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## A review on value-addition to agricultural wastes by extraction of carotenoids with microbial pectinases

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### Abstract

Pectinases are biosynthesized by plants, bacteria, algae, and fungi; animals, on the other hand, must consume them from their food. Based on their structural components, carotenoids are divided into two main classes: carotenes, comprised via carbon and hydrogen (for example  $\beta$ -carotene,  $\alpha$ -carotene, and lycopene), and xanthophylls, comprised via carbon, hydrogen, and moreover oxygen (for instance lutein,  $\beta$ -cryptoxanthin, zeaxanthin, astaxanthin and fucoxanthin). Pro-vitamin A, antioxidants, cancer prevention, obesity prevention, and anabolic effects on bone components are just a few of the positive effects that carotenoids have on human health. Carotenoids are currently being sold as nutrient supplements, feed additives, animal feed supplements, natural food colorants, and nutraceuticals for cosmetic and pharmaceutical use. By chemical synthesis, fermentation, or isolation from the few abundant natural sources, these compounds can be produced commercially. Additionally, commercial production of carotenoids from microorganisms competes primarily with chemical synthesis-based synthetic production. However, chemical synthesis is used to produce most commercially available carotenoids, such as beta-carotene, astaxanthin, and canthaxanthin.

**Key Words:** carotenoids, pectinases, fermentation, agro wastes.

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### Introduction

The use of synthetic colorants is decreasing, while the demand for natural colorants is increasing as a result of consumer concerns regarding the safety of food and stringent government regulations [1-4]. Carotenoids are one of the most significant normal food colorants and have drawn in by numerous analysts, due to their economically positive properties, like their normal beginning, wide conveyance, underlying variety, vital organic capability, invalid poisonousness furthermore, high adaptability [5-8]. Carotenoids give both lipo- and hydro-dissolvable colorants and have provitamin A movement. There are over 700 natural carotenoids that have been identified, and the number keeps rising annually. Algae, yeast, fungi, and photosynthetic bacteria can biosynthesize these 40 carbon-atom compounds in plants [9-14]. Carotenoids are categorized as xanthophylls (such as -cryptoxanthin, lutein, zeaxanthin, and canthaxanthin) and carotenes (such as -carotene, -carotene, and lycopene). Carotenoids have been credited

with valuable consequences for human wellbeing - upgrade of the invulnerable reaction. They show cell reinforcement exercises, which might forestall degenerative infections, for example, cardiovascular, dermatological, renal, aspiratory infections, the oxidative harms that are intended for maturing peculiarities, waterfall and macular degeneration, poisonous liver harm, metabolic condition, sepsis, immune system issues, diabetes, and a few kinds of disease, particularly, prostate, and gastrointestinal system cancers [15-19]. Additionally, it was discovered that the antimutagenicity is primarily associated with xanthophylls (lutein, -cryptoxanthin) and hydrocarbon carotenoids fractions (-carotene, lycopene), and that their supplementation can increase CD4 counts in HIV-infected patients. In addition, studies conducted by Bureau and Bushway (1986) demonstrated that retinol and several carotenoids (-carotene, -cryptoxanthin, zeaxanthin, lutein, capsorubin, capsanthin, lycopene, and capsanthol) were involved in the cytoprotective injury of the gastric mucosa

[20-24]. These carotenoids include capsanthin, lutein, capsorubin, caps. Carotenoids give to the tissues a yellow, orange, or red tone. According to different scientists they are frequently used as colorants in food and added directly to many products like butter, popcorn, salad dressings, and beverages or indirectly through animal uptake, such as chicken and fish. Provitamin A activity or antioxidant properties of beta-carotene have been shown to prevent atherosclerosis and multiple sclerosis, according to research. Additionally, in late examinations,  $\beta$ -carotene has been proposed to diminish cell expansion and prompt apoptosis of different disease cell lines by hindering  $\text{Ca}^{2+}$ /calmodulin-subordinate protein kinase IV. " $\beta$ -carotene has been reported to have anticancer stem cell actions on neuroblastoma," according to a different study, "and this anticancer action is enhanced by retinoic acid receptor." Because it prevents the formation of reactive oxygen species and has anti-inflammatory properties, carotene shields the skin from the damaging effects of ultraviolet (UV) light [25-39].

