

FOOD PIGMENTS: NUTRACEUTICAL COMPOUNDS WITH MULTIFUNCTIONS

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ABSTRACT

Natural pigments are compounds responsible for the diverse colours observed in living organisms, ranging from red, orange, yellow, green, blue and purple to brown and black shades. In raw and unprocessed foods, these pigments enhanced visual appeal and strongly influenced consumer perception of freshness, quality and acceptability. Natural pigments are widely distributed in plants, animals and microorganisms like algae, fungi and yeasts. Besides contributing to colour, these compounds perform numerous essential biological functions. In nature, pigments often act as attractants or mating signals helping organisms communicate and reproduce successfully. In some species, coloration provided camouflage and protection against predators. Several pigments also played critical physiological and metabolic roles. For example, chlorophylls and carotenoids are directly involved in photosynthesis, energy generation and biosynthetic pathways. Other pigments function as antioxidants, protecting biomolecules from oxidative damage caused by reactive oxygen species. Certain compounds are involved in vision, while others protect skin and cellular membranes from harmful effects of sunlight and radiation. Because of their multifunctional roles, interest in natural pigments extends beyond their colouring properties. In recent years, increasing attention has been given to their nutraceutical and functional benefits, including antioxidant, anti-inflammatory and health-promoting effects. Alongside natural pigments, synthetic and microbial food colourants are also widely used in the food industry to improve appearance and consumer appeal. However, concerns regarding the safety and health impacts of some synthetic pigments have encouraged greater research and utilization of natural alternatives. Therefore, natural, synthetic and microbial food pigments continue to be important not only for enhancing food aesthetics but also for their potential effects on human health and wellbeing.

Keywords: Food pigments, microbial food pigments, nanoencapsulation, melanin, phycobiliproteins and carotenoids

Food pigments are naturally occurring bioactive compounds responsible for the diverse colours observed in plants, microorganisms and animal-derived foods. These compounds selectively absorb light within specific wavelengths of the visible spectrum, thereby imparting characteristic colours to food materials. Beyond their visual role, food pigments have emerged as multifunctional constituents with significant nutraceutical, technological and industrial applications (Fernández-López *et al.*, 2020). Colour is one of the primary quality attributes influencing consumer perception, sensory acceptance and marketability of foods, often serving as an indicator of freshness, safety, ripeness and nutritional quality. Apart from improving aesthetic appeal, food pigments also contribute to product identification, brand differentiation and consumer trust in industrial food systems (Luzardo-Ocampo *et al.*, 2021).

The application of food pigments in functional foods and nutraceutical products has expanded into pharmaceutical, cosmetic, biomedical, textile and intelligent packaging industries owing to their diverse functional and bioactive characteristics. Current

scientific and industrial research is largely focused on improving the stability, bioaccessibility and processing suitability of food pigments through advanced extraction, encapsulation and delivery approaches, thereby enhancing their industrial applications and commercial significance (Ocampo *et al.*, 2021).

Synthetic food pigments are widely preferred in the food industry due to their high colour stability, intense colouring capacity, uniformity, cost-effectiveness and resistance to variations in pH, temperature, light and processing conditions. These properties enable manufacturers to maintain consistent product appearance and extended shelf life in processed foods and beverages. However, Consumer preference for naturally coloured foods has increased profoundly in recent years due to growing awareness regarding the potential adverse effects associated with synthetic pigments and the increasing demand for the natural pigments (Novais *et al.*, 2022). Therefore, continuous research is being directed toward the identification of novel, stable and safe pigment sources, particularly naturally occurring pigments derived from plants. These pigments are

increasingly associated with healthy dietary practices and sustainable consumption patterns and their utilization has the potential to reduce or replace the dependence on synthetic colourants in food and industrial applications. Furthermore, natural pigments possess considerable industrial significance in pharmaceutical, cosmetic, textile, nutraceutical and packaging sectors. Recent advances in green extraction technologies, encapsulation and stabilization strategies have further enhanced the applicability, bioavailability and storage stability of these compounds in food systems and industrial formulations (Masyita *et al.*, 2025).

Classification of food pigments

Pigments are broadly classified according to their origin into natural and synthetic pigments. Natural pigments are synthesized by living organisms including plants, animals, fungi and microorganisms, whereas synthetic pigments are chemically produced under laboratory conditions. Both natural and synthetic pigments primarily consist of organic compounds.

