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Review Article

Food-Grade Colorants: Prospects and Opportunities

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SUMMARY

Colour has long served as a primary cue through which consumers judge the quality, safety, and desirability of foods, and its significance has become even more pronounced with the expansion of processed and convenience products. While synthetic dyes historically dominated food coloration because of their intensity, stability, and cost advantages over the natural ones, growing concerns regarding safety, health implications, and consumer perception have reshaped expectations surrounding food-grade colorants. Today, consumers increasingly associate product quality with colours derived from natural and recognizable sources, prompting substantial changes in both research priorities and industrial practices. This review examines food-grade colorants within the context of these evolving expectations with a focus on their technological development, functional potential and market relevance. Progressive research in extraction, stabilization, and formulation technologies have improved the performance of natural pigments, mitigating long-standing challenges related to instability and limited processing tolerance. At the same time, growing evidence of the bioactive properties of many natural pigments has expanded their role beyond visual appeal, positioning them as contributors to health-oriented and functional food products. In this context, biotechnology, omics-based tools, and synthetic biology offer a practical bridge between the limitations of natural colorants and the concerns surrounding synthetic dyes. These approaches enable controlled pigment biosynthesis, improved stability, and scalable production while aligning with clean-label expectations. Collectively, they provide a viable framework for addressing longstanding technical, economic, and regulatory challenges in food coloration and represent a promising direction for the future development of food-grade colorants.

Keywords: synthetic dyes, functional food, biotechnology, food-colorants

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INTRODUCTION

Food-grade colorants are substances added to foods to impart, enhance, or restore colour lost during processing, storage, or preparation. Colour is one of the first quality attributes assessed by consumers and strongly influences perception of freshness, flavor, and overall acceptability. In many cases, consumers associate specific colours with expected tastes (e.g., red with strawberry, yellow with lemon), making colorants indispensable in modern food systems [43]. Beyond aesthetics, colour is a critical component of the organoleptic properties that determine food acceptance, selection, and eventual consumption. Although many foods possess inherent natural coloration, various processing and storage conditions, such as exposure to oxygen, light, metals, changes in pH, and variations in water activity can result in undesirable colour degradation or alteration [31]. These changes often reduce consumer appeal and market value, thereby necessitating the use of food-grade colorants to maintain visual quality and consistency.

Traditionally, food colorants were developed primarily for aesthetic purposes. However, growing public awareness regarding food safety, nutrition, and long-term health impacts has significantly reshaped this field. Synthetic food dyes, once dominant due to their stability and cost-effectiveness, are increasingly scrutinized because of reported associations with hyperactivity, allergic reactions, and possible carcinogenic risks in vulnerable populations [51]. In response to these concerns, regulatory agencies have implemented increasingly stringent guidelines governing the use, labeling, and permissible limits of food colorants to ensure consumer safety and adherence to good manufacturing practices.

Regulatory definitions further clarify the scope of food-grade colorants. According to the United States Food and Drug Administration (FDA), a food colorant is any pigment, dye, or substance that, when added to food or applied to the human body, is capable, either directly or through chemical interaction, of imparting colour [55]. This broad definition lays emphasis on the functional versatility of colorants and their potential interactions within complex food matrices. As a result, research and industry focus have shifted toward natural, sustainable, and functional food colorants.

Progressive research in food biotechnology, green extraction technologies, and microbial fermentation have expanded the scope of food-grade colorants beyond visual appeal to include health-promoting and functional roles. Contemporary classification systems recognize the diversity of food colorants based on origin, chemical structure, solubility, and functional performance. While colorants may be categorized as natural, natural-identical, or synthetic, the most widely adopted classification distinguishes them as natural or artificial. Natural colorants may be derived from plant sources such as curcumin, carotenoids, anthocyanins, betalains, and chlorophylls; animal-derived compounds such as carminic acid; microbial metabolites including carotenoids; or mineral sources like calcium carbonate. In contrast, artificial colorants are chemically synthesized and do not occur naturally [7].

Regardless of their source, food-grade colorants are formulated to avoid imparting undesirable aftertastes when used within approved concentrations.

They are extensively applied across multiple food sectors, including confectionery, bakery products, beverages, dairy items, and processed meat products [43]. Collectively, these developments set the foundation for understanding the scientific, industrial, and regulatory transformations shaping the future of food colorants.

Classification of Food-Grade Colorants (Synthetic Food Colorants)

Synthetic food colorants are chemically synthesized compounds, often derived from petroleum or coal tar intermediates. Common examples include Tartrazine (Yellow No. 5), Allura Red AC (Red No. 40), and Brilliant Blue FCF (Blue No. 1) [36]. These dyes have historically been favored in the food industry due to their strong tinting power, uniformity, resistance to heat and light, and long shelf life. From an industrial perspective, synthetic colorants offer consistent quality and predictable behavior across diverse food matrices, such as beverages, confectioneries, and baked goods. Their low production cost and ease of formulation make them attractive for large-scale food manufacturing [18].

In addition to these advantages, synthetic colorants are often preferred by manufacturers because they can be produced with high purity and reproducibility, offering a wide range of bright and stable shades that meet industrial requirements. Continuous scientific advancements have enabled the development of increasingly stable artificial dyes, further expanding their application across various food products [45]. Their physicochemical stability under different processing conditions, including thermal treatment and prolonged storage, has significantly contributed to their widespread use in the global food industry [41].

However, toxicological studies and epidemiological reports have raised concerns regarding their safety. Some synthetic dyes have been linked to behavioral disorders in children, hypersensitivity reactions, and potential genotoxic effects [37]. Consequently, several countries regularly reassess their food colorant regulations, with the aim of restricting or lowering the acceptable daily intake (ADI) of certain dyes. Regulatory frameworks differ considerably across regions: the United States and India apply relatively strict controls, authorizing only nine and eight food colorants respectively, while the European Union often revises approved limits or issues temporary ADI recommendations [3]. In other regions, including Brazil, there is a growing need to periodically re-evaluate the authorization of these substances to ensure the safety of processed foods intended for consumer use. This has created both a challenge and an opportunity for innovation in safer alternatives [38].

Adverse health effects associated with the consumption of certain synthetic food colorants, particularly azo dyes such as Tartrazine (E102), Sunset Yellow (E110), Carmoisine (E122), Ponceau 4R (E124), Allura Red (E129), and Amaranth (E123) [6]. Regulatory actions in the European Union have mandated specific warning labels on foods containing some of these additives, highlighting their potential effects on children's activity and attention. Tartrazine, one of the most widely used synthetic dyes, has been especially controversial, with studies linking its intake to hyperactivity, obsessive-compulsive behaviors, oxidative stress, immunotoxic effects, renal dysfunction, and DNA damage in experimental models. Similarly, other petroleum-derived dyes such as Sunset Yellow and Allura Red have been associated with pro-inflammatory responses and protein-binding interactions that may contribute to toxicity [26].

Differences in regulatory approval across regions further complicate the use of synthetic colorants, as some dyes permitted in the European Union are banned in the United States due to carcinogenic or genotoxic concerns. The availability of highly sensitive analytical methods for detecting synthetic dyes in food products has also increased scrutiny of their safety and long-term health implications.