The pharmaceutical, perfume, and cosmetic industries all make use of carotenoids. As a result of directives from the European Union (EU) and the Food and Drug Administration (FDA) favouring natural compounds over synthetic ones [40-43], natural compounds are increasingly being used in the food, cosmetic, and pharmaceutical industries. In recent times, it has been thought that some synthetic colorants are to blame for allergic and intolerance reactions. Due to their toxicity and potential to cause cancer, many nations have outlawed them. Cancer is linked to artificial food colouring, according to scientists [44-47]. Artificial food colouring has been linked to brain tumours, attention deficit hyperactivity disorder, and other disruptive behaviour, particularly in children, in other studies. The human body receives no nutritional or beneficial benefits from any of these additives. The worry is perfect to such an extent that the FDA has requested cautioning marks be put on food varieties containing counterfeit color and shading [44-49]. Therefore, the actual issue lies in the substitution of natural food colorants for synthetic ones.

### **Agriculture waste used for the pectinase production**

The agricultural sector is now growing rapidly. Investors have been more interested in the agricultural industry, estimated by the World Bank to be worth \$3.2 trillion, as a result of better economic growth and the rapid increase in population. The production of trash has expanded along with urbanisation and industrialization in the agriculture sector, causing several environmental concerns today. Around 5,000,000 metric tonnes of biomass are produced annually through agriculture [51-54]. If improperly handled and left unprocessed, this waste produced by agricultural industry acts as a breeding ground for harmful bacteria. Surprisingly, these agricultural waste products can be used as a starting point for the synthesis of valuable products or as a raw material for the creation of renewable energy. The EU's introduction of the terms "bio-economy" and "bio-refinery" The majority of waste produced in the agricultural sector is cellulosic in origin, which includes nutrients like pectin and proteins along with polysaccharides like hemi-cellulose, cellulose, and lignin. Industries need a consistent source of affordable raw materials to operate profitably and sustainably. Hence, the issue of trash accumulation will be resolved by successfully integrating waste streams from businesses with farm industries. Every year, almost one-third of the food that is produced for human consumption is wasted. Out of the 367 million tonnes of annual agricultural residue, 89 million tonnes are wasted each year, primarily from vegetables, fruits, tubers, and roots. Farmers use a portion of this for horticulture practises such diverse horticultural procedures, animal bedding, and animal fodder [55-59].

### **The following are examples of common agricultural wastes for carotenoids extraction:**

1. Wasted tomatoes: The strips, seeds, and mash of tomatoes are much of the time disposed of during handling; however, they contain elevated degrees of carotenoids like lycopene and  $\beta$ -carotene. These squanders can be handled to remove the carotenoids and utilized in different applications, for example, food shading and dietary enhancements [24-35].
2. Waste papaya: Carotenoids are found in abundance in the seeds and peels of papaya

fruit, as well as in the fruit itself. Carotenoids can be extracted from these wastes and used in a variety of products, including cosmetics, food colouring, and nutraceuticals [45,68,92].

3. Wasted carrots: Carotenoids like  $\beta$ -carotene and  $\alpha$ -carotene can be extracted from carrot tops, peels, and other waste products produced during processing. These carotenoids can be found in supplements and natural food colourings [35].

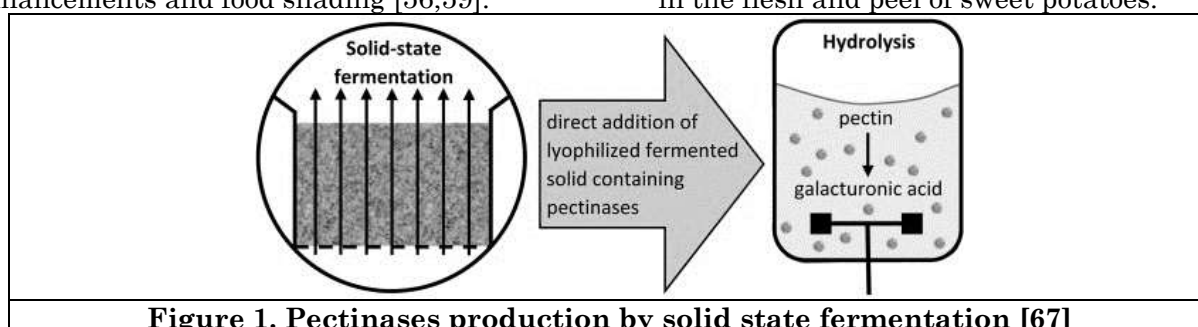
4. Waste orange peel: Carotenoids like zeaxanthin and cryptoxanthin can be found in abundance in orange peels. These squanders can be utilized for the extraction of carotenoids, which can then be utilized in different applications, for example, dietary enhancements and food shading [56,59].