Synthetic food pigments

Synthetic food pigments are chemically manufactured colourants produced through modification of precursor compounds using various industrial chemical processes. Unlike natural pigments, which are generally obtained from biological materials through extraction and purification techniques, synthetic pigments are synthesized under controlled laboratory conditions to achieve high purity, uniform quality and large-scale production. Certain food colourants are also semi-synthetic in nature, where naturally derived compounds undergo chemical modification to improve their colouring properties and stability. For example, indigo carmine is produced through sulphonation of indigo originally obtained from plants such as tropical indigo, woad or Chinese indigo (Konig, 2015).

Among synthetic food pigments, azo dyes constitute the largest and most widely utilized group. These compounds contain one or more azo ($-N=N-$) linkages connecting aromatic rings and are categorized into monoazo, diazo, triazo, tetraazo and polyazo dyes based on the number of azo groups present in their molecular structure. Azo dyes are

extensively used because they provide a broad spectrum of colours including yellow, red, blue and black, along with advantages such as high colouring intensity, low cost, tastelessness and greater stability against heat, light and chemical interactions compared to many natural pigments. Yellow and red azo dyes are particularly common in food processing industries due to their strong colouring efficiency and industrial demand (Lemoine *et al.*, 2020).

In addition to azo dyes, other important classes of synthetic food pigments include triarylmethane dyes such as Patent Blue, Brilliant Blue and Green S as well as related compounds including Quinoline Yellow, Erythrosine, Indigo Carmine and apo- β -8'-carotenal. The use of these pigments in foods is regulated under the European Commission Regulation (EC) No. 1333/2008, which governs the authorization and safety evaluation of food additives in Europe (European Commission, 2008). Although synthetic pigments are widely used because of their technological and economic advantages, certain colourants such as Tartrazine have raised concerns regarding possible adverse health effects leading to continuous scientific evaluation and regulatory scrutiny (Silva *et al.*, 2019).

Increasing concerns regarding the adverse effects of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have encouraged the replacement of synthetic additives with natural antioxidant pigments derived from food sources (Cunha *et al.*, 2018).

Presently authorised synthetic food colours in the European Union according to the European Commission Regulation (EC) No. 1333/2008 are E 102 (Tartrazine), E 104 (Quinoline Yellow), E 110 (Sunset Yellow FCF/Orange Yellow S), E 122 (Azorubine/ Carmoisine), E 123 (Amaranth), E 124 (Ponceau 4R/Cochineal Red A), E 127 (Erythrosine), E 129 (Allura Red AC), E 131 (Patent Blue V), E 133 (Brilliant Blue FCF), E 142 (Green S), E 151 (Brilliant Black BN/Black PN), E 154 (Brown FK), E 155 (Brown HT), E 160e (β -apo-8'-carotenal (C 30)) and E 180 (Litholrubine BK) (Konig, 2015).

Advantages

Synthetic food pigments are extensively used in the food industry because of their superior technological properties and economic advantages. These pigments exhibit high stability against heat,

light, pH fluctuations and processing conditions, thereby ensuring consistent colour retention throughout manufacturing and storage for maintaining product standardization and consumer appeal. Moreover, synthetic food pigments are commercially available in multiple formulations including powders, gels and liquid concentrates, allowing greater flexibility in different food applications (Maronpot *et al.*, 2020).

Limitations

Concerns regarding the safety and environmental impact of synthetic food pigments have increased considerably in recent years. Prolonged exposure to certain synthetic colourants has been associated with potential adverse health effects leading to continuous toxicological evaluation and strict regulatory scrutiny (Amchova *et al.*, 2024). Synthetic pigments generally lack the bioactive and nutraceutical properties associated with many naturally occurring pigments, limiting their functional contribution beyond colour enhancement. Furthermore, the production of synthetic pigments often involves petrochemical raw materials and chemical processing methods that may generate environmentally hazardous byproducts (Gosh, 2025).

Natural food pigments

The characteristic colours of foods are largely attributed to naturally occurring pigments such as carotenoids, anthocyanins, betalains and chlorophylls which may occur intrinsically in foods or be incorporated as food and feed additives (Rodriguez-Amaya, 2016).

