Bio-colours from vegetable crops - A Review

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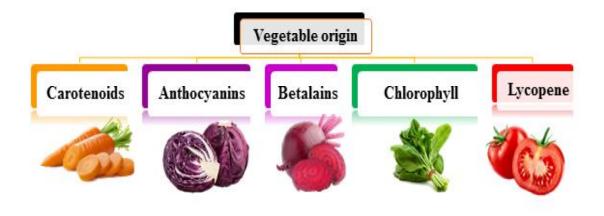
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Abstract

Pigments act as natural colourant which are observed to explore the world in all possible fields of lives. Natural pigments that are obtained from plants sources like chlorophyll, carotenoids, betacyanin etc., are used in medicines, preservation, food, textiles, molecular analysis that are probably safe towards the environment. Colours that come from natural sources are referred to as "Biocolours." Typically, they are obtained from various plant parts like fruits, vegetables, seeds, roots, as well as from microorganisms. Paprika and beet are some of the sources for vegetable based biocolours. The natural dyes are eco-friendly in nature because they are obtained from various plant sources.

Key words : Bio-colours, vegetable crops, carotenoids, Bioactive compounds, anthocyanins, Betalins, Lycopene.

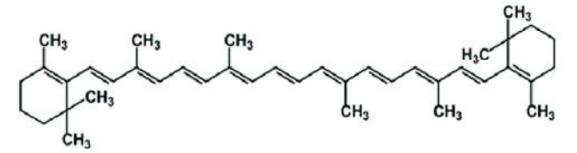
A natural colorant, often referred to as "Biocolour," encompasses dyes or pigments that, when incorporated into products⁵. The term "Biocolour" combines "bio," signifying its natural origin, and "colour," denoting its function in adding colour. Biocolourants are colouring agents sourced from biological materials, including plants, algae, insects, fungi, and animals, capable of imparting vibrant hues to food products. These biocolourants are derived from renewable sources, with the majority originating from plants. Beyond their role in food colouring, biocolourants possess bioactive properties and have found applications as therapeutic agents. Natural colours are preferable to artificial alternatives due to the adverse effects associated with artificial food colourings, which may include attention deficit hyperactivity disorder (ADHD), behavioral problems, depression, food allergies, headaches, and migraines. Desserts, doughnuts, fruit preserves, ice creams, colas, and sweets frequently include artificial food colorings for a better appearance, as mentioned by the European Food Safety Authority¹² (EFSA) in 2011. Biocolours is a viable alternative for artificial food colouring to avoid hazardous effects in human beings and environment. Specific pigments tend to dominate in certain plants, such as lycopene in tomatoes and capsanthin in red peppers,



2. Biocolours in vegetable crops : Carotenoids :

Carotenoids, which are orange-yellow pigments, are fat-soluble compounds found in a wide range of vegetables and fruits like carrots, pumpkins, mangoes, and oranges. The initial discovery of a carotenoid came from carrots, leading to its name "carotene." The vibrancy of a vegetable's colour often serves as an indicator of the concentration of carotenes within it. Lutein, violaxanthin, and neoxanthin are the most commonly found carotenoids, predominantly present in green leaves. Carotene and zeaxanthin, while widespread, are typically present in smaller quantities.

Carotenoids can also be found in most green leafy vegetables, coexisting with chlorophyll. However, their color is often concealed by the green pigment of chlorophyll. The fresh, yellow-green hue observed in spring leaves is a result of carotenoids, complemented by a small amount of chlorophyll. Carotenoids are commonly extracted from sources like annatto, saffron, paprika, tomatoes, and more, and are employed as natural food colorants. Extracts from carrots, butterfat, and palm contain oil with β -carotene, a precursor of vitamin A, imparting these extracts with vitamin A activity²³.



Structure of Beta carotene (Jeyakodi et al., 2008)

Clinical application

Carotenoids are photo-protectants. Lee *et al.*,²² suggested that supplementation with natural carotenoids may partially protect human skin from UV-A and UV-B induced erythema. Astaxanthin is a carotenoid that plays a significant role in the reproductive process as an intermediary compound.