5. Spinach waste: Spinach leaves contain elevated degrees of carotenoids like lutein and zeaxanthin, and the waste produced during handling can be utilized for the extraction of these shades. Dietary supplements commonly contain lutein and zeaxanthin, which are essential for healthy eyes [34].

6. Waste pumpkins: Carotenoids like  $\beta$ -carotene and lutein can be found in abundance in pumpkin seeds and pulp [67-72].

7. Waste mango: Carotenoids like lutein and  $\beta$ -carotene are abundant in mango seed kernels and peel [45-58].

8. Waste sweet potato: Carotenoids like  $\beta$ -carotene can be found in high concentrations in the flesh and peel of sweet potatoes.



**Figure 1. Pectinases production by solid state fermentation [67]**

With respect to pectinase production by SSF, although there have been numerous studies at laboratory scale [11], few attempts have been made to scale up this process. This is not surprising: since general strategies for scale-up of SSF processes are not yet available, processes must be scaled up on a case-by-case basis. In fact, studies of pectinase production involving more than 10 kg of dry substrate have only been carried out [12], [13] [14]. The last of these investigations was undertaken by our group and represents the largest scale for production of pectinases by SSF that has been used to date. In that work, *Aspergillus niger* was grown in a pilot-scale packed-bed bioreactor, using 30 kg (dry matter) of a substrate consisting of 90% wheat bran and 10% sugarcane bagasse. However, these fermentations suffered from problems with the formation of agglomerates of substrate particles and with shrinkage of the bed. These problems led to overheating of parts of the bed and a consequent lack of uniformity of pectinase levels within the bed at the end of the fermentation. With respect to the direct addition of fermented solids with enzyme activity in subsequent processes, this has only been done with lipases: several

groups have used "lipolytic fermented solids" to produce biodiesel through the esterification of fatty acids or the transesterification of triacylglycerols, using short chain alcohols [10], [15], [16], [17], [18], [19], [20], [21]. However, this strategy has not previously been used for the hydrolysis of pectin by pectinases. Many experiments have been done over the past ten years to use agricultural trash as a substrate for producing useful products. Nowadays, the industrial operations that use agricultural waste as a raw material are the manufacture of bio-ethanol and other biofuels. The pre-treatment of the waste is followed by saccharification and fermentation in the process. From industrial perspective, they find a range of applications biorefinery, brewing, baking, detergents, textile, paper, and pulp industry. pectinases are mostly produced from *Trichoderma reesei* or its improved strains for industrial use [60-62]. Apart from this, several microorganisms have been explored that produce cellulases such as *Clostridium thermocellum*, *Schizophyllum commune*, *Bacillus circulans*, *Melanocarpus* sp., *Proteus vulgaris*, *Aspergillus* sp., *Klebsiella pneumonia*, *Penicillium* sp., *Escherichia coli*, *Fusarium*

sp. and *Cellulomonas* sp. To produce pectinases using various microbial species, numerous agricultural residue varieties have been tried and tested. The effectiveness of apple and grape pomace as suitable substrates to produce pectinase has been extensively investigated in studies [54-64]. Researchers devised a novel strategy, in which they used wheat bran as a carbon source to produce pectinase and supplemented the fermentation media with Neurobion® tablets (a multivitamin) and polygalacturonic acid. It was reported that the maximum enzyme activity was 8050 U/g dry substrate. To produce pectinase from the filamentous fungus *Penicillium viridicatum* RFC3, a 1:1 mixture of orange bagasse and wheat bran was used as a medium. When the fermentation process was carried out in polypropylene packs, the highest enzyme activities were observed—0.7 and 8.33 U mL<sup>-1</sup> for endo- and exo-polygalacturonase, respectively, and 100 U mL<sup>-1</sup> for pect. As potential media additives for the production of pectinase, seedless sunflower heads and spent barley grain are lignocellulosic wastes from food processing [66-71].