Carotenoids

Carotenoids are a diverse class of pigments composed of eight isoprenoid units arranged in various structural configurations. Structurally, all carotenoids are considered derivatives of lycopene ($C_{40}H_{56}$) formed through reactions such as cyclization, hydrogenation, dehydrogenation, oxygen incorporation, methyl migration and modifications in carbon chain length. Carotenoids represent one of the most extensively distributed pigment groups in nature and are present in both photosynthetic and non-photosynthetic organisms including higher plants, algae, fungi, bacteria and several animal species. Carotenoids are responsible for the bright yellow, orange and red colours observed in fruits, vegetables,

flowers, crustaceans, insects, birds and aquatic organisms (Pachaiyappan *et al.*, 2020).

The number of identified carotenoids has increased considerably over the years from 300 carotenoids in 1972 to 600 by 1992 particularly due to discoveries from marine organisms. Naturally occurring carotenoids are estimated to be produced in 10x tons/year globally with fucoxanthin, lutein, violaxanthin and neoxanthin being among the dominant forms in nature. Although hundreds of carotenoids have been identified, only a limited number possess significant biological activity and are categorized into provitamin A and non-provitamin A carotenoids. Carotenoids capable of serving as vitamin A precursors must contain at least one unsubstituted β -ionone ring and a polyene chain of 11 carbon atoms (Mezzomo and Ferreira, 2016).

Apart from their colouring properties, carotenoids are widely recognized for their nutritional and health-promoting functions. They are extensively used in food fortification because of their provitamin A activity and antioxidant potential. Studies have demonstrated their beneficial role in immune enhancement, prevention of degenerative disorders, reduction of oxidative stress and management of obesity and lipid-related disorders. Chemically, carotenoids are hydrophobic and lipophilic compounds that are insoluble in water but soluble in organic solvents such as acetone, alcohol and chloroform which contribute to their broad application across food and biological systems (Liu *et al.*, 2022).

β -Carotene

β -Carotene is an orange-coloured carotenoid sensitive to heat, light and oxygen exposure. It is widely known for its provitamin A activity and antioxidant properties. β -carotene contributes to lowering the risk of cardiovascular diseases and cancers to a certain limit by inhibiting oxidation of low-density lipoprotein (LDL) cholesterol, a major factor involved in atherosclerosis development (Lopez *et al.*, 2023).

The red-orange coloured organic pigment mostly extracted from the β -carotene rich algae, *Dunaliella salina*. The production of β -carotene through fermentation of *Blakeslea trispora* produces a pigment equivalent to pigments produced through

a chemical process and is an accepted colouring agent. It is used in a variety of food items ranging from red to yellow in colour. The human body converts β -carotene to vitamin A via body tissues as opposed to liver thus avoiding a buildup of toxins in the liver. Vitamin A is essential for the human body as it assists the body's immune system and helps battle eye diseases like cataracts and night blindness, various skin ailments such as acne, signs of aging and certain forms of cancer (Singh and Sambhyal, 2022).

Lycopene

Lycopene is a non-oxygenated carotenoid characterized by a symmetrical structure containing 11 conjugated double bonds. Due to its highly unsaturated structure, lycopene exhibits exceptionally strong antioxidant activity and is considered one of the most effective singlet oxygen quenchers among carotenoids. It has exceptional antioxidant activity by donating electrons to neutralize singlet oxygen and other reactive oxidative species before they can damage cellular components and exceed that of β -carotene significantly (Schweiggert *et al.*, 2016)

Epidemiological and clinical studies have associated lycopene-rich diets with reduced risks of prostate, lung and ovarian cancers along with lower incidence of cardiovascular and chronic degenerative diseases. Interestingly, consumption of lycopene through tomato-based foods appears to provide greater protective effects than purified lycopene supplements possibly due to synergistic interactions with other bioactive compounds present in the food matrix (Patthawaro *et al.*, 2020).

Lutein and Zeaxanthin

Lutein and zeaxanthin are xanthophyll carotenoids primarily concentrated in the retina and lens of the human eye. Higher dietary intake of these pigments, particularly from foods such as spinach, broccoli and egg has been associated with reduced risk of age-related macular degeneration (40 %) and other vision-related disorders (20%) (Maoka, 2020)

Astaxanthin

Astaxanthin is a xanthophyll pigment naturally present in aquatic organisms including shrimp, lobster, crab and salmon. Since aquatic animals cannot synthesize astaxanthin independently, it is

commonly supplemented through feed in aquaculture and poultry industries to improve pigmentation and consumer acceptability. Astaxanthin possesses remarkably high antioxidant activity reportedly 10 times greater than β -carotene and 500 times over vitamin E. Additionally, it has demonstrated anti-inflammatory, antitumor and protective effects against oxidative damage, lipid peroxidation and ultraviolet radiation-induced cellular injury. Supplementation of astaxanthin in aquaculture feed has been shown to enhance colour intensity in salmonids and crustaceans (Oslan *et al.*, 2021).