Extraction and encapsulation of bioactive compounds from $carrot^{29}$:

The primary objectives of this study were to optimize the extraction and encapsulation processes for carotenoids obtained from carrots. Freeze-dried carrots underwent extraction through conventional solvent extraction (CSE) employing four different solvents: ethanol, acetone, ethyl acetate, and hexane. Among these, hexane and ethyl acetate yielded the highest carotenoid contents, measuring 18.27 and 15.73 mg β -carotene per 100 grams, respectively. However, for further investigations, acetone and ethanol were chosen, even though they yielded slightly lower carotenoid contents (14.52 and 11.45 mg βcarotene per 100 grams). This decision was based on their enhanced compatibility with food applications and their higher polyphenol content, which measured 88.86 and 66.21 mg GAE (Gallic Acid Equivalents) per 100 grams, particularly when utilizing a lower solid-tosolvent ratio of 1:10 w/v.

To achieve the highest carotenoid encapsulation efficiency (EE), ethanol and acetone carrot extracts were subjected to encapsulation using various carriers via freezedrying. Wall materials such as maltodextrin, whey, and soy protein were employed, resulting in carotenoid EE ranging from 41.95% to 100%. Over the course of a two-month storage period at ambient temperature, both under light and dark conditions, the encapsulated β -carotene content was monitored. Generally, carotenoid retention was significantly better in dark conditions, with the highest retention (ranging from 65.94% to 87.32%) observed in samples encapsulated with maltodextrin and soy protein.

Extraction of bioactive compound from some fruits and vegetables (Pomegranate peel, carrot and tomato)³¹:

In this study, the extraction of Betacarotenes from carrot roots was conducted under various conditions, including different temperatures, sample treatments, and solvent choices (ethanol and methanol). Carrot roots were subjected to extraction at three different temperatures: 20°C, 40°C, and 60°C. The samples were analyzed at different stages, including immediately after harvest, after cold storage (held at 5°C), and after freezing at $-5^{\circ}C$.

The most efficient extraction was achieved by freezing the samples and using an extraction temperature of 60°C for a duration of 2 to 4 hours. After 5 hours of extraction from fresh samples, the carotene yield was 1.58 mg/100 g, with the extraction rate notably slowing down. When the extraction was carried out at 40°C, the carotene yield increased to 2.45 mg/100 g compared to the 20°C extraction, but the highest yield was obtained at 60°C. At 60°C, the maximum extraction occurred in the second hour, resulting in a yield of 4.28 mg/100 g. Beyond this point, the extraction yield of β-carotene decreased, likely due to carotene degradation and loss, a phenomenon previously reported by Sharmin *et al.*,³¹.

Beta carotene content of some commonly consumed vegetables and fruits available in Delhi, India²⁵ :

The majority of dietary vitamin A in developing countries is derived from plantbased sources. This study was conducted with the aim of assessing the β -carotene content in a total of 26 types of green leafy vegetables, tubers, other vegetables, and fruits obtained from four wholesale markets in Delhi, India, utilizing High-Performance Liquid Chromatography (HPLC) for analysis.

The study revealed a considerable variation in β -carotene content among green leafy vegetables, with mean levels ranging from 2199 µg/100 g in *Basella rubra* to 7753 µg/100 g in *Amaranthus gangeticus*. Similarly, a wide range of β -carotene content was observed in fruits, including various mango varieties. Levels ranged from undetectable in strawberries to 808.60 µg/100 g in Totapuri mango, with alphonso mango registering the highest level at 11789 µg/100 g.

These findings have practical implications, indicating that consuming approximately 65 grams of green leafy vegetables would meet the daily β -carotene requirements of a preschooler, while an older child or adult would require approximately 100 grams to meet their daily needs.

Production and application of natural dye from skin of yellow pumpkin vegetable¹⁶ :

In this study, a natural dye was extracted from vegetable waste, specifically pumpkin skin, and utilized for dyeing cotton fabric under various dyeing conditions using mordants. Additionally, a comparative analysis was conducted with a chemical dye.

