

# Advance Ecological Approach to New Assessment in Red Plant Pigments

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## ABSTRACT

Natural food colour production is increasing all over the world. Food sources have been colored with pigments like betalains, chlorophylls, anthocyanin's and carotenoids. The current situation is more focused on using the prodigious diversity of natural colour pigment supplies for use in food products, pharmaceuticals, and textiles, rather than their synthetic counterparts, in order to preserve and prolong human health as well as life on Earth. Since natural pigments are less stable than synthetic colourants, there are challenges relayed with colour loss throughout food handling, storage, and commercialization. The addition of copigment materials, which are identical to polymers, phenolic mixtures and metals, encapsulation technology included in the stabilising techniques. The stability is also increase by reducing the toxic effect of plant secondary metabolites. Recently, researchers have discovered that newly exposed auronidins could be used as possible food pigments in the future. Combining processes and evaluating new materials that can stabilise anthocyanins and improve their ability to be exploited as important attribute of natural food pigments.

**Key words:** Natural pigments, Anthocyanin, Extraction technologies, Color loss, Stability of anthocyanin.

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## INTRODUCTION

Plants have a fundamental and essential function in supporting life on the planet. Plant pigments are described as specific compounds found in plants that have the ability to absorb light of specific wavelength and thus appear as colorful.<sup>[1]</sup> Plant pigments which are secondary metabolic compounds exploit specific colour effect in various plant tissues, these are termed as biochromes. Due to the presence of coloring properties of anthocyanin, they are extensively used in food manufacture, pharmaceutical industry, cosmetic establishments, and animal feed producing industries. In large variety of food industries, the anthocyanin primarily used to store the natural colour which is vanish during

processing or can also be to standardize the coloration in numerous food products like fruit juices, pasta preparation, candies, margarine and cheese of different variety. They are additionally precursors of a number of vital compounds which impart flavor to some foods, such as alkaloids and other volatile compounds.

Consumer acceptance of processed foods and beverages containing natural colorants is higher. The three pigment classes liable for the natural exhibit of red colors in plants are carotenoids, anthocyanins and betacyanins. The three classes are mainly very distinct and have a wide range of characteristics; each of them can give an astonishing and pleasant red colour towards the plants. These pigments through colourant and pharmacological properties are significant to food researchers, dietitians, and the food industry due to their positive impact on human health. Standard food colourants have now found their niche in high-volume food applications. With popular colourants like carotenoids or anthocyanins, single-stage colouring systems like baking products (solid phase) or beverages (liquid phase) have been successfully tested.<sup>[2]</sup>

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Extraction is needed for the preparation of specific pigments as well as for gaining natural colourant extracts. Solvent or aqueous extraction, ultrasonic and microwave aided extraction, enzymatic extraction, fermentation or supercritical liquid extraction are some of the techniques used for the extraction of plant pigments.<sup>[3]</sup> To separate the pure pigments, solid phase extraction and subsequent chromatographic partition procedures are used to purify the extracts. After a technical extraction, the colourants can be applied to food systems or they may be needed for the colored crude content. While few of the natural bioactive mixtures which chemically create these colorants may be lost because of storing as well as handling conditions in the matrix, few of them can be condensed to exploit their biological and technical properties. In order to increase the concentration of natural colorants, a genetical modification had to be investigated, there is an increased concern in using these methodologies to increase the plant's output yield of colourants and discover more reasonable applications that can be used in food applications, as well as innovative treatments to stabilise these colourants.<sup>[4]</sup>

Colorants that are encapsulated are easier to manage and have improved physicochemical properties like stability, flow and solubility.<sup>[4]</sup> To minimise anthocyanin complexation with ascorbic acid, biopolymers like heat-denatured when protein isolate, can be added to preserve their colouring properties. Various systems include Using cysteine, cysteine derivatives, glutathione and dihydrolipoic acid to protect calcium carbonate and anthocyanidins sugars as pH-modifiers, aromatic acyl groups to acylate the 30 position of metal particles or anthocyanin to form anthocyanidins complex suspended in polysaccharide matrices.