Anthocyanins

Anthocyanins are water-soluble flavonoid pigments responsible for the red, purple and blue colours of many fruits, flowers and plant tissues. Chemically, they belong to flavonoids and are glycosylated derivatives of polyhydroxy or polymethoxy 2-phenylbenzopyrylium compounds (Bendokas *et al.*, 2019). Besides contributing to plant coloration and environmental adaptation, anthocyanins possess significant biological activities. Consumption of anthocyanin-rich fruits and vegetables has been associated with protective effects against cardiovascular diseases, obesity, neurological disorders and used cancer therapy. These health benefits are largely attributed to their strong antioxidant and free radical scavenging properties as well as their ability to intracellular antioxidant action through modulation of cellular antioxidant defence systems (Alappat and Alappat, 2020).

Anthocyanins are distributed among fruits and vegetables thoroughly; however, berries represent one of the richest dietary sources. Their concentration and composition vary according to cultivar, maturity stage, environmental conditions, temperature and light exposure (Juric *et al.*, 2020).

Betanin

Beetroot (*Beta vulgaris* L.) is the main source of betanin and recognized as a rich dietary source of nitrate compounds (NO_3^-) with potential cardiovascular benefits through nitric oxide generation. Betanins are heterocyclic compound with water-soluble nitrogen-containing pigments and are classified into betacyanins (betanin, prebetanin, isobetanin and neobetanin) which impart red-violet colouration and betaxanthins (Indica xanthin, vulgaxanthin I and II)

responsible for yellow-orange hues (Saponjac *et al.*, 2016). Betanin, chemically identified as betanidin 5-O- β -D-glucoside, is the predominant betacyanin in beetroot and is approved as a natural food colourant under the code E162 in Europe and by the United States Food and Drug Administration (Silva *et al.*, 2019).

Betanin has protect biological systems such as cell membranes and low-density lipoproteins from oxidative damage due to its strong antioxidant activity and potential therapeutic properties. Moreover, betanin exhibits anti-inflammatory, hepatoprotective and antiproliferative activities in various cellular models. Studies have shown that betanin can activate the Nrf2-mediated antioxidant response pathway thereby regulating the expression mRNA and protein levels of detoxifying and antioxidant enzymes including glutathione S-transferases, NAD(P)H quinone dehydrogenase 1 and heme oxygenase-1 which contribute to its hepatoprotective and anticarcinogenic effects (Paunovic *et al.*, 2020)

Chlorophyll

Chlorophyll is the principal green pigment present in plants and plays a fundamental role in photosynthesis. The term chlorophyll originates from the Greek words *chloros* meaning green and *phyllon* meaning leaf. Several forms of chlorophyll occur naturally, among which chlorophyll *a* is the primary photosynthetic pigment responsible for converting absorbed light energy into chemical energy during photosynthesis (Fernandez *et al.*, 2020) Chlorophyll *a* predominantly absorbs light in the blue region around 430 nm and in the red region near 660 nm while reflecting green wavelengths thereby imparting the characteristic green colour to plants. This pigment is present in most green plants as well as in blue-green and certain red algae. Other chlorophyll forms function mainly as accessory pigments by transferring absorbed light energy to chlorophyll *a*. Chlorophyll *b* occurs together with chlorophyll *a* in higher plants and green algae whereas chlorophyll *c* is found in brown algae and diatoms. Chlorophyll *d* is present in some species of red algae. All chlorophyll forms are lipophilic and exhibit solubility in oils and organic solvents (Chen and Roka, 2018).

Chlorophyll derivatives such as chlorophyllin are widely utilized as food additives and colouring agents. Chlorophyllin, commonly identified as natural green 3 and designated with the food additive code

E141 is frequently incorporated into confectionery products, desserts, sauces, condiments, cheese and soft drinks. The acceptable daily intake (ADI) established for copper complexes of chlorophylls and chlorophyllins is 15 mg/kg body weight/day (Ibrhimi *et al.*, 2023).