The natural dye and chemical dye were subjected to decolorization and degradation processes using Bacillus licheniformis and Aspergillus niger. Both microorganisms effectively degraded the natural and chemical dyes enzymatically, converting them into nontoxic products. Bacillus licheniformis exhibited optimum decolorization at 37°C, while Aspergillus niger showed optimal decolorization at 30°C. Under these conditions, Bacillus licheniformis produced enzyme levels of 13.1 IU/ml for pumpkin dye and 5.1 IU/ml for the chemical effluent. Aspergillus *niger*, under similar conditions, produced enzyme levels of 6.5 IU/ml, 8.3 IU/ml, 7.3 IU/ ml, 11 IU/ml, 9.1 IU/ml, and 6.1 IU/ml for different samples.

Following the degradation process, the water containing natural dye, chemical dye effluent, and their respective treated waters were used to cultivate chickpeas under controlled conditions. Morphological studies of the grown plants showed variations. Chickpea seeds treated with pumpkin dye waste resulted in the growth of 9 out of 10 seeds, while only 1 out of 10 seeds grew in the chemical dye waste. The number of leaves also varied significantly, with 69 leaves in the pumpkin dye-treated group and 22 leaves in the chemical dye-treated group. This study highlights that chemical dyes have a detrimental impact on the environment, affecting soil fertility and plant growth.

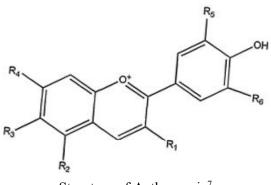
Furthermore, chemical effluents pose risks to animals and humans when they come into contact with contaminated resources. The findings emphasize the potential benefits of using natural dyes, which are eco-friendly as they are derived from plant sources, as a more sustainable alternative to chemical dyes.

Anthocyanin :

The term "anthocyanin" is derived from Greek word "anthos," which means flower, and "kyanos," signifying dark blue. Anthocyanin is a water-soluble plant pigment and polyphenolic compound used widely in the international food and beverage industries as a natural food colorant. It imparts a spectrum of colors, ranging from orange, red, pink, and purple to blue. Anthocyanins serve as essential food colorants in beverages, food products, confectioneries, and fruit preparations. The colour they provide depends on the pH of the environment; anthocyanins can appear red, violet, or blue. At acidic conditions, many anthocyanins exhibit a red hue, while at less acidic conditions, they tend to shift towards blue.

These pigments are present in various parts of plants, including fruits, stems, and leaves, and their levels may vary. In plants, one of the primary roles of anthocyanins is to act as a form of natural sunblock. These dark pigments serve to protect plants from sun damage, reducing the risk of sunburn and other harmful effects caused by intense sunlight exposure³³.

Anthocyanins in flowers make them bright red to help birds, bees, and other organisms find them easily and assist in pollination.



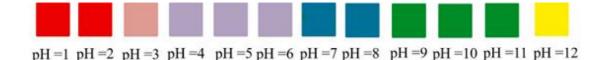
Structure of Anthocyanin⁷

Extraction methods :

- ✓ Solvent extraction
- ✓ Pulsed Electric Field (PEF) processing
- ✓ High Pressure Carbon Dioxide (HPCD) extraction
- ✓ Pressurized Liquid Extraction (PLE)
- ✓ Ultra Sound Assisted Extraction (UAE)
- ✓ Microwave Assisted Extraction (MAE)

Stability :

Stability is defined as the incapability of flavylium cations in transforming into colourless forms of carbinol pseudobases and chalcones⁶. The chemical structure of anthocyanins has an impact on both their stability and colour¹¹. The formation of chalcone might be considered the primary stage for the deterioration of anthocyanins³⁹. Since anthocyanins are highly unstable and very susceptible to degradation, in spite of being abundant in nature, they cannot be applied in the food industry³⁰. Their stability is affected by various factors such as pH, temperature, enzymes, light, oxygen, and ascorbic acid¹¹. (807)



Processing techniques :

- ✓ Spray drying
- ✓ Freeze drying
- ✓ Gelatin
- ✓ Lipid based particles
- ✓ Electrohydrodynamic process

Applications of anthocyanin :

Anthocyanins find wide use in food and beverage industries, including soft drinks, instant beverages, fruit juices, liquors, confectionery, fruit jellies, jams, and more.