Natural Food Colorants

Natural food colorants are derived from plant, animal, or mineral sources and include pigments such as carotenoids, anthocyanins, chlorophylls, betalains, and curcuminoids. Examples include annatto (orange-red), turmeric (yellow), beetroot extract (red-purple), and spirulina (blue-green) [23]. Historically, food coloration relied exclusively on naturally sourced pigments such as saffron, cochineal, caramel, beetroot extract, and indigo. Although synthetic dyes later replaced many of these traditional colorants due to superior stability and lower cost, advancements in extraction, purification, stabilization, and standardization techniques have renewed interest in natural pigments. Modern processing technologies now allow natural dyes to be used either directly or in chemically modified forms to achieve a broad spectrum of hues ranging from green and yellow to red, blue, and violet [1]. These colorants are widely perceived as safer and more acceptable to consumers, especially in the context of clean-label and organic food movements. In addition to colouring properties, many natural pigments possess bioactive functions, such as antioxidant, anti-inflammatory, and antimicrobial activities [17].

Several natural colorants are already extensively utilized in the food industry. Curcumin (E100), extracted from *Curcuma longa*, imparts an orange-yellow colour and is widely used in mustard, dairy products, baked goods, and salad dressings [4]. Carminic acid (E120), derived from *Dactylopius coccus* insects, produces a stable bright red colour and is valued for its technological performance, although its animal origin limits its use in vegan and kosher foods. Chlorophylls and their copper-stabilized derivatives (E140 and E141) are extracted primarily from alfalfa and are applied in soups, beverages, dairy products, and confectionery. Annatto (E160b), obtained from *Bixa orellana* seeds, provides yellow-to-orange hues and is widely used in dairy products, baked goods, snacks, and meat products [43].

Carotenoids such as β-carotene, lutein, lycopene, astaxanthin, and fucoxanthin are among the most widely applied natural pigments, contributing yellow, orange, and red colours to sauces, beverages, dairy products, and seasonings [33]. Anthocyanins (E163), a diverse group of over 700 identified compounds, provide red, purple, and blue shades and are commonly used in soft drinks, fruit preparations, and confectionery. Betalains, particularly betanin (E162) from beetroot, have gained industrial importance as replacements for synthetic red dyes due to their high-water solubility and stability in acidic and neutral pH environments [21].

Natural colorants face significant limitations despite their advantages. They are often sensitive to environmental conditions such as pH, oxygen, temperature, and light, leading to colour degradation. Variability in pigment concentration due to seasonal and geographical factors also complicates standardization [24]. Addressing these challenges is a major research focus in food science. Stability remains a critical factor, as degradation of natural pigments can result in undesirable organoleptic changes and consumer rejection. Consequently, extensive research efforts are directed toward improving extraction methods, developing stabilization strategies, and identifying novel pigment sources capable of competing with synthetic colorants in terms of performance and shelf life.

A notable example is crocetin, a key pigment and bioactive compound concentrated in the stigmas of *Crocus sativus*, where the production of approximately 20 g of crocetin from 1 kg of dried stigma requires between 110,000 and 170,000 flowers and more than 400 hours of manual labor. This challenge is further compounded by the slow growth rate and exclusively vegetative propagation of the plant. Plant cell culture technology offers a mechanistically driven alternative for pigment production, enabling the synthesis of secondary metabolites under tightly controlled in vitro conditions [26].

Unlike field cultivation, *in vitro* systems are independent of seasonal and geographical constraints and allow continuous production with improved reproducibility. Enhanced pigment yields can be achieved through the selection of high-producing cell lines and systematic optimization of cultivation parameters, including nutrient composition, environmental conditions, cell immobilization strategies, and bioreactor design for scale-up (figure 2). In addition to these conventional approaches, emerging strategies such as elicitation, mimicking biotic or abiotic stress signals to activate biosynthetic pathways, and metabolite exudation, which facilitates the continuous release of pigments into the culture medium, represent promising extensions for increasing productivity and process efficiency [7].

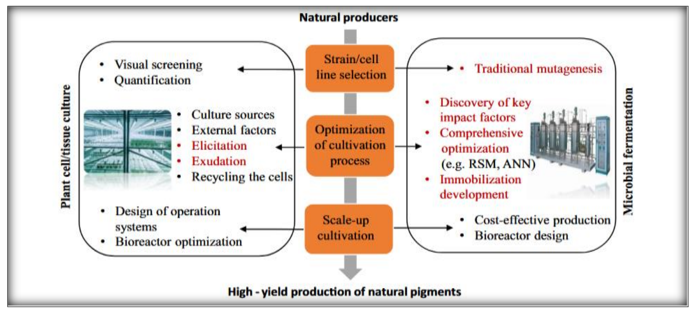

Figure 1: Routes and strategies for improving production of pigments from natural producers, by optimization of traditional methods including plant cell/tissue culture (left) and microbial cultivation (right) [35].

Microbial and Bioengineered Colorants

Microbial colorants are pigments produced by microorganisms including bacteria, fungi, yeast, and microalgae. Examples include carotenoids from *Blakeslea trispora*, phycocyanin from *Spirulina platensis*, and prodigiosin from *Serratia* species [48]. Microbial production offers several advantages over plant-derived sources. Fermentation-based processes allow controlled production, higher yields, independence from climatic conditions, and scalability. Moreover, microbial pigments can be engineered through metabolic and genetic modifications to enhance stability, yield, and safety [13].

Microorganisms represent an important alternative source of natural pigments, as they can synthesize colour compounds that are difficult or impossible to obtain from botanical sources. Bacteria, in particular, are attractive due to their rapid growth rates, genetic manipulability, and suitability for large-scale fermentation [16]. Metabolic engineering has enabled the production of pigments such as anthocyanins in genetically modified *Escherichia coli* at industrially relevant concentrations. Fungal species, including *Monascus* spp., produce a wide range of yellow-to-red pigments, although concerns over toxin co-production have limited regulatory approval in Western markets despite long-standing use in Asia [15].

An important breakthrough in natural blue colorants achieved through phycocyanin extracted from *Spirulina* has been reported [20]. Due to the instability of blue anthocyanins across varying pH conditions, phycocyanin has emerged as a viable alternative and is currently the only approved natural blue dye in certain regions, including the United States and parts of Asia [62]. While its application was initially limited to confectionery, technological advancements have expanded its use to beverages, dairy products, and cosmetics, although low pH stability remains a constraint. Recent advances in precision fermentation and synthetic biology enable the design of microorganisms that produce food-grade pigments identical to natural compounds. This represents a promising intersection between biotechnology and sustainability, opening new commercial opportunities while reducing reliance on agricultural resources.

Despite concerns regarding pigment stability and consumer acceptance of microbially derived ingredients, many natural and microbial colorants have demonstrated performance comparable to synthetic dyes. Importantly, several of these pigments offer additional functional benefits, such as antioxidant and preservative effects, thereby providing multiple advantages when incorporated into food products. The growing demand for functional foods has further accelerated industry efforts to replace artificial colorants with natural and bioengineered alternatives that align with health-conscious consumer preferences [9].