Chlorophylls possess significant biological and therapeutic properties. Chlorophyll and its derivatives exhibit antioxidant activity in dark conditions by scavenging free radicals, although under light exposure they may also display pro-oxidant behaviour. These pigments have been associated with numerous health-promoting effects including immune stimulation, detoxification, liver protection, reduction of body odour, maintenance of intestinal health and potential anticancer activity (Perez- Galvez *et al.*, 2020). Chlorophyllin has also been reported to inhibit the early formation of calcium oxalate kidney stones and may contribute to alleviation of anaemia, normalization of blood pressure and reduction of inflammatory conditions such as sinusitis and skin irritation (Martins *et al.*, 2023)

Advantages

Natural food pigments are biodegradable, environmentally sustainable, and less likely to produce toxic residues during processing or disposal. In addition to their coloring properties, many natural pigments possess important biological activities such as antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects, thereby contributing additional therapeutic and functional value to foods (Novais *et al.*, 2022). Pigments such as astaxanthin, α -carotene, lutein, fucoxanthin and phycocyanin contribute nutritional enrichment and may help protect against oxidative stress, inflammation, cardiovascular disorders and degenerative diseases. Consequently, these pigments are increasingly incorporated into functional foods, nutraceutical beverages, dietary supplements, dairy products and fortified formulations to improve both sensory quality and nutritional profile (Benucci *et al.*, 2022).

Limitations and Regulatory Concerns

Despite their advantages, natural pigments have several limitations compared with synthetic colorants. They offer a relatively limited color range and often lack the intensity and uniformity provided by synthetic dyes restricting their application in certain food systems. Natural pigments exhibit lower

physicochemical stability and are highly sensitive to factors such as pH, temperature, light, oxygen, and moisture. These conditions can cause degradation, fading, oxidation or undesirable colour changes during processing and storage thereby affecting product shelf life and consumer acceptability (Negi *et al.*, 2026).

Economic factors also limit their widespread industrial use. Natural pigments generally involve higher production costs due to lower extraction yields, expensive purification processes and the requirement for stabilization technologies. Plant-derived pigments are further influenced by seasonal availability and raw material variability (Singh *et al.*, 2023).

Although many natural pigments are considered relatively safe, comprehensive toxicological and safety evaluations remain necessary before approval for food applications, particularly for newly identified pigments and novel microbial sources. Regulatory authorities require extensive studies on toxicity, allergenicity, metabolic fate, acceptable daily intake and long-term health effects prior to commercialization. Regulatory approval systems for natural pigments also vary considerably across countries, creating challenges for global commercialization. The absence of harmonized international regulations slows industrial adoption and market expansion (Azmin *et al.*, 2022).

Microbial pigments

Microbial pigments are naturally occurring coloured secondary metabolites synthesized by bacteria, fungi, yeasts, cyanobacteria and microalgae. These pigments have attracted considerable scientific and industrial interest due to their biodegradability, eco-friendly nature, safety profile and multifunctional biological activities. Microbial pigments preferred over synthetic pigments as safer and sustainable alternatives (Shen *et al.*, 2018).

Microbial pigments are considered superior to many plant-derived pigments because microbial fermentation is independent of seasonal variations, requires less land and water, allows rapid biomass production and facilitates genetic manipulation for enhanced pigment yield. Many microbial pigments possess important therapeutic properties including

antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral, immunomodulatory and photoprotective activities, thereby enabling their utilization not only as food colorants but also as nutraceutical and pharmaceutical ingredients. The global market for natural pigments is expanding rapidly because consumers increasingly prefer naturally derived additives in foods and beverages. Microbial pigments such as carotenoids, riboflavin, Monascus pigments, phycocyanin, astaxanthin, violacein and prodigiosin are currently among the most promising alternatives to synthetic dyes in the food industry (Silva *et al.*, 2019; Sajjad *et al.*, 2020).

Carotenoids from microbial sources

Carotenoids are among the most extensively studied microbial pigments and constitute a large class of lipid-soluble tetraterpenoid compounds composed of eight isoprene units forming a C40 polyene backbone. Their characteristic yellow, orange and red colours arise from long chains of conjugated double bonds that absorb light in the visible spectrum. Microbial carotenoids include canthaxanthin, lycopene, lutein and zeaxanthin.