Clinical application :

Enhancement of sight acuteness, treatment of various blood circulation disorders resulting from capillary fragility³⁸, radiation protective action¹, to examine the anti-cancer effects³⁵.

Genotypic differences for anthocyanins in different parts of eggplant (Solanum melongena L.)²⁰ :

Anthocyanins are a crucial group of secondary metabolites responsible for the diverse colours found in fruits. They play a vital role in protecting plants from UV radiation and insect attacks. In humans, anthocyanins are believed to offer various health benefits, including anti-inflammatory, antiulcer, antioxidant, and antimutagenic properties. Specifically, Brinjal is recognized as a rich source of anthocyanins, which are responsible for the purple coloration seen in its fruits. In this study, 50 different brinjal genotypes bearing fruits of varying colours (purple, pink, green, and white) were assessed for their anthocyanin content in the peel, flesh, and whole fruit, using fresh tissue samples in both 2012 and 2013.

Consistently, in both years, the highest anthocyanin content was found in the peel, followed by the whole fruit, and then the flesh part. Fruits with green and white colours exhibited either low or negligible anthocyanin content in their peels. Among the genotypes evaluated, those with the highest anthocyanin content in the peel, flesh, and whole fruit were SR-312 (purple), SR-308 (green), and SR-303 (purple), respectively. Interestingly, there was a negative correlation between the weight of the fruit and the anthocyanin content present in the peel. Conversely, a positive correlation was observed between the anthocyanin content in the peel and the content in the whole fruit.

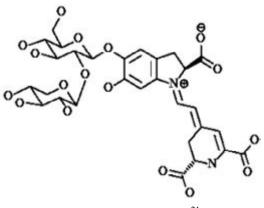
Evaluation of red pigment extracted from purple carrots and its utilization as antioxidant and natural food colorants⁴:

Purple carrots are found to contain approximately 168.7 mg of anthocyanin per 100 grams on a fresh weight basis. The primary components of these anthocyanins include Cyanidin-3-xylosyl-glucosyl-galactoside acylated with ferulic acid (making up 33.65% of the total), followed by Cyanidin-3-xylosyl-glucosyl-galactoside acylated with coumaric acid (29.85%), and Cyanidin-3-xylosyl-galactoside (28.70%). These constituent percentages were determined using High-Performance Liquid Chromatography (HPLC).

In terms of carriers, dextrin was found to be the most effective for preserving the anthocyanin pigment from purple carrots, followed by cellulose, soluble starch, and glucose. The anthocyanin pigment derived from purple carrots exhibited the highest colour stability within a pH range of 1.0 to 4.0 and at temperatures between 40 and 80°C. However, when exposed to higher temperatures, particularly 100°C, a degradation ratio of 15% of the total pigments was observed after a duration of 180 minutes.

Betalains :

Khan and Giridhar¹ conducted a study to estimate the annual production potential of plant betalains from various edible sources, including red beetroot, Swiss chard petiole, cactus pear fruit, pitaya fruit, and amaranth seed. Their findings showed that beetroot had the highest production potential compared to the other sources. Betalains are water-soluble pigments responsible for red and yellow colors found in red beet, cactus fruits, and certain flowers. These pigments remain stable within a pH range of 4-6 but are susceptible to degradation during thermal processing. Due to their sensitivity to various factors, the use of betalains as food colorants is limited. They are best suited for foods with a short shelflife, minimal heat treatment, and packaging that protects them from light, oxygen, and humidity^{28,37}.



Structure of Betalains²¹

Extraction methods³⁶:

- \checkmark Aqueous extraction
- \checkmark Acid extraction
- ✓ Alkali extraction

Drying method :

- ✓ Spray drying
- ✓ Freeze drying

Extraction of natural colour from beetroot (Beta vulgaris) its phytochemical analysis and antibacterial activity⁴⁰:

Beetroot (*Beta vulgaris*) is a rich source of antioxidants and essential minerals like sodium, potassium, iron, and magnesium. It is low in calories (about 45 Kcal per 100g) and contains no cholesterol. Food colour is a crucial factor in enhancing food quality. Beetroot possesses various medicinal properties, including anti-hypertensive, antimicrobial, antiinflammatory, hepato-protective, anti-cancer, and diuretic effects. This study aimed to promote the use of natural colorants in food and enhance overall nutritional quality. The colour extraction process was carried out through aqueous extraction. The highest colour yield from beetroot was achieved at 40°C for 20 minutes, with a yield of 12.7%. Chemical analysis revealed the presence of phytochemicals associated with the health benefits of beetroot. These findings indicate that beetroot possesses valuable phytochemical properties, making it a beneficial addition to both food products and overall health.

