of a diverse group of sponges with brilliant colors because of the presence of carotenoids. Mostly aryl carotenoids are found in the sponges such as renierapurpurin, isorenieratene and renieratene [72]. Aryl carotenoids are also found in green sulfur bacteria other than sea sponges. So, it has been assumed that the aryl carotenoids in sponges originate from symbiotic bacteria. Bastaxanthins, a class of acetylenic carotenoids have been isolated from sea sponge *Ianthella basta* [73], which are thought to be metabolites of fucoxanthin, a carotenoid found in microalgae.

6.5.2 Coelenterates

In jellyfishes, the most dominant form of carotenoid found is astaxanthin, which comes from the zooplanktons that they feed on. Other forms like the diadinoxanthin, pyrrhoxanthin and peridinin in some corals [74] come from the symbiotic dinoflagellates. Sea anemones are another group of brightly colored coelenterates that possess carotenoids. For example, *Actinia equina* and *Tealia feline* [73] possess two unique carotenoids: 2-nor astaxanthin and actinioerythrin, respectively.

6.5.3 Arthropods

The crustaceans have carapace with bright color due to the presence of carotenoids, mostly astaxanthin. They feed on algae from where they acquire β-carotene and metabolize it to astaxanthin via other intermediates, namely echinenone, 3-hydroxyechinenone, canthaxanthin and adonirubin [70]. This astaxanthin exists in carapace in the form of carotenoproteins like crustacyanin and results in yellow, purple and blue color formation.

Accumulation of carotenoids by insects to acquire color in their body, eggs and even galls are seen widely. The green color in some insects and the purple-blue color in other arthropods are due to the presence of carotenoids. This alteration in color is due to the reaction with specific carotene protein or some of the chlorophyll degradation products like pterobilin [75]. Blue pigments like pterobilin combine with carotenoids to impart cryptic coloration in insects [76]. Studies have shown that carotenoids are responsible for the various intraspecific color morphs in aphids. Torulene imparts red coloration to the color morphs of aphid [77]. The bright red color of elytra in several ladybirds is also found to be derived from carotenoids [78]. Carotenoids are extracted from hair tuft and body of several lepidopterans [79] including the monarch butterflies where it confers the dramatic yellow stripes of caterpillar [80]. The green color of butterfly larva and stick insect (*Dixippus morosus*) is due to the combination of carotenes with other blue pigments [81]. Carotenoids, along with chlorophyll degraded products, impart green color to many lepidopteran larvae.

6.5.4 Molluscans

Chitons are molluscan herbivores, which mainly feed on algae. Carotenoids found in chitons are zeaxanthin, fucoxanthin, lutein and some of their metabolites [82]. Carotenoids found in sea snails like *Haliotis discus* and *Turbo cornutus* are zeaxanthin, fucoxanthin, lutein, α-carotene and β-carotene [74]. Apart from the herbivores, there are carnivores like the sea snails which feed on corals and zooplankton. The carotenoids found in them are mostly dependent on their diet as in the case of *Charoniasauliae,* which feeds on starfish, has been found to have 7,8,7′,8′-tetradehydroastaxanthin, 7,8-didehydroastaxanthin and astaxanthin which are some of the specific carotenoids of starfish. Diadinoxanthin and peridinin present in corals are also found in the sea snails (*Drupella fragum)* which prey upon these corals [74]. In spindle shells (*Fushinus perplexus)*, (3*S*)-adonirubin and (3*S*,3′*S*)-astaxanthin are the major carotenoids [83]. Sea hares and sea slugs are herbivores who feed on red and brown algae. Apocarotenoids are found in sea hares and sea slugs [75]. In *Aplysia kurodai*, apocarotenoid derived from zeaxanthin, lutein and β-carotene are found [84]. Bivalves modify the carotenoids they accumulate from dietary microalgae. Major carotenoids reported in bivalves are the metabolites of diadinoxanthin, diatoxanthin, alloxanthin and fucoxanthin [70]. Oxidative metabolites of alloxanthin and diatoxanthin like 4-hydroxyalloxanthin, 4-ketoalloxanthin, pectenolone and pectenol are widely distributed among ark shells and scallops [70, 71]. Bright red and orange colors are found to be present in some edible clams. Major carotenoids found in *Meretrix petechialis*, *Ruditapes philippinarum* and *Mactra chinensis* are mostly fucoxanthinol 3-ester and fucoxanthin [85]. In cuttlefish and octopus, major carotenoids found are astaxanthin and its esters.