### Anthocyanins

Anthocyanins are water soluble vacuolar polyphenolic pigments elements of the flavonoid cluster. Anthocyanins are a type of coloured pigment derived from natural plants. Plant pigments that are red in colour are used as indicator metabolites in both evolutionary and metabolic findings of plant cell growth and differentiation. Their presence in exclusive plant organs confers the leaves, flowers and fruits colors from red-orange to blue-purple.<sup>[5]</sup> Anthocyanin are very prominent natural food-colorants allowing to various pH reliant color gradients, utilized in extremely prevalent nutrition like beverages, desserts, ice cream and dairy products.<sup>[2]</sup>

### Anthocyanins as natural colorants

Anthocyanins have appealing properties as natural food colourants, they have been utilized in the food

industry. Since the molecular shape of these pigments is ionic, it is well recognized that the color of anthocyanin extracts modification with the pH of the solution. For pH ranges of 1 to 3, a typical red colour is shown. Due to their comparative instability and exchange with other food matrix ingredients, anthocyanins use as colourants in food is very restricted. As a result of their abundance and possible applications, chemical stabilisation of anthocyanins has become a major subject of recent research. The stability of these molecules is influenced by their molecular structure. In general, increasing hydroxylation reduces stability while methylation increases it.<sup>[6]</sup>

Anthocyanin colour is thought to be maintained and improved through copigmentation interactions with non-colored molecules in pigment solution. Copigmentation is demonstrated by changing the overall absorbance wavelength marginally or growing the colour concentration. Acylation is believed to improve the stability of anthocyanins via causing intramolecular copigmentation. The increase is primarily due to physicochemical and biochemical factors. Non-acylated anthocyanins are found primarily in plants, although acylated anthocyanins are found primarily in flowers and vegetables.<sup>[7]</sup> The widespread usage of vegetable-based anthocyanin extracts as food colourants is due to the higher stability of acylated anthocyanins.

### Carotenoids

Carotenoids are divided into xanthophylls and those with only carbon chains based on their functional groups (carotene, lycopene, among others). They are normally extracted using organic solvents due to their hydrophobicity and depending on the natural source, the raw material can require a series of pretreatments.<sup>[2]</sup> They are difficult to use as colourant additives and functional ingredients due to their water insolubility, instability and low bioavailability, so suitable alternatives have been developed, like carotenoid delivery in water-dispersible products, colloidal suspensions, emulsions, and colloidal dispersions.

### Carotenoids as natural colorants

Carotenoids rich plant extract are often used like natural food colorants and decent source of bioactive or functional compounds. Carotenoids are hydrophobic compounds that bind with lipids and other hydrophobic structures such as membranes. Some plant species have their carotenoid content significantly increased as a results of gene splicing, leading to carotenoid rich plants which will be used as direct dietary sources of

nutrients or as raw materials for extracting natural red, orange and yellow pigments.

The shape and utility of a carotenoid, also as its stability within the membrane are determined by factors like the shape and structure of the carotenoid and the presence of functional groups. Carotenoid pigments are stable in natural plant conditions, but when plant cells are damaged, like during cutting, carotenoid pigments become more fragile and they become much more fragile when extracted or dissolved in organic solvents.<sup>[8]</sup>

### Different concepts for carotenoid sequestration

Carotenoids should be metabolically engineered to account for processes that facilitate carotenoid aggregation and sequestration. For example, de novo biosynthesis and accretion of carotenoids accompanied the transformation of the green chloroplast to the red-colored and extremely carotenogenic chromoplast. After Carotenoids are biosynthesized these hydrophobic pigments that accumulate in membranes or other lipophilic structures like plastoglobules. Carotenoid sequestration could be regulated in a controlled manner to regulate downstream bioactivities in the plant. For example, Apocarotenoid glycosylation is thought to reduce apocarotenoid bioactivity in plants.<sup>[9]</sup>

### Betalains

Betalains are water-soluble pigments that are categorised into red violet betacyanins and yellow betaxanthins based on their chemical structure. The first food and drug administration-approved betalain was betanin derived from red beetroot (*Beta vulgaris*). These pigments are light and high-temperature sensitive and they can impart an unpleasant earthy flavour to food. Betalains display higher water solubility, increased colouring potential and better neutral pH stability compared to anthocyanin.