*Extraction of natural dye and preparation of herbal gulal from beetroot (Beta vulgaris)*³⁶ :

This study aimed to extract more colouring components while maintaining an environmentally friendly extraction process without relying heavily on organic solvents. Three extraction techniques were employed: aqueous, acidic, and alkaline techniques, to extract natural dye from beetroot. In total, six samples of beetroot extract were prepared. The extraction methods included the use of different concentrations of NaOH in the alkaline technique, specifically 0.10M, 0.20M, 0.30M, and 0.40M.

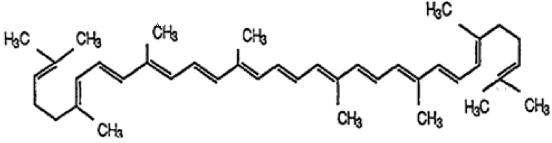
Study on optimization and pigment analysis of beetroot (beta vulgaris) and their application as natural dyes³:

Natural sources like fruits and vegetables, abundant in nutrients, eco-friendly pigments, and antioxidants, are gaining attention. Notably, the often-discarded peels of these sources are being re-evaluated for their environmental applications. Utilizing a simple aqueous extraction method, valuable natural colorants can be efficiently processed. The study focused on beetroot, revealing its richness in betacyanin pigments, which are water-soluble compounds. Through rigorous experimentation, including temperature variations (room temperature to 110p C), different heating durations (1 to 10 minutes), and diverse pH levels (2 to 9), optimal conditions for betacyanin extraction and application were determined. Additionally, the study assessed pigment stability, enhancing its suitability for future analytical applications. The aim of this research is to provide an eco-friendly, traditionally obtained natural dye, abundant in environmentally friendly pigments, offering significant value.

Lycopene :

Lycopene is the pigment principally responsible for the characteristic deep red colour of ripe tomato fruits and tomato products; it is also found in watermelon, papaya, pink grapefruit and pink guava. Processed tomato products are more available dietary sources of lycopene than fresh tomatoes. Lycopene is a member of the carotenoid family; it is a natural fat-soluble pigment found in certain plants and microorganisms, where it serves as an accessory light gathering pigment and to protect these organisms against the toxic effects of oxygen and light²⁷. The average daily intake of lycopene is approximately 25 mg; 50% of this is in the form of processed tomato products.

(810)



Structure of Lycopene¹⁹

Sources of lycopene

Common name	Туре	Species
Tomato	Fruit	Lycopersicon esculentum
Watermelon	Fruit	Citrullus lanatus
Pumpkin	Fruit	Cucurbita pepo
Plum	Fruit	Prunus domestica
Carrot	Root	Daucus carota

(Choksi et al., 2007)

*Extraction methods of lycopene*¹³ :

- ✓ Solvent extraction
- ✓ Enzyme Aided Extraction
- ✓ Supercritical Fluid Extraction (SCFE)

Extraction of bioactive compound from some fruits and vegetables (pomegranate peel, carrot and tomato)³¹:

In a study conducted by Sharmin *et al.*, 2016, the extraction of lycopene from tomatoes was explored by varying extraction times and using different solvents, including hexane, petroleum benzene, and a ternary mixture of hexane, ethanol, and petroleum benzene in a 50:25:25 ratio. The researchers

found that the highest lycopene recovery occurred when the extraction time was set at 1 hour. Under these conditions, lycopene yields were 9.4 mg/100g in hexane, 8.7 mg/100g in petroleum benzene, and notably higher at 12.3 mg/100g in the ternary mixture.