A variety of wastes have also been used to make pectinases. Biz and others (2016) came up with a clever way to produce pectinases using sugar cane bagasse and citrus peel in solid-state fermentation mode without worrying about overheating. This was done with a packed bed reactor that was pilot-scale. Pectinase activity ranged from 34 to 41 U/mL throughout the bed. Because

sugarcane bagasse is highly porous, combining citrus peel and bagasse prevented bed shrinkage and agglomerate formation while maintaining temperature control. The fermentative microbe that was used was *Aspergillus oryzae* [72-29].

#### The principles and enzymes used in EAE

The plant material is pretreated with enzymes like protease, pectinase, pectinesterase, cellulase, hemicellulase, cellobiase, -amylase, and fructosyltransferase in order to hydrolyze the cell walls and release the phytochemicals that are bound to lipid and carbohydrate chains within the cell in EAE. For the purpose of extracting volatile compounds, hydrophilic and hydrophobic pigments, phenolic compounds, and other bioactive compounds from plant samples, this pretreatment is followed by solvent (water) extraction or pressurized hot water extraction [42-45]. The following elements are necessary for the EAE: the sort, measurement, and required state of the catalysts; a combination of the process's time and temperature; the chemical composition, particle size, and water content of the plant material; and the ratio of solvent to solid. Enzyme-assisted extraction offers several advantages, including the ability to scale up the process, a higher extraction yield, a higher quality of the extract, green extraction (since the water and enzymes used are of natural origin), and a reduced need for additional extract filtration and purification. Anthocyanins from saffron tepals were recovered using EAE by [51-54].

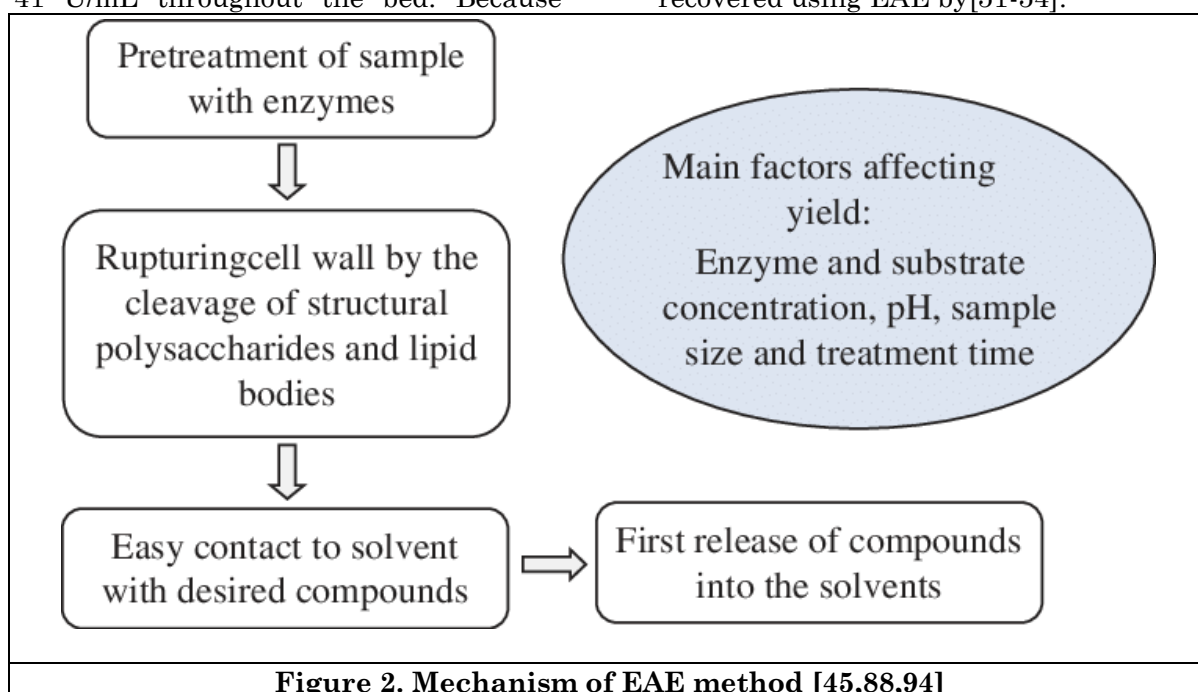


Figure 2. Mechanism of EAE method [45,88,94]