α -carotene (C40H56) is a hydrocarbon carotene consisting of two β -ionone rings connected through a conjugated polyene chain. Astaxanthin (C40H52O4) and canthaxanthin (C40H52O2) are oxygenated carotenoids (xanthophylls) containing hydroxyl and keto functional groups that enhance antioxidant activity. Lycopene is an acyclic carotenoid with an extended conjugated double-bond system responsible for its intense red coloration and strong singlet oxygen quenching ability. Microbial carotenoids are synthesized by organisms such as *Dunaliella salina*, *Blakeslea trispora*, *Haematococcus pluvialis*, *Rhodotorula* spp., *Paracoccus carotinifaciens* and *Agrobacterium aurantiacum* (Rana *et al.*, 2021).

Carotenoids are extensively utilized as natural colorants and nutraceutical ingredients in foods. α -carotene is widely incorporated into margarine, butter, cheese, beverages, bakery products, confectioneries, breakfast cereals and infant formulas to impart yellow-orange color while simultaneously providing vitamin A activity. Astaxanthin is predominantly used in aquaculture feed for salmon, shrimp and trout to improve flesh pigmentation. It is also incorporated

into dietary supplements and functional beverages because of its antioxidant properties. Lycopene is used in beverages, sauces, meat products and health supplements. Canthaxanthin is approved for use in poultry feed to enhance egg yolk and skin coloration (Sen *et al.*, 2019).

Phycobiliproteins

Phycobiliproteins are water-soluble fluorescent chromoproteins synthesized mainly by cyanobacteria and red algae. They include phycocyanin (blue), phycoerythrin (red) and allophycocyanin (blue-green) pigments. These pigments function as accessory photosynthetic pigments involved in light harvesting (Dufosse *et al.*, 2018).

Phycobiliproteins are composed of protein subunits covalently attached to open-chain tetrapyrrole chromophores called phycobilins. Phycocyanin contains phycocyanobilin as its chromophore and is mainly produced by *Arthrospira* (*Spirulina*) species, whereas phycoerythrin contains phycoerythrobilin and is synthesized by *Porphyridium cruentum* and related algae. These pigments are highly water-soluble, making them especially suitable for aqueous food systems (Cuellar-Bermudez *et al.*, 2015).

Phycocyanin exhibits strong antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation. It also demonstrates anti-inflammatory activity through inhibition of cyclooxygenase-2 (COX-2) and reduction of inflammatory mediators. Phycocyanin exerts hepatoprotective and nephroprotective effects by reducing oxidative stress and cellular apoptosis. Phycobiliproteins exhibit immunomodulatory activity, enhance macrophage function and improve antioxidant enzyme systems such as superoxide dismutase and catalase. Antitumor activity has also been reported through induction of apoptosis and inhibition of tumour cell proliferation (Sen *et al.*, 2019).

Phycocyanin is among the few commercially available natural blue food pigments widely used in confectionery products, chewing gums, ice creams, dairy beverages, smoothies, jellies, candies and sports drinks. Because synthetic blue dyes such as Brilliant Blue FCF have faced increasing scrutiny, phycocyanin provides a desirable natural alternative for clean-label formulations (Sen *et al.*, 2019).

Prodigiosin

Prodigiosin is a red linear tri pyrrole pigment produced mainly by *Serratia marcescens*, *Pseudoalteromonas* spp and *Streptomyces* spp. Prodigiosin consists of three pyrrole rings linked together to form a tripyrrolic structure. The methoxy-substituted bipyrrole and monopyrrole moieties are responsible for its deep red coloration and hydrophobic nature (Choi *et al.*, 2021).

Prodigiosin exhibits remarkable pharmacological properties. It possesses broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria and also demonstrates antifungal and antimalarial activities. Prodigiosin induces apoptosis through mitochondrial membrane depolarization, caspase activation, DNA fragmentation and disruption of intracellular pH homeostasis. It has shown cytotoxic effects against leukemia, breast cancer, colon cancer and lung cancer cell lines. Prodigiosin exhibits immunosuppressive activity through inhibition of T-cell proliferation and modulation of immune signaling pathways. The pigment also possesses antioxidant activity that contributes to cellular protection against oxidative stress. Prodigiosin has been explored as a natural red food colorant in dairy products such as yogurt and flavored milk as well as in soft drinks and confectioneries. However, commercial food applications remain limited because of incomplete toxicological evaluation and regulatory approval requirements (Minf *et al.*, 2022; Agarwal *et al.*, 2023).