As the extraction time extended beyond one hour, there was a progressive reduction in lycopene yields. This indicated that the enzymatic degradation of cell wall components was rapid, primarily occurring within the first hour of extraction. Consequently, most of the lycopene molecules initially enclosed within protective chromoplast structures were quickly released into the external environment, where they were susceptible to rapid oxidative degradation. Regarding the choice of solvents, hexane and petroleum benzene showed similar extraction efficiencies, while the ternary mixture of hexane/petroleum benzene/ethanol 50:25:25 proved to be more effective. This suggests that petroleum benzene and ethanol played auxiliary roles in facilitating solvent penetration by swelling the plant tissue, enhancing overall extraction. These beneficial effects were particularly noticeable when the tomato pulp's structural integrity was preserved, resulting in lycopene recoveries of 12.3 mg per 100g at 1 hour using the hexane/ petroleum benzene/ethanol mixture.

Lycopene content, antioxidant capacity and colour attributes of selected watermelon (Citrullus lanatus) cultivars grown in India²⁴:

This study investigated the variability in lycopene, ascorbic acid, total phenolics, antioxidant capacity, and colour attributes among 12 watermelon cultivars grown in India. The evaluation of antioxidant capacity involved four *in vitro* assays: ferric reducing antioxidant power, cupric reducing antioxidant capacity, Trolox equivalent antioxidant capacity, and 2,2diphenyl-1-picryl hydrazyl.

Among the watermelon cultivars studied, significant differences (p < 0.05) were observed in terms of lycopene content and antioxidant capacity. Lycopene content ranged from 3.46 to 8.00 mg/100 g of fresh weight. The colour of watermelon flesh was assessed using an optimized colour index (CI). Notably, cultivars such as 'PWM25-4,' 'Arun,' 'Kiran,' and 'Kareena' displayed the highest lycopene content, antioxidant capacity, and CI values.

These findings highlight watermelon as a valuable dietary source of lycopene, with significant variations among cultivars. This diversity can be harnessed to develop highquality watermelon cultivars with enhanced nutritional and antioxidant properties.

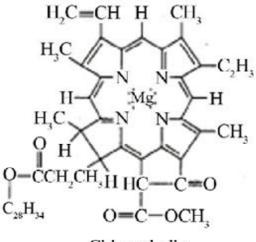
*Effect of different parameters on enzyme assisted extraction of lycopene from tomato processing waste*²⁶ :

This study aimed to optimize the extraction process for lycopene using different solvents and explore the impact of enzyme treatment on lycopene recovery. Various parts of tomato fruits, including the whole tomato, peel, pulp, and industrial waste, were assessed for their lycopene content. Lycopene extraction was conducted using four different solvents to assess their efficiency. For enzyme-assisted extraction, a two-step process was followed. In the first step, waste samples were treated with cellulase and pectinase enzymes, while the second step involved lycopene extraction with a solvent. Optimization studies were conducted for enzyme concentrations and incubation times. Additionally, the influence of particle size and cooking methods on lycopene recovery was investigated.

The findings showed that tomato peel had the highest lycopene content (376.17 μ g/ g), followed by industrial waste (176.17 μ g/g), whole tomatoes (83.9 μ g/g), and pulp (47.6 μ g/ g). Tri-mixture solvent (acetone, ethanol, and hexane) yielded the highest lycopene recovery compared to other solvents. Maximum lycopene recovery was achieved with 1.5% cellulase and 2% pectinase during a 4-hour incubation period. Finer particle sizes resulted in higher lycopene recovery, while all cooking methods led to a reduction in lycopene recovery. This optimized extraction process could benefit small-scale entrepreneurs, contributing to improved socio-economic conditions, as reported by Ranveer *et al.*,²⁶.