Technological Advances and Opportunities

(Extraction, Stability, and Technological Innovation)

A central limitation in the application of natural food-grade colorants is their comparatively lower stability when exposed to food processing and storage conditions. Natural pigments are often sensitive to environmental factors such as heat, light, oxygen, and pH fluctuations, which can lead to colour degradation and loss of consumer appeal [32]. In contrast, synthetic dyes are chemically engineered for high stability, presenting a significant technological gap that natural colorants must overcome. To address this disparity, substantial advances have been made in extraction technologies aimed at improving pigment yield while preserving structural integrity. Techniques such as supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction enable efficient pigment recovery with reduced solvent consumption and minimized thermal degradation, thereby improving both sustainability and pigment quality [19]. In living plant tissues, pigments exhibit remarkable stability due to their localization within specialized biological environments, where they are often complexed with proteins, organic acids, or polysaccharides that limit uncontrolled degradation. Once pigments are isolated from these protective matrices, however, they become highly vulnerable to physicochemical stress [35]. Recent technological strategies have therefore focused on mimicking these native stabilization mechanisms. Microencapsulation using food-grade biopolymers such as alginate, maltodextrin, and chitosan has been shown to significantly reduce pigment sensitivity to light, oxygen, and moisture, while nanotechnology-based delivery systems enhance dispersibility, matrix compatibility, and controlled release within complex food systems [61].

Despite extensive patent activity and academic research proposing stabilization strategies, many reported methods remain unsuitable for food applications due to economic constraints, regulatory non-compliance, or safety concerns. Chemical modification of pigments can improve stability but often compromises their classification as “natural,” directly conflicting with clean-label requirements. Consequently, current technological innovation increasingly prioritizes biologically inspired and food-compatible stabilization approaches that preserve both pigment functionality and consumer acceptability.

Functional and Health-Promoting Colorants

Beyond their role in visual enhancement, many natural food-grade colorants possess intrinsic biological activities that contribute to human health. The human di*et al*ready includes substantial quantities of natural pigments particularly anthocyanins, carotenoids, and chlorophylls, derived from fruits and vegetables, with annual intake levels far exceeding those introduced through food coloration alone. Although colorants are added at comparatively low concentrations, their incorporation still contributes functional value when delivered through frequently consumed food matrices [16].

Anthocyanins have been widely recognized for their antioxidant, anti-inflammatory, and cardioprotective effects [60], while carotenoids are essential for immune modulation, visual health, and protection against oxidative stress. Betalains and curcuminoids similarly demonstrate antioxidant and antimicrobial activities [57]. In addition to direct health benefits, several pigments exhibit technological functionality, including the ability to delay lipid oxidation and suppress microbial growth, thereby extending shelf life. This multifunctionality has repositioned food-grade colorants as nutraceutical or functional ingredients rather than purely aesthetic additives.

The growing consumer demand for functional and minimally processed foods has accelerated interest in pigments that simultaneously enhance colour, nutritional value, and product stability. As a result, food manufacturers are increasingly replacing synthetic dyes with natural pigments to meet regulatory expectations and capitalize on market demand for health-oriented, plant-based, and fortified products.

Major natural pigments and advances in biotechnological production

Natural food pigments can be broadly grouped into five main classes based on their chemical structures: tetrapyrroles, carotenoids, flavonoids, curcuminoids, and betalains. Each class differs in molecular architecture, biological function, coloration properties, and biosynthetic origin.

Tetrapyrroles (Structure, classification, and function)

Tetrapyrroles, often referred to as the "pigments of life," constitute a relatively small yet biologically essential class of natural pigments that are widely distributed across living organisms. Structurally, they are composed of four pyrrole-derived units linked through methine bridges, forming either linear bile pigments or cyclic porphyrin structures [35]. Variations in oxidation state, side-chain substitutions, and the nature of centrally chelated metal ions contribute to the remarkable structural diversity within this group. Cyclic tetrapyrroles include chlorophylls, hemes, cytochrome c, vitamin B_{12}, and coenzyme F_{430}, collectively responsible for a broad spectrum of natural colours ranging from yellow and red to green, blue, and purple [35].

In addition, the breakdown of cyclic tetrapyrroles gives rise to linear forms such as bilins, which result from the loss of one bridge carbon. Among the most well-known tetrapyrroles are chlorophyll, which imparts the characteristic green colour to plants and drives photosynthesis, and heme, the iron-containing pigment responsible for the red colour of animal blood. Functionally, tetrapyrroles are indispensable in core metabolic processes, including light harvesting and electron transport in plants, as well as oxygen transport and redox reactions in animals. Beyond their physiological roles, many tetrapyrroles exhibit antioxidant, anticancer, antimutagenic, and anticlastogenic activities, underscoring their growing relevance in food, nutraceutical, and pharmaceutical applications [28].

Biosynthetic pathway

Tetrapyrrole biosynthesis proceeds through a highly conserved multistep pathway that can be divided into six major stages [33]. These include the formation of the universal precursor 5-aminolevulinic acid (ALA), followed by its conversion into the pyrrole unit porphobilinogen. Subsequent condensation reactions yield uroporphyrinogen III, which serves as the fundamental carbon framework for porphyrins. This intermediate undergoes decarboxylation to form coproporphyrinogen III, which is then oxidized to protoporphyrinogen IX. The final step involves dehydrogenation to generate protoporphyrin IX, a fully conjugated ring system responsible for the characteristic coloration of tetrapyrrole pigments [65]. Figure 3 represents the biosynthesis of tetrapyrrole.

Typical product: heme

Heme is one of the most prominent members of the tetrapyrrole family, playing critical biological roles in oxygen transport, electron transfer, gene regulation, protein stability, and cellular differentiation. Its intense red colour has historically supported its use as a meat additive, while its high bioavailability has led to widespread application as an iron supplement in healthcare products [36]. More recently, heme has gained significant interest in the development of plant-based meat alternatives, where it enhances meat-like colour and flavor attributes [60].

Traditionally, free heme has been extracted from animal-derived materials, particularly blood, a process constrained by low yields and ethical concerns related to animal welfare [12]. Consequently, microbial and biotechnological production strategies have emerged as more sustainable alternatives. Heme biosynthesis begins with ALA formation via either the C_4 pathway, predominant in animals, fungi, and some bacteria, or the C_5 pathway, which operates in plants and most bacteria. This is followed by a series of cyclization, modification, and metal incorporation reactions leading to the final heme molecule [36].

Early metabolic engineering efforts demonstrated the feasibility of reconstructing the entire heme biosynthetic pathway in *Escherichia coli* by assembling genes from multiple organisms, resulting in detectable intracellular heme accumulation [22]. Subsequent studies improved yields through co-expression of key enzymes involved in ALA synthesis and central carbon metabolism, as well as enhancement of precursor supply [14]. More advanced strategies focused on system-level optimization, including comparative evaluation of the C_4 and C_5 pathways, elimination of metabolic bottlenecks, suppression of competing pathways such as acetate and lactate formation, and inhibition of heme degradation via targeted gene knockouts. These interventions significantly increased intracellular heme levels [36].