Violacein

Violacein is a violet indole-derived pigment produced by *Chromobacterium violaceum*, *Janthinobacterium lividum*, *Pseudoalteromonas* spp and several aquatic bacteria. Violacein is chemically classified as an indolocarbazole derivative synthesized from two tryptophan molecules through oxidative condensation reactions. Its highly conjugated aromatic structure contributes to its intense purple coloration (Wu *et al.*, 2017).

Violacein possesses potent antibacterial activity against pathogenic microorganisms including *Staphylococcus aureus* and multidrug-resistant bacterial strains. It also demonstrates antifungal and antiparasitic effects. Violacein induces apoptosis through mitochondrial dysfunction, reactive oxygen

species generation and activation of programmed cell death pathways. It has demonstrated cytotoxicity against leukemia, melanoma, colon cancer and breast cancer cells (Atalah *et al.*, 2020). Its ability to interfere with quorum sensing also contributes to antimicrobial action and biofilm inhibition. Although violacein possesses attractive colouring properties, its use in foods is still under investigation due to limited toxicological data. Potential applications include nutraceutical beverages, specialty confectioneries and functional food formulations requiring natural purple pigments (Huang *et al.*, 2024).

Riboflavin

Riboflavin is a yellow fluorescent vitamin pigment produced by microorganisms such as *Ashbya gossypii*, *Candida guilliermondii* and *Bacillus subtilis*. Riboflavin consists of an isoalloxazine heterocyclic ring attached to a ribitol side chain. The conjugated ring structure imparts yellow fluorescence and light absorption properties. It is used in dairy items, breakfast cereals, baby foods, sauces, fruit and energy drinks. It had a special affinity for cereal-based products, but its use in these applications is somewhat limited due to its slight odour and naturally bitter taste. Riboflavin fermentation is classified into three categories as weak overproducers (100 mg L⁻¹ or less, e.g., *Clostridium acetobutylicum*), moderate overproducers (up to 600 mg L⁻¹, e.g., yeasts such as *Candida guilliermondii* or *Debaryomyces subglobosus*) and strong overproducers (over 1 g L⁻¹, e.g., the fungi *Eremothecium ashbyii* and *A. gossypii*) (Nigam and Luke, 2016)

Riboflavin functions as a precursor for FMN and FAD coenzymes involved in oxidative metabolism and mitochondrial electron transport. It plays essential roles in ATP production, fatty acid oxidation, amino acid metabolism and antioxidant defense. Adequate riboflavin intake supports nervous system function, red blood cell formation, skin health and vision. Riboflavin also contributes to regeneration of glutathione, an important endogenous antioxidant (Dufossé, 2018).

Riboflavin is widely used as both a nutritional fortificant and natural yellow food colorant in breakfast cereals, infant formulas, sports drinks, dairy products,

bakery products, sauces and nutritional supplements and highly valued in fortified foods as it combines coloring and nutritional functionality (Ramesh *et al.*, 2019).

Melanin: Melanin is a dark brown or black polymeric pigment produced by various fungi, bacteria and yeasts including *Streptomyces*, *Bacillus* and *Cryptococcus* species. Melanin is formed through oxidative polymerization of phenolic and indolic precursors such as tyrosine and dihydroxy-phenylalanine (DOPA). The pigment consists of highly heterogeneous aromatic polymers with extensive cross-linking (Ragab *et al.*, 2019).

Melanin exhibits strong antioxidant and radioprotective activities because of its ability to absorb ultraviolet radiation and neutralize free radicals. It also binds heavy metals and toxic compounds thereby reducing oxidative cellular injury. The pigment possesses antimicrobial activity and may contribute to immune defense mechanisms. Its UV-absorbing capacity has generated interest in photoprotective and dermatological applications (Wu *et al.*, 2022).

Melanin has potential applications as a natural black colorant in beverages, confectioneries, sauces and bakery products. However, commercial utilization remains limited due to challenges associated with extraction, purification and standardization (Lopes and Ligabue-Braun, 2021).

Monascus pigments

Monascus pigments are secondary metabolites produced mainly by *Monascus purpureus* during fermentation. These pigments belong primarily to the azaphilone group and include yellow pigments (ankaflavin and monascin), orange pigments (rubropunctatin) and red pigments (monascorubramine) (Zhou *et al.*, 2020).