Anthocyanins were extracted at 40°C for 20–180 minutes with a mixture of enzymes (Pectinex) containing cellulase, pectinase, and hemicellulase at various concentrations (1%–10%). At an enzyme concentration of 5% and a treatment time of 60 minutes, the highest anthocyanins recovered by EAE were 6.7 mg/g dry basis, 40% higher than those obtained by solvent (ethanol) extraction. When compared to the anthocyanins produced by the conventional method, those produced by EAE underwent three times less degradation during storage [56-63]. Additionally, HPLC analysis revealed that EAE produced 80% more cyanidin-3-glucoside than the conventional method. Because enzymes release proteins from plant material, they destroy plant cells, making enzyme-assisted extraction more effective. Numerous studies have also been conducted on the extraction of bioactive compounds from various other plant sources [72-74]. Gunther et al. [28] extracted total phenols and lutein from the marigold flower using EAE. At 40°C for 120 minutes, the maximum yield of total phenols (84 mg/g) and lutein (5.6 mg/g) was achieved with a concentration of 1.4% (w/w) enzyme. EAE produced a yield that was 65% greater than the conventional method. Other extraction methods like SFE, MAE, and ionic liquids have also been used in conjunction with enzyme-assisted extraction. Pomegranate peel phenolic compounds were extracted using enzyme-assisted extraction in conjunction with SFE. Cellulase, pectinase, alkalase, protease, and viscozyme enzyme pretreatment of the pomegranate peel was followed by CO<sub>2</sub>-mediated SFE with ethanol as a solvent to obtain the bioactive compounds. A double harvest of phenolics, namely It was possible to obtain vanillic acid, syringic acid, and ferulic acid [75-82].

#### **Enzymes extraction process and advantage**

Solid State fermentation is widely used for the pectinase production from the Agro wastes. These biomolecules are mostly metabolites generated by agro wastes grown on solid support selected for this purpose. In this fermentation process, different solid substrates such as Wheat bran, Rice bran, coconut oil cake, vegetable waste, gram husk, orange peel, sugarcane bagasse etc were used with pure cultures [23-27]. In recent years there has been a renewed interest in solid-state fermentation (SSF) processes to

produce bioactive compounds. Enzymes production by SSF using bacterial spp. has been reported for many enzymes such as xylanase and amylase but reports on [37-39] pectinase production by SSF using bacterial spp. is lacking in the literature. SSF has been reported to be more advantageous than submerged (SmF) as it allows cheaper production of enzyme having better physicochemical properties than that produced by SmF. Pectinases comprises a heterogeneous group of [45] enzymes that catalyse the breakdown of pectin containing substrates. Pectic substances are characterized by long chains of galacturonic acid residues. On these residues are carboxyl groups, which are sometimes modified by the addition of methyl groups, forming methoxyl groups. Pectic enzymes act by breaking glycosidic bonds of the long carbon chains (polygalacturonase, pectin lyase and pectate lyase) and by splitting off methoxy groups (pectin esterase). In submerged fermentation, enzymes and other reactive compounds are submerged in a liquid such as alcohol, oil, or nutrient broth. Culture is sited in a small closed flask containing the rich nutrient broth with high volume of oxygen [82-88]. The in-situ production of enzymes results in production of bioactive molecule. Batch Fed fermentation method is used commonly which utilizes the sterilised nutrients under optimized conditions along with fungal endophytes which increase in density. The growth rate of fungal endophytes is maintained by the addition of nutrients, also reduces risk of overflow of metabolism. Fruits, vegetables, and the agro-industrial waste products from these are the most significant natural sources of carotenoids. A potential low-cost source of carotenoids, particularly carotene (beta-carotene), lycopene, -cryptoxanthin, violaxanthin, and lutein, is tomato skin, orange peel, and tangerine peel. The chemical and physical characteristics of the component you wish to extract from the plant material must be considered before creating a carotenoids extraction. The basis for most extractions is the solubility of the target compounds [88-92]. The physiologically active molecules were distributed throughout a wide range of materials and fluids, making it impossible to adopt a widely accessible extraction process that can be used globally as a standard procedure. The varied architectures of substances that are biologically active are to

blame for this. Even people in the same class have differences, performance thin layer chromatography (HPTLC), ultraviolet-visible (UV-Vis), infrared (IR) and Raman spectrometry have been employed as an analytical technique to identify and quantify low levels and various forms of carotenoids [90-92].