A major breakthrough was achieved by coupling pathway optimization with enhanced heme export through overexpression of specific transporters, enabling not only higher total production but also efficient secretion into the culture medium [55]. Collectively, these studies demonstrate that integrated metabolic engineering, combining precursor pathway reinforcement, flux redistribution, suppression of degradation routes, and improved secretion offers a highly promising route for large-scale, sustainable production of heme and other tetrapyrrole-based colorants.

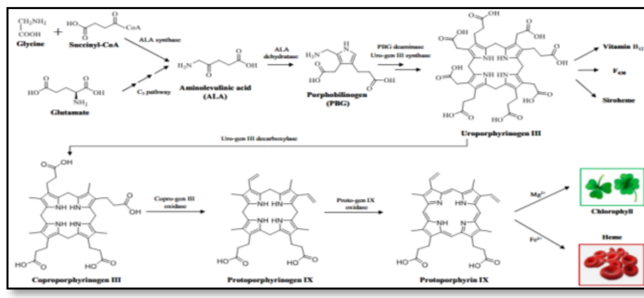

Figure 3: Schematic for the biosynthetic pathways of tetrapyrroles. Tetrapyrroles, including vitamin B 12, F430, siroheme, chlorophyll and heme, are initiated from condensation of glycine and succinyl-CoA or synthesized through C5 pathway from the C5-skeleton of glutamate [36].

Carotenoids (Structure, classification, and functions of Carotenoids)

Carotenoids are a large group of lipophilic isoprenoid colorants synthesized by a wide range of microorganisms, including bacteria (e.g., *Corynebacterium*, *Micrococcus*, *Bradyrhizobium*, *Dietzia*), fungi (e.g., *Blakeslea trispora*, *Rhodotorula* spp., *Xanthophyllomyces dendrorhous*), and microalgae such as *Chlorella*, *Dunaliella*, *Coelastrella*, and *Haematococcus* [51]. In addition, recent evidence suggests that certain insects, including aphids and gall midges, possess the unusual ability to synthesize carotenoids de novo, whereas higher animals, including humans, rely entirely on dietary intake [50].

From a structural perspective, carotenoids are formed through the sequential condensation of C_5 isoprenoid units, giving rise to more than 600 known carotenoid structures, with C_{40} carotenoids being the most prevalent in nature [11]. They are broadly classified into two major categories: carotenes, which are hydrocarbon carotenoids such as lycopene, α-carotene, and β-carotene, and xanthophylls, which are oxygenated derivatives including lutein, zeaxanthin, astaxanthin, and canthaxanthin. Carotenoids exhibit colours ranging from yellow and orange to deep red, and in some cases appear colourless. Functionally, they are recognized for their strong antioxidant, anticancer, and anti-inflammatory properties. Economically, carotenoids represent a significant segment of the global colorant market, which was valued at approximately USD 1.5 billion in 2017 and is projected to approach USD 2.1 billion by 2025 [42].

Biosynthetic pathway of Carotenoids

Carotenoid biosynthesis generally proceeds through four key stages (Figure 4): (1) generation of the universal C_5 precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP); (2) chain elongation via sequential condensation reactions to form polyprenyl diphosphates; (3) cyclization of linear intermediates to establish the carotenoid backbone; and (4) structural modification to yield diverse carotenoid end products. Among these, β-carotene and astaxanthin are of particular commercial importance [36].

The synthesis of IPP and DMAPP occurs via two alternative pathways: the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. The MVA pathway, present in many bacteria, fungi, and the cytosol of plants, originates from acetyl-CoA and involves several enzymatic steps, with 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) acting as a major rate-limiting enzyme. In contrast, the MEP pathway, found predominantly in bacteria, green algae, and plant plastids, begins with glyceraldehyde-3-phosphate and pyruvate, and is regulated by enzymes such as DXS, DXR, and IPPI, which are also considered bottlenecks [36].

Downstream of IPP/DMAPP formation, lycopene is synthesized through the action of enzymes such as geranylgeranyl diphosphate synthase, phytoene synthase, and phytoene desaturase. Lycopene subsequently undergoes cyclization and functional modification to form α- and β-carotene, as well as xanthophylls including lutein, zeaxanthin, and astaxanthin [30].

Typical product: β-carotene

β-Carotene is the most extensively studied carotenoid and is widely distributed in vegetables and other plant-derived foods. It is commercially important due to its role as a provitamin A compound and its potent antioxidant activity, contributing to immune function, vision, and overall human health [51].

Conventional production of β-carotene relies on extraction from plant materials, algae, or cultivation of native carotenoid-producing fungi and yeasts. However, plant-based extraction is limited by long growth cycles, environmental variability, and complex downstream processing. Although microalgae offer higher productivity, large-scale cultivation remains challenging due to stringent growth requirements and pigment degradation issues [29].

Microbial fermentation has therefore emerged as a promising alternative, enabling continuous production and greater process control. Natural producers such as *Blakeslea trispora* and *Rhodotorula glutinis* have been improved through medium optimization, mutagenesis, and use of stimulatory additives [25].Nevertheless, limited genetic tools have constrained precise metabolic engineering in these organisms. As a result, attention has shifted to model hosts such as *Escherichia coli* and *Saccharomyces cerevisiae*. In *E. coli*, strategies including reinforcement of the MEP pathway, introduction of the heterologous MVA pathway, cofactor engineering, and membrane remodeling have significantly enhanced β-carotene accumulation, reaching up to 44.8 mg/g dry cell weight. Despite these achievements, food safety concerns and regulatory limitations have hindered commercial deployment [51].

In contrast, *S. cerevisiae*, a GRAS organism, has been widely explored for carotenoid biosynthesis. Advances include overexpression of rate-limiting MVA enzymes, deletion of competing pathways, transcription factor engineering, and dynamic regulation of pathway flux, collectively leading to substantial improvements in β-carotene yield. More recently, the oleaginous yeast *Yarrowia lipolytica* has demonstrated exceptional promise, achieving β-carotene titers as high as 6.5 g/L through pathway balancing and acetyl-CoA flux optimization [25].

Typical product: astaxanthin

Astaxanthin, a red ketocarotenoid, is highly valued for its exceptional antioxidant capacity and is extensively used in aquaculture feed, particularly for salmon pigmentation [8]. The global astaxanthin market was valued at over USD 1.3 billion in 2020 and is expected to grow rapidly in the coming years. Due to limitations associated with algal extraction and chemical synthesis, microbial biosynthesis has gained increasing attention [40].

Heterologous production of astaxanthin has been successfully demonstrated in several microbial hosts, including *E. coli*, *S. cerevisiae*, *Y. lipolytica*, and *Corynebacterium glutamicum*. Engineering strategies typically focus on enhancing β-carotene precursor supply and optimizing the downstream conversion of β-carotene to astaxanthin by balancing the activities of β-carotene hydroxylase and ketolase enzymes. Approaches such as promoter and ribosome binding site engineering, gene copy number adjustment, protein engineering, and construction of multi-enzyme complexes have proven effective [27].

In addition to pathway-level interventions, host cell properties such as oxidative stress tolerance and membrane composition have been shown to influence astaxanthin accumulation. Random mutagenesis, CRISPRi-based regulation, and omics-guided target identification have further expanded the engineering toolbox. Overall, successful microbial astaxanthin production relies on coordinated optimization of precursor generation, enzymatic conversion efficiency, and host robustness [2].