### Betacyanins as natural colorants

Betacyanins have a number of advantages over other natural red pigments as colouring agents. Their water-soluble property make their incorporation into the easier as majority foods are hydrophilic by nature. Several studies have been performed for biotechnological conception of red beet from cell cultures for betanin manufacture,<sup>[6]</sup> but due to the low cost of agricultural red beet production, this approach is less economically successful.

When extracts of betacyanin are used as food colouring agents, colour stability is the major concern. Although betacyanin extracts are stable across a wide range, from

3 to 7, betacyanin is more stable at pH 4 to 5. This pH-dependent stability builds betacyanins identical useful in low acidic and natural foods.

### Extraction method of carotenoid and anthocyanin pigments

Chemical solvents like acetone, chloroform, hexane, isopropanol, methanol, methylene chloride and diethyl ether are used to extract carotenoids and anthocyanins. A broad range of solvent combinations have been used, resulting in a synergistic effect on pigment extraction.

One of the most important factors in extracting carotenoids and anthocyanins is choosing the right solvent or solvent combination. Selection of the appropriate one is not always easy, as the functional group (polarity) and chain length of the existing pigment. Cell disruption, whether by physical, chemical, or mechanical means, are needed for efficient intracellular pigment extraction. The disruption of the cell wall allows solvents to enter the cell and solubilize intracellular carotenoids.

### Soxhlet extraction

Franz Von Soxhlet invented the extractor, which consists of a thimble containing plant materials and a round-bottom flask containing extraction solvent. As the solvent is heated, the vapour moves through the distillation route of extractor before condensing back onto the plant materials. The extract vapour returns to the round bottom flasks. The procedure is carried out again and again until the extraction is completed. A condenser with running water attached to the extractor for cooling reduced target compound degradation. This technology is mainly used to evaluate the effectiveness of other conventional approaches. It is also suitable for extracting thermostable compounds due to the high temperatures involved.<sup>[10]</sup>

Carotenoids were extracted from freeze-dried carrots using 50 mL hexane and reused for 4 hr. Soxhlet extraction yielded 1832 mg b-carotene per g dry carrot. B-carotene was also extracted from freeze-dried aloe vera skin powder samples using soxhlet extraction with 100 mL petroleum ether for 8 hr.<sup>[11]</sup> In addition to Soxhlet extraction, various extraction methods were used. Pretreatment had a major impact on yield, according to the findings. Cooking also shattered the bond in the carotenoid-protein complex. Soxhlet obtained a high yield of astaxanthin using hexane, isopropanol and acetone.<sup>[12]</sup>

Only instruments that are based on one of these concepts and are specifically designed for Soxhlet extraction. For example, in the exhaustive extraction

method, distillation with a solvent commonly used in organic chemistry has only been compared to Soxhlet extraction. The well-known benefits of the techniques include a significantly shorter heat-up and extraction time, a high yield, ease of equipment use, low consumption of solvent and the sample.