Monascus pigments exhibit antioxidant, anti-inflammatory, antimicrobial, antitumor and cholesterol-lowering effects. Some *Monascus* strains also produce monacolin K, a naturally occurring HMG-CoA reductase inhibitor that reduces cholesterol biosynthesis. The pigments additionally demonstrate hepatoprotective and anticancer activities by modulating oxidative stress and apoptosis pathways (Silbir and Gokungur, 2019).

Monascus pigments have been traditionally used in East Asian fermented foods including red yeast rice, sausages, fish products, rice wine, soy products and fermented bean curd. They are commercially important because they provide stable red coloration and flavor enhancement simultaneously. However, careful strain selection is necessary because certain *Monascus* strains produce citrinin, a nephrotoxic mycotoxin (Daud *et al.*, 2020).

Advantages of microbial pigments over synthetic and other natural pigments

Microbial pigment production is eco-friendly and biodegradable, whereas synthetic dyes often generate toxic and carcinogenic industrial waste. Microbial fermentation reduces dependency on petroleum-based raw materials (Cortez *et al.*, 2017). Unlike plant pigments, microbial pigments can be produced throughout the year under controlled fermentation conditions without climatic limitations. Microorganisms possess rapid growth rates and can utilize inexpensive agro-industrial residues such as molasses, fruit peels, sugarcane bagasse, rice husk and whey as fermentation substrates, thereby reducing production costs (Lopes and Ligabue-Braun, 2021). Microbial strains can be genetically engineered to improve pigment yield, stability and safety profiles through metabolic engineering approaches. Unlike synthetic pigments that primarily provide color, microbial pigments frequently possess extensive therapeutic benefits. Modern consumers increasingly prefer clean-label and naturally derived food additives, making microbial pigments commercially attractive alternatives to synthetic colorants (De Santos *et al.*, 2018).

Limitations and regulations of microbial pigments

Despite their advantages, microbial pigments also face several challenges that limit their widespread industrial utilization. Many microbial pigments exhibit poor stability under varying pH, temperature, oxygen exposure and light conditions which can reduce shelf life and affect food quality. Extraction, purification and stabilization of pigments from microbial biomass remain technically challenging and economically expensive (Sen *et al.*, 2019). Certain pigment-producing microorganisms may simultaneously produce toxic metabolites or mycotoxins (*Monascus* strains produce citrinin)

necessitating strict safety evaluation and strain selection. Only a limited number of microbial pigments have received approval from regulatory agencies such as FDA and EFSA for food applications and some microbial pigments may impart undesirable flavour or odour changes in food products, limiting their application range (Ramesh *et al.*, 2019)

Microencapsulation, nano emulsions and the formation of nano formulations to increase the stability of food pigments:

Microencapsulation involves enclosing solid, liquid, or gaseous active compounds within protective wall materials ranging from millimeter to nanometer scale used to improve the stability, solubility and delivery efficiency of natural pigments in food systems like sensitive anthocyanins and carotenoids. extending shelf life and maintaining colour quality (Singh *et al.*, 2023). In this process, the pigment acts as the core material while the surrounding shell material provides protection and stability. Suitable wall materials should possess emulsifying ability, biodegradability, film-forming properties, low viscosity, low hygroscopicity, gastrointestinal resistance and cost-effectiveness. Commonly used encapsulating materials for microbial pigments include maltodextrin, modified starch, inulin and furcellaran. Several encapsulation techniques such as spray drying, freeze drying, coacervation, and emulsion formation are widely employed in the food industry (Sui *et al.*, 2023).

Nanoencapsulation and nano emulsion systems further enhance pigment functionality by producing droplets generally below 100 nm in size. Nano emulsions consist primarily of water, oil and emulsifiers where emulsifiers stabilize the system by reducing interfacial tension and preventing droplet aggregation (Aslam *et al.*, 2021). Compared with conventional emulsions, nano emulsions possess greater surface area, improved kinetic stability, enhanced absorption and superior resistance to physicochemical degradation. Their extremely small droplet size allows transparent dispersion of poorly water-soluble pigments in aqueous systems without affecting food appearance or taste. Nano emulsions are considered non-toxic, versatile, and cost-effective because they improve pigment bioavailability while reducing the quantity of colorant required to achieve the desired colour intensity (Bhandari *et al.*, 2024).

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