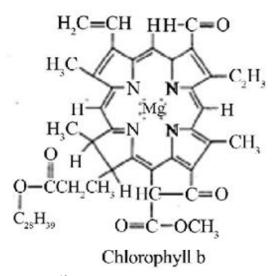
Chlorophyll :

Chlorophyll is green pigment found in algae and plants. When Chlorophyll and Chlorophyllins are used as a food additive it is known as E140. It is usually extracted from nettles, grass and alfalfa and used in pasta, absinthe, cheeses, preserved vegetables, jams, jellies and marmalade². Although there are five



Chlorophyll a

different types of chlorophylls, only two, chlorophylls a and b, are utilized in the food industry as colorants. The main challenge in using chlorophylls lies in their intricate structure, which makes stabilization difficult. The industry has been exploring methods to retain or substitute the magnesium ion within their structure. Commercial chlorophyll colorants are typically extracted from alfalfa and are used in a variety of food products such as dairy items, soups, beverages, and sugary confections.





Clinical applications :

Chlorophyll plays a role in promoting a healthy gut microbiome, which in turn enhances our immune system function. Chlorophyll has great antioxidant capacity and therefore it helps neutralize free radicals and limit oxidative damage within the body. Numerous animal and *in vitro* research studies have demonstrated that chlorophyll and its derivatives possess anti-carcinogenic properties, meaning they can help prevent or slow down the development of cancer. Additionally, research has shown that chlorophyll can enhance the health of blood cells by increasing the uptake of oxygen in the bloodstream. Austrian scientists have found that chlorophyll and its derivatives successfully slow down the oxidation of LDL (the 'bad' cholesterol) which is a major contributor to cardiovascular disease³². Variation in chlorophyll and carotenoid contents in kale (Brassica oleracea var. Sabellica) as influenced by cultivars and harvesting dates³⁴:

This study focused on assessing the levels of total chlorophyll and carotenoids in kale leaves of three commercial cultivars: Siberian Kale, Khanyari, and Japanese Green. The research took place at the Central Institute of Temperate Horticulture in Srinagar, Jammu & Kashmir, spanning two consecutive years (2009-2010). During each year, kale transplants were planted in the field, and three harvests were conducted at 5, 7, and 9 weeks after planting.

Throughout the study, the average chlorophyll content in 100g of fresh weight ranged from 136.18 g in Siberian Kale to 172.10 g in Japanese Green. In all cultivars, chlorophyll content significantly increased from the first to the second harvest, with the most substantial increase (3.5%) observed in Japanese Green. Subsequently, chlorophyll levels decreased in the third harvest. Similarly, the levels of carotenoids varied significantly among different cultivars, with the highest concentration (23.50 mg/100g) found in Japanese Green and the lowest (18.99 mg/ 100g) in Siberian Kale. The study also noted significant differences related to harvest dates and the interaction between harvest date and cultivars.

Anthoxanthins :

Anthoxanthin is a pigment that shares similarities with anthocyanin, but it is found in a less oxidized state because the oxygen on the central group is uncharged. It is actually a composite of compounds known as flavones, flavanols and flavanones. Anthoxanthins are white, pale yellowish, water-soluble pigments found in a plant's cell sap. Anthoxanthins are responsible for the cream and white colouration in vegetables like cauliflower, onions, white potatoes, and turnips. It's important to note that these pigments can become brownish-grey when subjected to prolonged cooking⁹.

Clinical applications :

Anthoxanthins are believed to offer health benefits because of their strong antioxidant capacity, as demonstrated in both *in vivo* and *in vitro* studies. These pigments are thought to promote human health by stimulating protective enzyme systems and have shown potential protective effects against conditions like cardiovascular diseases, cancers, and other age-related illnesses, as indicated by various epidemiological studies¹⁰.

The food industry is increasingly shifting its attention away from synthetic food colorants due to growing concerns about their safety-in-use, leading to an uncertain future for these synthetic additives. Instead, there is a noticeable trend towards embracing biocolours, which are natural colorants with a focus on plant-based sources. This transition is driven by heightened consumer awareness of the health risks associated with synthetic colours.

Biocolourants, in contrast to their synthetic counterparts, are prepared from renewable resources, primarily sourced from plants. They not only impart vibrant colours to food products but also offer additional benefits as antimicrobials and antioxidants, contributing to the prevention of various diseases and health disorders in human consumers. One of the key advantages of biocolours is their eco-friendliness, as they are obtained from diverse plant sources, aligning with the growing demand for sustainable and natural food additives in the modern food industry.

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