### Enzyme extraction

The enzyme-based extraction method is primarily determined by the assortment of enzymes (pectinase, cellulase), the operating conditions (pH, Temperature), and the substrate (material). The use of aqueous extraction and enzymatic treatment to improve the extraction of oil from fruits and oil seeds has been published.<sup>[13]</sup> Before using the traditional extraction process, enzymes were used to pretreatment of the plant material. The use of enzymes in the extraction of oil from oil seeds such as sunflower, soybean, rapeseed, corn, coconut, olives, avocado, and rice bran oil, are well known in the literature. Plant materials that have the ability to be a rich source of flavor, such as vanilla, pepper, mace, mustard, fenugreek, rose, and citrus peel, have been studied for enzyme-assisted flavor extraction. Plant materials such as marigold, safflower, grapes, paprika, tomato, alfalfa and cherries have also been tested for enzyme-assisted extraction of colour.

The following are some of the advantages of enzymatic extraction: decreased extraction time, increased extract ability/yield, reduced amount of solvent used in extraction, complete removal of solvent when vegetable oils are used as solvents, environmentally friendly and does not cause controversy, sustainable (enzyme can be purified and reused), enzymes are less expensive than organic solvents, enzymes are versatile and precise, a small number of enzymes can perform a large number of reactions. The key drawbacks of this method are the high cost of enzymes and their vulnerability to degradation, so it is important not to beat the maximum operating temperature specified by the manufacturer.<sup>[14]</sup>

### Challenges associated with stability of pigments

The stability of anthocyanins is influenced by a number of physicochemical factors, including the pH of the substance, light exposure, temperature, and complexation with other compounds in the matrix which may cause loss or colour variation.

### Environmental elements affect the stability of plant pigment

#### pH levels

Some colourants reduce or change to a different, less stable colour at certain pH levels. Annatto will

precipitate at a pH less than 4.0, and carmine will degrade at a pH less than 3.5 in their natural color; however, by changing their shape, these colorants may be used in acidic applications. At low pH, an annatto mixture repels precipitation. At pH less than 3.8, anthocyanins are most stable and provide red hues, while at higher pH levels, the colour is either vanished or changes to unstable blue and/or purplish tones.

#### Ascorbic acid

Ascorbic acid caused browning as well as bleaching of anthocyanin pigment. Anthocyanin pigment and ascorbic acid are supposed to degrade through a direct condensation mechanism. On the positive side, ascorbic acid is an oxygen scavenger and helps prevent the fading of colors like carmine and beta carotene. Ascorbic acid is primarily a problem in beverages, especially those that are Vitamin C fortified.

#### Heat

The ability of a colour to withstand heat is determined by the manufacturing conditions of the finished product, precisely the temperature, time, and point of colour addition. Although natural colorants degrade when exposed to heat for long periods of time, they are frequently used in products with less intense heat treatment, such as cookies and extruded cereals.

#### Light

If exposed to enough light, all natural colors will fade with time. Annatto, carmine and some anthocyanins have adequate to strong light stability. Caramel colors have good light stability and absorb UV light, which helps to prevent the degradation of UV-sensitive ingredients in a product, such as nutraceuticals. Color encapsulation or emulsions, packaging products with UV barriers, and shelf-life control are all methods for reducing light degradation. In the presence of light, the colour of annatto can fade in days or weeks, but an encapsulated type of annatto can have a shelf life of a year or more.

#### Oxygen

Colorants like carmine, carotenoids and paprika can disappear in the presence of oxygen. Antioxidants such as ascorbic acid and tocopherols can help prolong the shelf life of colour and keep the desired shade in the final product. By scavenging residual oxygen, ascorbic acid in a beverage protects those colours. Natural rosemary extract is used to increase the oxidative stability of carotenoids and has antioxidant properties.<sup>[15]</sup>



## Outlook regarding issues

Flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, metals and even other anthocyanins have been studied as copigmenting agents for anthocyanins, all with the goal of minimising colour degradation. This copigmentation method frequently results in a red-shift with an increase in absorbance and a more stable colour over time.<sup>[16]</sup> In addition, a number of methods for stabilising anthocyanins have been published, including the addition of chemical reagents and modified processing.<sup>[17]</sup>