6.5.5 Echinodermatans

Echinenone, an oxidative metabolite of β-carotene, is found in the gonads of sea urchin [70]. Starfishes mainly feeding on small crustaceans and bivalves have been reported to acquire astaxanthin, 7,8,7′,8′-didehydroastaxanthin and 7,8-didehydroactaxanthin. *Acanthaster planci* also called the crown-of-thorns starfish is a nocturnal sea star which preys on coral polyps. Four new carotenoids, namely 3′- epigobiusxanthin, 7,8-dihydrodiadinoxanthin, 4-keto-4′-hydroxydiatoxanthin and 4-ketodepoxyneoxanthin are found in *A. planci* as the minor components and 7,8-didehydroastaxanthin, peridininol and astaxanthin as significant components [86]. Ophioxanthin is reported in *Ophioderma longicaudum*, the brittle star. Astaxanthin and canthaxanthin are found in the gonads of sea cucumber as the major carotenoids [87]. Lutein, zeaxanthin and astaxanthin have been isolated from spiny sea star *Marthasterias glacialis* [88].

6.5.6 Protochordates (Tunicates)

Being filter feeders, phytoplankton and diatoms, are the main sources of food of tunicates. Carotenoids originating from diatoms, like the metabolites of fucoxanthin, alloxanthin and diatoxanthin are mostly found in the tunicates [70].

6.5.7 Pisces

Predominant carotenoids found in fishes are tunaxanthin (yellow), α-β-dordexanthin (yellow), β-carotene (orange), lutein (greenish yellow), canthaxanthin (orange-red), zeaxanthin (yellow-orange), taraxanthin (yellow), eichinenone (red) and astaxanthin (red). These carotenoids are the metabolites of other carotenoids which fishes accumulate in their body through diet. Astaxanthin is the primary carotenoid found in ornamental as well as exotic fishes which causes their red and pink coloration. Accumulation of carotenoids mainly occurs in the gonads and integuments of fishes. Interestingly, salmonids accumulate astaxanthin in muscles. Salmonidae and Perciformes fishes cannot synthesize astaxanthin. Hence, the astaxanthin present in their body comes solely from the dietary zooplanktons. Perciformes fishes are found to possess tunaxanthin which imparts the bright yellow color to the skin and fins of these marine fishes [70, 89]. Cyprinid fish oxidatively metabolize zeaxanthin and synthesize 3S,3’S-astaxanthin. Micropteroxanthins, some unique apocarotenoids, are found in the integuments of *Micropterus salmoides* [90].

6.5.8 Birds

Carotenoids impart the red, yellow and orange coloration to the plumage in the majority of birds [91, 92]. As birds cannot synthesize carotenoids *de novo*, they modify dietary carotenoids biochemically [92]. The main dietary carotenoids for most of the birds are β-carotene, zeaxanthin & β-cryptoxanthin and these are converted biochemically to red ketocarotenoids and yellow canary xanthophylls which get deposited in the feathers and some bare parts [92]. It has been found that wild type canaries deposit xanthophylls in the feathers that impart the yellow coloration unique to this species [93].

6.5.9 Mammals

Marine mammals like dolphins are reported to have β-carotene and lutein [94]. Whales accumulate astaxanthin as a result of their preferred feeding habit on krill, which are small crustaceans carrying this carotenoid type in their body.

6.6 Carotenoids as Food Colorants

The range of colors of carotenoids varies from yellow to dark red. β-carotene is the most widespread carotenoid which occurs naturally in many foods like egg yolk, fish, milk, butter, spinach, tomato, corn, oranges, pineapples, mangoes, etc. β-apo-8′ carotenal (apo-carotenal) is found in citrus fruits, spinach, marigold, grass, other green plants and animal tissues. Canthaxanthin (4,4′-diketo-β-carotene) is another carotenoid which is distributed in algae, crustaceans, sea trout, edible mushrooms, etc. Therefore, it is relevant that carotenoids are used as food coloring agents artificially as well [95, 96].

The β-carotene is used in several forms to color different food items. 30% β-carotene liquid suspension is used for coloring oil and fat products, cheese, margarine, frozen egg yolk, winter butter, etc. 24% β-carotene semisolid suspension is used in margarine and other fat based products. 22% β-carotene HSR liquid suspension is used for coloring cooking oil (heat stressed) and in fat based foods where the stability of carotene is required in a greater way. 3.6% β-carotene liquid emulsion is used for colouring fruit juice blends especially orange color drinks. Dry 10% β-carotene beadlets are used to color water-based foods, beverages and reconstituting dry products in warm water. Dry 2.4% β-carotene beadlets is used for coloration of beverages, reconstitution of water based liquid and dry foods in water [95, 96].