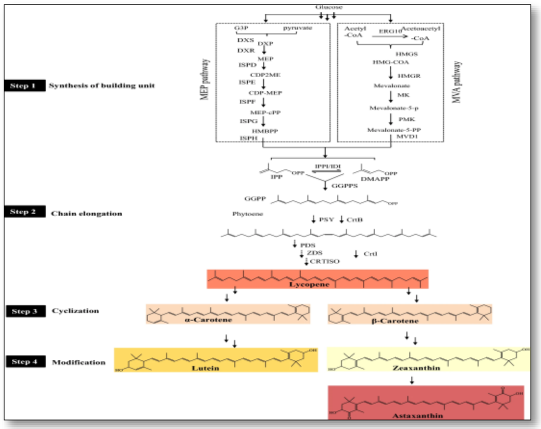

Figure 4: Schematic of carotenoids biosynthesis. Most carotenoids go through four synthetic steps consisting generation of building unit, chain elongation, cyclization, and modification. DXS, 1-deoxy-d-xylulose-5-phosphate synthase; DXR, 1-deoxy-d-xylulose-5-phosphate reductoisomerase; ISPD, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase; IspE, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; IspF, 2C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; IspG, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase; IspH, 1-hydroxy-3-methyl-2-(E)-butenyl-4-diphosphate reductase; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; MVD1, mevalonate diphosphate decarboxylase; IPPI/IDI1, isopentenyl-diphosphate isomerase; GGPPS, GGPP synthase; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; CRTISO, carotenoid isomerase; CrtB, phytoene synthase; CrtI, phytoene desaturase; DXP, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-C-methyl-D-erythrito-l4-phosphate; CDP2ME, 4-diphosphocytidyl-2-C-methylerythritol; MEP-cPP, 2-C-methyl-D-erythritol-2,4-cyclo-diphosphate; HMBPP, (E)-4-hydroxy-3-methylbut-2-enyl-diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate [36].

Flavonoids (Structure, classification, and function of Flavonoids)

Flavonoids constitute a large and structurally diverse group of plant-derived secondary metabolites originating from the phenylpropanoid pathway. They share a common C_6–C_3–C_6 backbone composed of two aromatic rings linked by a heterocyclic ring. Structural diversification through hydroxylation, glycosylation, methylation, and other modifications gives rise to six principal subclasses: flavanones, flavones, isoflavones, flavonols, catechins, and anthocyanins [36]. These compounds are widely distributed in fruits, vegetables, grains, and flowers, where they play essential roles in pigmentation, plant defense, and ecological interactions. While many flavonoids impart yellow hues, anthocyanins are responsible for red, purple, and blue coloration. Beyond their role in plants, flavonoids are associated with numerous health benefits in humans, including antioxidant, anti-inflammatory, cardioprotective, and neuroprotective effects [54]. Their vivid coloration, water solubility, and bioactivity make flavonoids, particularly anthocyanins, highly attractive as natural food colorants. The global flavonoid market is projected to exceed USD 1 billion by 2025, driven largely by demand for anthocyanin-based colouring agents [42].

Biosynthetic pathway of Flavonoids

Flavonoid biosynthesis is illustrated in figure 5. This begins with L-phenylalanine or L-tyrosine, which are converted into p-coumaric acid. Subsequent activation by 4-coumarate-CoA ligase and condensation with malonyl-CoA via chalcone synthase yields chalcones, which are then isomerized into flavanones such as naringenin. From this central intermediate, a series of tailoring enzymes generate the diverse array of flavonoids, including anthocyanins through the coordinated action of F3H, DFR, ANS, and glycosyltransferases [65].

Typical product: anthocyanins

Anthocyanins are water-soluble pigments producing red to blue coloration and have been approved for food use by the European Food Safety Authority [5].Although plant metabolic engineering has achieved notable success in enhancing anthocyanin content, production remains constrained by environmental variability and slow growth cycles. Microbial biosynthesis offers a scalable alternative, with early studies demonstrating heterologous anthocyanin production in *E. coli*. Subsequent advances in precursor supply, enzyme balancing, pathway integration, and cultivation optimization have led to titers exceeding 400 mg/L [57].

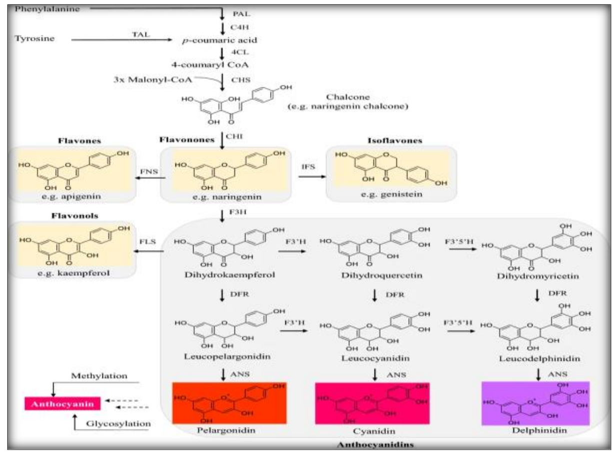

Figure 5: Schematic of flavonoids biosynthesis. PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, p-coumaric acid: CoA ligase; TAL, tyrosine ammonia lyase; CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavone synthase; IFS, isoflavone synthase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; F3′H, flavonoid 3′-hyroxylase; F3′5′H, flavonoid 3′,5′-hyroxylase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase [36].

More recently, de novo anthocyanin biosynthesis has been achieved in both *E. coli* and *S. cerevisiae*, eliminating the need for costly precursor supplementation [42]. Representative examples of engineered plant and microbial systems are summarized in Table 1

.

Table 1. Typical examples of metabolic engineering of plants and microorganisms for enhanced anthocyanin production [36].

Target compound	Hosts	Strategies	Substrate	Yield	Year
Pelargonidin 3-O-glucoside (P3G) Cyanidin 3-O-glucoside (C3G)	*E. coli*	Introduction of F3H, DFR, ANS and 3GT	Naringenin, eriodictyol	5.6 μg/L, 6.0 μg/L	2005

PEG and C3G	*E. coli*	Gene screening, enhancement of UDP-glucose supply, culture medium pH adjustment, protein fusion	Afzelechin, catechin	78.9 mg/L of P3G, 70.7 mg/L of C3G	2007
C3G	*E. coli*	Improvement of catechin uptake and C3G secretion; increase of ANS and 3GT expression; enhanced intracellular availability of UDP-glucose; optimization of culture and induction conditions	Catechin	350 mg/L	2015
P3G	*E. coli*	Polycultures; 15 exogenous enzymes from diverse sources were divided into four *E. coli* strains	Glucose	9.5 mg/L	2017
C3G	*E. coli*	Promoter engineering for balancing ANS and 3GT expression	Catechin	439 mg/L	2019
Pyranoanthocyanins	*E. coli*	Co-culture of 4-vinylphenol or 4-vinylcatechol producing *E. coli* strains with cyanidin-3-O-glucoside producer recombinant *E. coli*	Catechin	19.5 mg/L of pyranocyanidin-3-O-glucoside-phenol, and 13 mg/L of pyranocyanidin-3-O-glucoside-catechol	2019
Total anthocyanin	Tomato	Expression of the Delila (*Del*) and Rosea1 (*Ros1*) genes from the snapdragon *Antirrhinum majus*	/	2.83 mg/g (fresh weight)	2008
C3G	*Corynebacterium Glutamicum*	Expression optimization of ANS and 3GT, improved UDP-glucose availability, process optimization	Catechin	40 mg/L	2018