In standard pH-4 solutions containing ascorbic acid, biopolymer nanoparticles were tested as carriers for anthocyanins. The other methods also used like, a mixture of polymers was used, with a second heating stage of the two polymers added to allow for denaturation and accumulation of the molecules.<sup>[17]</sup> A 2:1 part of whey protein separate and beet pectin was used to make small anionic nanoparticles, and all particle filled with anthocyanin-rich extract at a total concentration of 300 or 600 g/mL. The efficiency of anthocyanin incorporation measured before and after thermal treatment. When anthocyanins introduced after thermal processing, the integration efficacy reached a high of around 35 percent, while it around 55 percent before thermal processing. This increased efficacy in integration means that the anthocyanin extracts can be encapsulated more effectively when heat is applied.

According to the study, this may be due to phenolic compounds having easier contact to exposed hydrophobic groups on the protein, which they can well bind to throughout the heating process. Despite the fact that heat degrades anthocyanin colour, improved productivity is not necessarily a favorable. The chroma of the encapsulated anthocyanin decreased by 43 percent or more after processing the sample with the lowest ascorbic acid content of 0.3 mg/mL. Using this data, it appears that single-polymer encapsulation methods were more successful than multipolymer heat-treated encapsulation methods in preventing colour degradation. The nanoparticles are only physically stable between pH 3.8 and 4.2, which extremely restricting the use of this form of food nanoparticles process.

An aromatic acyl group is added to the third position of an anthocyanin to increase its stability. Anthocyanins became more stabilised and bluer as more aromatic acyl groups modified them. Acylation improves the stability of anthocyanin molecules by intramolecular and intermolecular copigmentation, as well as nature association reactions.<sup>[7]</sup> Anthocyanins are currently obtained from

crude extracts of fruits or waste products from the beverage industry. These are not standardised in terms of which anthocyanins they contain or how much of each anthocyanin is present in the extract.<sup>[18]</sup>

## Opportunities and future perspectives

As a result of potential health benefits and consumer demand, food colorants derived from natural sources have risen in popularity. Potential food is expected to follow this need for safety considerations. The food industry faces a major challenge in meeting customer demands for safer, healthier and more useful functional food items, such as food colorants, as a result of this phenomenon. As a result, research into overcoming natural pigment persistence and bioavailability limitations will continue in the future to assemble the need for synthetic colorants to be substituted.<sup>[19]</sup> Nature offers a wealth of plant-based colour pigments with enormous potential for coloration and functional abilities that are currently under utilised. Natural pigments with enhanced properties, like new colours and upgraded stability, have been exposed and their uses have prolonged to include food, medicinal, nutraceutical and cosmeceutical applications.

In the future, new relevant and efficient regulations will include a broader range of natural colourants, as well as enhanced stability characteristics. It is important to improve a strong blue anthocyanin for viable use, and it should be investigated as a safe natural food colorant. Modern outcome on newly discovered red auronidin pigments suggest that it may be the significant to creating much stable and powerful natural plant-based colorants. Auronidins have not yet been investigated for use in food colouring, but due to their water solubility and yellow/orange colour, they may give a new source of pigments suitable for use in food and beverages. If auronidins prove to be useful as natural colourants, our research into how plants regulate and produce auronidin pigments will be critical for scaling up the production of these pigments.<sup>[20]</sup>

## CONCLUSION

This is remarkable significant to confirm long-term sustainability and respond to worries about food colours consumed in the industry. Understanding of natural colourant stability and degradation prevention will be useful not only in the food industry, but also in agriculture, cosmetics, pharmaceuticals and nutraceuticals. Massive research on current practices to improve the stability of plant-based pigments is needed to ensure attractive appearances of food products. New

approaches involving genetic modification with the aim of expanding anthocyanin synthesis are currently being investigated. These recent methods could be exploited by the food and agro industries to boost anthocyanin yields during extraction, and they could be more economical for the food industry. Many recent advances in plant-based pigments have been made; but in order to enhance food technology, many more must be evaluated for coloration properties and promising bioactive properties.

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