Similarly, apo-carotenoid is utilised in many forms for food coloration. Apo-carotenal is a good replacement for oleoresin of paprika in many dressing products. It not only improves the color stability but also offers a uniform composition as well as colour. French dressings are also coloured readily with apo-carotenal. 20% Apo-carotenal liquid suspension is used in fat and oil product coloration, cheese and salad dressing. 2% carotenal solution is also used for salad dressing and coloring some fat-based products. Moreover, it is also used for plating dry spices and other breading mixers [95, 96].

Canthaxanthin is also used as a food colorant. Roxanthin Red 10® dry canthaxanthin beadlets is used for the coloration of tomato products, meat products, dry or water based products which are reconstituted in warm water. 10% Canthaxanthin SD is also used for colouring water based products, tomato products and any other products for reconstitution in water [95, 96].

6.7 Carotenoids as Dyes

One of the most critical methods for the isolation of lipophilic pigments (e.g., lutein & zeaxanthin) from crude plant extracts involves high-speed counter-current chromatography (HSCCC). However, the pigments neoxanthin, violaxanthin, β-carotene, chlorophylls a and b can also be isolated by this process [97]. Another mode of extraction of carotenoids is by using magnetic stirring, which is an alternative to ultrasound extraction techniques [98].

Owing to their toxicity, nowadays, natural pigments are being explored as potential textile dyes. Fungi are significant sources of carotenoids. In a recent study, a fungal strain *Talaromyces verruculosus* from spoiled mango and capable of producing pigments suitable for textile dyeing, was isolated. The extracted pigment was applied to cotton fabric following a standard dyeing procedure and it exhibited adequate color yield. However, the exact nature and structure of the extracted pigment are yet to be investigated [99].

In another study, carotenoids extracted from orange peels were investigated of their potency as textile dyes. Effect of combination of different solvents like acetone, ethanol & hexane and their mixtures on the carotenoid yield from the orange peel wastes was studied. It was observed that acetone-hexane-ethanol (55–45-4%) mixture was able to extract more carotenoids than other mixtures. 3% alum of the net weight fraction of cotton and copper sulfate were used as mordants. Carotenoids dye isolated from orange peels were used as dyes for cotton fabrics with potent fastness properties [100].

6.8 Carotenoids in Industry

Many industrial sectors like food, textile and cosmetics are leaning towards biocolorants from synthetic artificial colors as they are more stable, less toxic and cheaper than the synthetic ones [101]. Pigments with microbial origin are gradually gaining special interest over synthetic dyes due to their higher yield, easy downstream processing and higher shelf life [102]. The current market share of carotenoids is not clearly known, but several market research reports have shown a clear increasing trend of carotenoid market growth. BBC research has stated that the global carotenoid market had reached $1.5 billion in 2017 and it is expected to touch $2.0 billion by 2022 (www.bccresearch.com). Mordor Intelligence (www.mordorintelligence.com) has predicted the total global feed carotenoid market to cross $2100 million in 2020 in their report 'Feed Carotenoids Market - By Type, Animal Type and Geography-Trends and Forecasts (2017–2022). 60% of the total market share of carotenoids are made up of β-carotene, astaxanthin and lutein [103]. According to Grand View Research (www.grandviewresearch.com), the leading industrial source of β-carotene is from algae and fruit-vegetable. They have also presented a possible β-carotene market application showing dietary supplements and food & beverages as the prime consumption area.

6.9 Conclusion

Carotenoids act as important coloring agents. They are found to impart color in different types of microbes, plants and animals. The mechanism of color generation is dependent on the structure of different types of carotenoids. They impart colors in the range of yellow to red to orange, i.e., within a wavelength range of 440 to 500 nanometers. Due to growing health consequences, the use of nature identical colors is increasing day by day. The use of microbial pigments is one of the major developing industries in the food sector. The industrial production of natural pigments is relatively low to compensate for the vast number of synthetic dyes that are being used. So, it is necessary to discover novel and new natural pigments. For large scale productions optimising several factors like metabolic engineering, biotechnological manipulations and strain specific fermentation can make better result in quality, quantity and cheap production of the pigments. Nano-pigments could be a solution for carotenoids with low solubility or stability, increasing its shelf life.

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