MARKET AND INDUSTRIAL PERSPECTIVES OF FOOD COLORANTS

Consumer Trends and Clean-Label Demand

Consumer perception advertently shapes the food colorants market. There is increasing resistance toward ingredients perceived as artificial, chemically complex, or unfamiliar [52]. In contrast, colorants derived from recognizable food sources such as fruits, vegetables, and spices align strongly with clean-label and transparency expectations. Historically, many natural colorants were accepted due to long-standing tradition, despite originating from sources not commonly consumed in modern diets [59]. However, introducing new natural colorants of similar origin today may face consumer skepticism. To address this, some manufacturers are shifting from the use of "colour additives" to "colouring foods," where pigments are obtained from minimally processed food ingredients. This strategy represents a significant market opportunity, as it allows compliance with clean-label standards while maintaining visual appeal.

Market Growth and Commercial Opportunities

The global food colorants market continues to expand, with natural colorants growing at a faster rate than synthetic counterparts. This growth is driven by urbanization, increased consumption of processed foods, and heightened health awareness [43]. Major users of natural colorants include manufacturers of confectionery, beverages, dairy products, baked goods, and dressings. Pigmented by-products from food processing, such as grape skins or tomato peels, represent underutilized resources that can provide natural coloration without regulatory complications. Selective breeding of crops with enhanced pigment content and improved processing conditions that preserve natural colour may further reduce the need for added colorants [37].

Regulatory and Safety Considerations

It is important to take note that food-grade colorants are subject to rigorous regulatory oversight to ensure safety and consumer protection. Approval processes require extensive toxicological evaluation, determination of acceptable daily intake levels, and compliance with good manufacturing practices. While natural colorants are generally perceived as safer, they are not exempt from concerns related to contamination, allergenicity, or batch-to-batch variability. Regulatory frameworks differ significantly across regions, creating challenges for global harmonization, particularly for microbial and bioengineered pigments. The high cost and lengthy timelines associated with regulatory approval limit the introduction of new colorants. Transparent labeling, proper quality control, and compliance with international standards are therefore essential to building consumer trust and facilitating market expansion.

Challenges and Future Directions of food colorants

Despite significant progress, several challenges continue to limit the widespread adoption of natural and bioengineered food-grade colorants. These include reports of insufficient stability under processing conditions, restricted colour range compared to synthetic dyes, higher production costs, and inconsistent supply chains. Additionally, the lack of universal stabilization strategies, due to the diverse chemical nature of pigments, necessitates case-by-case solutions [41].

Future research must focus on understanding pigment behavior in natural biological environments and translating this knowledge into food-compatible stabilization techniques. Integrated approaches combining biotechnology, advanced processing, and thoughtful food formulation offer the most promising pathway forward. Continued collaboration between academia, industry, and regulatory authorities will be important to unlock the full prospects and opportunities of food-grade colorants.

CONCLUSION

Food-grade colorants have evolved from being purely aesthetic additives to becoming strategically important components of modern food systems. Growing consumer awareness, stricter regulatory scrutiny, and increasing demand for clean-label and health-oriented products have collectively driven a shift away from synthetic dyes toward natural, sustainable, and functional alternatives. This transition has redefined the prospects and opportunities within the food colorant sector. Progressive research on extraction, stabilization, biotechnology, and systems-level approaches have significantly improved the feasibility of natural colorants, addressing long-standing challenges related to instability, limited colour range, and processing compatibility. In particular, the integration of green technologies, micro- and nano-encapsulation strategies, and omics-driven production platforms has expanded both the performance and application scope of food-grade pigments. Additionally, the recognition of bioactive pigments as dual-purpose ingredients, providing colour and health benefits, positions food colorants at the intersection of food technology and nutraceutical innovation. Despite these advances, technical, economic, and regulatory barriers remain. Variability in raw materials, high production costs, and the complexity of global regulatory frameworks continue to limit large-scale adoption and commercialization of novel colorants. Nonetheless, opportunities abound in microbial fermentation, valorization of food-processing by-products, plant-based and vegan foods, and regionally sourced pigments.

Author’s Contribution

Conceptualization, extensive literature search, data curation, preparation of draft manuscript by MUO. Supervision, critical review of the manuscript, and intellectual input to improve content quality and structure by ONM. Supervision, technical guidance and critical revision of manuscript by CEE. Supervision, final proofreading of the manuscript by MAP. All authors contributed to the refinement of the manuscript, reviewed and approved the final version for submission, and agree to be accountable for all aspects of the work.

REFERENCES

1. Aggarwal, S. (2025). Advancement in extraction and characterization techniques of natural dyes from dye yielding plants sources: a review. *Pigment & Resin Technology*, *54*(2), 293-301.
2. Alberts, A., Lungescu, I. A., Niculescu, A. G., & Grumezescu, A. M. (2025). Natural Products for Improving Soft Tissue Healing: Mechanisms, Innovations, and Clinical Potential. Pharmaceutics, 17(6), 758.

3. Anjum, V., Dixit, V., Jha, P., & Potoroko, I. (2024). Food products regulations in the European Union. In *Global Regulations of medicinal, pharmaceutical, and food products* (pp. 255-287). CRC Press.
4. Awulachew, M. T. (2024). Spices in the Food Processing and Food Safety. *Herbs and Spices-New Perspectives in Human Health and Food Industry: New Perspectives in Human Health and Food Industry*, 63.
5. Ayvaz, H., Cabaroglu, T., Akyildiz, A., Pala, C. U., Temizkan, R., Ağçam, E., ... & Diaconeasa, Z. (2022). Anthocyanins: Metabolic digestion, bioavailability, therapeutic effects, current pharmaceutical/industrial use, and innovation potential. Antioxidants, 12(1), 48.
6. Banc, R., Filip, L., COZMA-PETRUȚ, A., CIOBÂRCĂ, D., & MIERE, D. (2024). Yellow and red synthetic food dyes and potential health hazards: A mini review. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology*, *81*(1), 2024.
7. Bashir, I., Pandey, V. K., Dar, A. H., Dash, K. K., Shams, R., Mir, S. A., ... & Zahoor, I. (2024). Exploring sources, extraction techniques and food applications: a review on biocolours as next-generation colorants. *Phytochemistry Reviews*, *23*(4), 1-26.
8. Behnam, B. M., Mahmoud, H., Shafigh, S., Sadegh, S., & Esmaeil, P. K. (2025). The Red Gold of Aquaculture: The Dual Role of Astaxanthin in Aquatic Nutrition and Human Health. Aquaculture Research, 2025(1), 7000490.
9. Bensid, A., El Abed, N., Houicher, A., Regenstein, J. M., & Özogul, F. (2022). Antioxidant and antimicrobial preservatives: Properties, mechanism of action and applications in food–a review. *Critical Reviews in Food Science and Nutrition*, *62*(11), 2985-3001.
10. Bhagat, N., Gupta, G. K., Minhas, A. K., Chhabra, D., & Shukla, P. (2025). Artificial Neural Network-Multi-Objective Genetic Algorithm based optimization for the enhanced pigment accumulation in Synechocystis sp. PCC 6803. BMC biotechnology, 25(1), 23.
11. Britton, G. (2022). Getting to know carotenoids. In Methods in enzymology (Vol. 670, pp. 1-56). Academic Press.
12. Cao, Z., Khan, I. M., Niu, X., Hu, Y., Duan, Z., Fang, Q., ... & Yang, J. (2025). Edible domestic animal blood products for biomedical and disease treatment applications. Current Research in Biotechnology, 100308.
13. Chavan, A., Pawar, J., Kakde, U., Venkatachalam, M., Fouillaud, M., Dufossé, L., & Deshmukh, S. K. (2025). Pigments from microorganisms: a sustainable alternative for synthetic food colouring. *Fermentation*, *11*(7), 395.
14. Chen, Y. Y., Huang, J. C., Wu, C. Y., Yu, S. Q., Wang, Y. T., Ye, C., ... & Huang, H. (2025). A comprehensive review on the recent advances for 5-aminolevulinic acid production by the engineered bacteria. Critical Reviews in Biotechnology, 45(1), 148-163.
15. Deshmukh, S. K., Lagashetti, A., Singh, S. K., Badgujar, H. F., & Kumar, U. (2022). Fungal pigment research