### Applications

Carotenoids, the basic ingredient in the pigments of plants that make yellow, orange, and red. They are found in all living things, including algae, bacteria, yeast, and higher plants and animals. More than 700 naturally occurring pigments make up this group of compounds that plants, algae, fungi, and bacteria biosynthesize *de novo* [1-9]. Most of them are found in higher plants, particularly in the leaves, flowers, and fruits of those plants. Carotenoids cannot be produced by animals, so they must be obtained them from the previously mentioned. Carotenoids, on the other hand, cannot be made by animals, so they are present in food sources like pink salmon flesh and the plumage of many birds. Be that as it may, they should ingest carotenoids from food and use them for use in physiological capabilities. According to Bonnie, carotenoids are widely used in food applications. According to Liu et al., carotenoids like lycopene and beta-carotene have a lot of scientific and commercial value). Carotenoids have traditionally been utilized in the nutraceutical, food, and feed industries. According to Lee and Schmidt, the recent discoveries of carotenoids' health-beneficial properties have sparked a great deal of interest in the production of structurally diverse carotenoids for pharmaceutical applications. Carotenoids are currently utilized commercially as natural food colorants, nutrient supplements, feed additives [11-19], animal feed supplements, and nutraceuticals for cosmetic and pharmaceutical purposes [20] Carotenoids are utilized industrially as colorants in foods and cosmetics, nutraceuticals, pharmaceuticals, and animal feed additives [24].

Carotenoids are primarily found in vegetables and fruits in the human diet. According to Rao, a typical human diet contains approximately 40. Even though carotenoids are found in a wide variety of common human foods, the primary sources come from deeply colored fruits, juices, and

vegetables [44-49]. Yellow-orange vegetables and fruits provide the majority of the -carotene and -carotene, orange fruits provide -cryptoxanthin, dark green vegetables provide lutein, and tomatoes and tomato products contain lycopene [68-71]. Average food sources and measures of carotenoids present. In many nations, the utilizing of food added substances (counting colorants) is administered by severe guideline. According to Mortensen (2006), the law specifies the colorants that can be used, their sources, their purity, which foods they can be added to, and how much color can be added to a particular food. The natural and nature-identical colorants are examples that are permitted in the EU and the United States (in this instance, colorants that are only permitted in fish or chicken feed for the purpose of pigmenting flesh and/or eggs are excluded). In food application, carotenoid specifically, lycopene is permitted in the EU as a food colorant and has as of late become permitted in the USA too. Tomatoes (*Lycopersicon esculentum*, which means wolf peach) are the only permitted source. According to Mortensen, in addition to lycopene, a tomato oleoresin also contains significant quantities of carotene, phytoene, and phytofluene [82-89]. According to Mortensen, this compound is rarely utilized as a colorant since it is a rather costly pigment and is extremely susceptible to oxidative degradation much more so than -carotene. Food colorants has forever been focussing of grumbles of the food business buyers, mostly due to the awful popularity of the underlying engineered shades that just have a corrective worth and were related with wellbeing harm. According to Johnson and Schroeder, only a small number of carotenoids (-carotene, lycopene, astaxanthin, canthaxanthin, capsanthin, lutein, annatto, -apo-8-carotenal, and -apo-8-carotenal-ester) can be commercially produced through chemical synthesis, fermentation, or isolation from the small number of abundant natural sources. Business creation of carotenoids from microorganisms contends primarily with engineered produce by substance systems [91-92]. Microbes are expected to accumulate carotenoid by effectively stimulating carotenoid biosynthesis. According to Bhosale and Bernstein, canthaxanthin and astaxanthin are also of commercial interest to the pharmaceutical and food industries and play a significant

role in aquaculture for salmonid and crustacean pigmentation.

### Conclusion

Carotenoids, which are the primary pigments found in plants and are yellow, orange, and red, are abundant in nature. It has long been known that carotenoids have some medicinal properties. They are frequently used in food and nutritional supplements. Chemical synthesis is currently only able to produce a small number of carotenoids for commercial use. Carotenoids made commercially from microorganisms compete primarily with those made synthetically through chemical synthesis now. However, chemical synthesis is used to produce many commercially available carotenoids, such as  $\beta$ -carotene, astaxanthin, and cantaxanthin. Additionally, strict regulations govern the application of carotenoid as food additives (including colorants) in most nations, including the United States and the European Union.

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