in India: An overview. *Progress in Mycology: Biology and Biotechnological Applications*, 519-544.

16. Dhiman, S., Kaur, S., Thakur, B., Singh, P., & Tripathi, M. (2025). Nutritional Enhancement of Plant-Based Fermented Foods: Microbial Innovations for a Sustainable Future. *Fermentation*, *11*(6), 346.
17. Di Salvo, E., Lo Vecchio, G., De Pasquale, R., De Maria, L., Tardugno, R., Vadalà, R., & Cicero, N. (2023). Natural pigments production and their application in food, health and other industries. *Nutrients*, *15*(8), 1923.
18. Dunford, E. K., Galligan, T. M., Taillie, L. S., & Musicus, A. A. (2025). All the Colours of the Rainbow: Synthetic Dyes in US Packaged Foods and Beverages in 2020. *Journal of the Academy of Nutrition and Dietetics*.
19. Fatima, I., Munir, M., Qureshi, R., Hanif, U., Gulzar, N., & Sheikh, A. A. (2024). Advanced methods of algal pigments extraction: A review. *Critical Reviews in Food Science and Nutrition*, *64*(27), 9771-9788.
20. Fernandes, R., Campos, J., Serra, M., Fidalgo, J., Almeida, H., Casas, A., ... & Barros, A. I. (2023). Exploring the benefits of phycocyanin: From Spirulina cultivation to its widespread applications. *Pharmaceuticals*, *16*(4), 592.
21. Gangopadhyay, A., Keshri, S., Panigrahi, S. P., Goswami, T. D., & Panda, N. (2022). A general study of stability enhancement factors, formulations, and pharmacological application of glycosidic dye betalain. *Journal of Pharmaceutical Negative Results*, *13*(9).
22. Ge, J., Wang, X., Bai, Y., Wang, Y., Wang, Y., Tu, T., ... & Zhang, J. (2023). Engineering Escherichia coli for efficient assembly of heme proteins. Microbial Cell Factories, 22(1), 59.
23. Ghosh, S., Sarkar, T., Das, A., & Chakraborty, R. (2022). Natural colorants from plant pigments and their encapsulation: An emerging window for the food industry. *Lwt*, *153*, 112527.
24. Guo, C., Li, Y., Zhang, H., Zhang, Q., Wu, X., Wang, Y., ... & Xia, X. (2024). A review on improving the sensitivity and colour stability of naturally sourced pH-sensitive indicator films. *Comprehensive Reviews in Food Science and Food Safety*, *23*(4), e13390.
25. Guo, Q., Peng, Q. Q., Li, Y. W., Yan, F., Wang, Y. T., Ye, C., & Shi, T. Q. (2024). Advances in the metabolic engineering of Saccharomyces cerevisiae and Yarrowia lipolytica for the production of β-carotene. Critical Reviews in Biotechnology, 44(3), 337-351.
26. Hegazy, A. A., Abdel Haliem, W. A. H. R., El-Bestawy, E. M., & Ali, G. M. E. (2023). Brief overview about tartrazine effects on health. *Eur. Chem. Bull*, *12*, 4698-4707.
27. Hou, Y., Guan, A., Fan, X., & Qin, J. (2025). Harnessing synthetic biology for astaxanthin production: recent advances and challenges. Synthetic and Systems Biotechnology.
28. Hu, M., Lu, X., Qin, S., Liu, R., Wang, Q., Lu, C., & Li, W. (2024). Research progress on the biosynthesis, activity and application of natural tetrapyrrole compounds. Arabian Journal of Chemistry, 17(5), 105736.
29. Jiang, X., Hong, X., Wang, Z., Liu, J., Zhong, H., Ren, J., & Zhou, B. (2024).

Phospholipid biosynthesis regulation for improving pigment production by Monascus in response to ammonium chloride stress. Applied and Environmental Microbiology, 90(10), e01146-24.

30. Jiang, Y., Ye, J., Hu, Y., Zhang, J., Li, W., Zhou, X., ... & Hu, Y. (2024). Extraction and synthesis of typical carotenoids: lycopene, β-carotene, and astaxanthin. Molecules, 29(19), 4549.
31. Joshi, S., Appukuttan, J., Chandrapala, J., & Majzoobi, M. (2025). Impact of Conventional and Advanced Techniques on Stability of Natural Food Colorants. *Foods*, *14*(18), 3187.
32. Jurić, S., Jurić, M., Król-Kilińska, Ż., Vlahoviček-Kahlina, K., Vinceković, M., Dragović-Uzelac, V., & Donsì, F. (2022). Sources, stability, encapsulation and application of natural pigments in foods. Food reviews international, 38(8), 1735-1790.
33. Kobayashi K and Masuda T (2016) Transcriptional Regulation of Tetrapyrrole Biosynthesis in Arabidopsis thaliana. Front. *Plant Sciences*. 7:1811. doi: 10.3389/fpls.2016.01811
34. Koop, B. L., Maciel, A. G., Soares, L. S., Monteiro, A. R., & Valencia, G. A. (2022). Natural colorants. *Natural Additives in Foods*, 87-122.
35. Lin, L., Zhang, T., & Xu, J. (2023). Genetic and environmental factors influencing the production of select fungal colorants: challenges and opportunities in industrial applications. Journal of Fungi, 9(5), 585.
36. Lyu, X., Lyu, Y., Yu, H., Chen, W., Ye, L., & Yang, R. (2022). Biotechnological advances for improving natural pigment production: A state-of-the-art review. *Bioresources and bioprocessing*, *9*(1), 8.
37. Malabadi, R. B., Kolkar, K. P., & Chalannavar, R. K. (2022). Plant natural pigment colorants-health benefits: toxicity of synthetic or artificial food colorants. *Int. J. Innov. Sci. Res. Rev*, *4*, 3418-3429.
38. Miller, M. D., Steinmaus, C., Golub, M. S., Castorina, R., Thilakartne, R., Bradman, A., & Marty, M. A. (2022). Potential impacts of synthetic food dyes on activity and attention in children: a review of the human and animal evidence. *Environmental Health*, *21*(1), 45.
39. Mota, I. G. C., Neves, R. A. M. D., Nascimento, S. S. D. C., Maciel, B. L. L., Morais, A. H. D. A., & Passos, T. S. (2023). Artificial dyes: Health risks and the need for revision of international regulations. *Food Reviews International*, *39*(3), 1578-1593.
40. Mussagy, C. U., Kot, A., Dufossé, L., Gonçalves, C. N., Pereira, J. F., Santos-Ebinuma, V. C., ... & Pessoa Jr, A. (2023). Microbial astaxanthin: from bioprocessing to the market recognition. Applied Microbiology and Biotechnology, 107(13), 4199-4215.
41. Negi, A. (2025). Natural dyes and pigments: sustainable applications and future scope. *Sustainable Chemistry*, *6*(3), 23.
42. Newsome, A. G., Murphy, B. T., & Van Breemen, R. B. (2013). Isolation and characterization of natural blue pigments from underexplored sources. In *Physical methods in food analysis* (pp. 105-125). American Chemical Society.

43. Novais, C., Molina, A. K., Abreu, R. M., Santo-Buelga, C., Ferreira, I. C., Pereira, C., & Barros, L. (2022). Natural food colorants and preservatives: A review, a demand, and a challenge. *Journal of agricultural and food chemistry*, *70*(9), 2789-2805.
44. Eichenberger M, Hansson A, Fischer D, Dürr L, Naesby M., (2018). De novo biosynthesis of anthocyanins in Saccharomyces cerevisiae. *FEMS Yeast Res* 18(4)
45. Ochoa-Viñals, N., Alonso-Estrada, D., Pacios-Michelena, S., García-Cruz, A., Ramos-González, R., Faife-Pérez, E., ... & Iliná, A. (2024). Current Advances in Carotenoid Production by Rhodotorula sp. Fermentation, 10(4), 190.
46. Pizzicato, B., Pacifico, S., Cayuela, D., Mijas, G., & Riba-Moliner, M. (2023). Advancements in sustainable natural dyes for textile applications: a review. *Molecules*, *28*(16), 5954.
47. Pognan, F., Beilmann, M., Boonen, H. C., Czich, A., Dear, G., Hewitt, P., ... & Newham, P. (2023). The evolving role of investigative toxicology in the pharmaceutical industry. *Nature reviews drug discovery*, *22*(4), 317-335.
48. Prabowo, C. P. S., Eun, H., Yang, D., Huccetogullari, D., Jegadeesh, R., Kim, S. J., & Lee, S. Y. (2022). Production of natural colorants by metabolically engineered microorganisms. *Trends in Chemistry*, *4*(7), 608-626.
49. Rather, L. J., Mir, S. S., & Islam, Z. (2025). Introduction to Microbial Colorants. *Microbial Colorants: Chemistry, Biosynthesis and Applications*, 1-32.
50. Shaaban, H. A., Abdel-Gawad, H. H., & Sleem, M. M. (2024). Composition and Health Benefits of Carotenoids in Foods and Feeds. Carotenoids: Trends and Advances, 227-244.
51. Sharma, C., Kamle, M., & Kumar, P. (2024). Microbial-derived carotenoids and their health benefits. Microbiology Research, 15(3), 1670-1689.
52. Siddiqui, S. A., Pahmeyer, M. J., Mehdizadeh, M., Nagdalian, A. A., Oboturova, N. P., & Taha, A. (2022). Consumer behavior and industry implications. In *The age of clean label foods* (pp. 209-247). Cham: Springer International Publishing.
53. Singh, T., Pandey, V. K., Dash, K. K., Zanwar, S., & Singh, R. (2023). Natural bio-colorant and pigments: Sources and applications in food processing. *Journal of Agriculture and Food Research*, *12*, 100628.
54. Stachelska, M. A., Karpiński, P., & Kruszewski, B. (2025). A comprehensive review of biological properties of flavonoids and their role in the prevention of metabolic, cancer and neurodegenerative diseases. Applied Sciences, 15(19), 10840.
55. Su, H., Chen, X., Chen, S., Guo, M., & Liu, H. (2023). Applications of the whole-cell system in the efficient biosynthesis of heme. International Journal of Molecular Sciences, 24(9), 8384.
56. Sultana, S., Rahman, M. M., Aovi, F. I., Jahan, F. I., Hossain, M. S., Brishti, S. A., ... & Sharma, R. (2023). Food colour additives in hazardous consequences of human health: An overview. *Current Topics in Medicinal Chemistry*, *23*(14), 1380-1393.

57. Wan, S., Liu, X., Sun, W., Lv, B., & Li, C. (2023). Current advances for omics-guided process optimization of microbial manufacturing. Bioresources and Bioprocessing, 10(1), 30.
58. Wijesinghe, V. N., & Choo, W. S. (2022). Antimicrobial betalains. *Journal of Applied Microbiology*, *133*(6), 3347-3367.
59. Withanachchi, R. K., Jayatilleke, Y. D., & Silva, O. T. D. (2024). An Anthropological Study on the Use of Natural Flavoring Agents in Sri Lankan Traditional Food Culture. *Vidyodaya Journal of Humanities and Social Sciences*, *9*(02).
60. Xing, Y., Gao, S., Zhang, X., & Zang, J. (2022). Dietary heme-containing proteins: Structures, applications, and challenges. Foods, 11(22), 3594.
61. Yadav, S., Sharma, A., Bishnoi, S., Gaur, M., Tomar, D., & Kumar, A. (2026). The Role of Anthocyanins in Cardiovascular Health: A Review. *Current Cardiology Reviews*, *22*(2), E1573403X372621.
62. Yan, C., Kim, S. R., Ruiz, D. R., & Farmer, J. R. (2022). Microencapsulation for food applications: A review. *ACS Applied Bio Materials*, *5*(12), 5497-5512.
63. Yuan, B., Li, Z., Shan, H., Dashnyam, B., Xu, X., McClements, D. J., ... & Cao, C. (2022). A review of recent strategies to improve the physical stability of phycocyanin. *Current Research in Food Science*, *5*, 2329-2337.
64. Zhang, J., Yang, Y., Mao, Z., Yan, Q., Chen, Q., Yi, M., & Shao, Y. (2022). Inactivation of MrSir2 in Monascus ruber influenced the developmental process and the production of Monascus azaphilone pigments. Applied Biochemistry and Biotechnology, 194(12), 5702-5716.
65. Zhang, Y. W., Nie, F., Zheng, X. Y., & Zhao, S. J. (2022). L-tyrosine metabolic pathway in microorganisms and its application in the biosynthesis of plant-derived natural products. World Journal of Traditional Chinese Medicine, 8(3), 386-394.