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Research Article

PLANT NATURAL PIGMENT COLORANTS-HEALTH BENEFITS: TOXICITY OF SYNTHETIC OR ARTIFICIAL FOOD COLORANTS

[1,*] Ravindra B. Malabadi, [2]Kiran P. Kolkar, [1]Raju K. Chalannavar

[1]Department of Applied Botany, Mangalore University, Mangalagangotri-574199, Mangalore, Karnataka State, India
[1]Miller Blvd, NW, Edmonton, Alberta, Canada.
[2]Department of Botany, Karnatak Science College, Dharwad-580003, Karnataka State, India.
***E-mail**: rbmalabadi_b3g@yahoo.com

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ABSTRACT

This literature review paper highlights about health benefits of **plant natural pigment** colorants and **toxicity** of **synthetic** or artificial food colorants. **Fruits** and **vegetables** are colourful pigment-containing food sources. **Natural food colours** or biological pigments originate from a wide range of sources like vegetables, fruits, plants, minerals and other edible natural sources. These natural pigments are essential secondary metabolites, which play multiple roles in the whole life cycle of plants and are characterized by powerful medicinal properties. Owing to their nutritional benefits and phytochemicals, they are considered as **functional food ingredients**, capable of providing additional health benefits, like prevention or delaying onset of chronic diseases, as well as meeting basic nutritional requirements. **Colour additives** are widely used by the food industry to enhance the appearance, as well as the nutritional properties of a food product**. Synthetic colours** are added to foods to replace natural **colour lost** during processing to reduce batch-to-batch variation and to produce products with consumer appeal where no natural colour exists. However, some of these substances may pose a **potential risk to human health**, especially if they are consumed excessively and are regulated. **Synthetic colorants** become **toxic** after prolonged use, causing health problems such as indigestion, anemia and allergic reactions as asthma and urticaria, pathological lesions in the brain, kidney, spleen and liver, tumours and **cancer paralysis**, mental retardation, abnormalities in offsprings, growth retardation, **carcinogenic effect** and eye defects resulting in **blindness**.

Keywords: Anthocyanins, carotenoids, colorants, dyes, food color, flavonoids, natural colors, plant pigments, synthetic colors, toxicity.

INTRODUCTION

Plant natural pigments plays an important role in plant metabolism and visual attraction in nature (1-14, 16, 18-24). Plant pigments are important clues to humans and animals in identifying the plant and plant parts such as leaf, fruit, flower, seed, root, and tubers (1-20). **Pigments** are responsible for imparting colours to various plant parts (1-20). In fact, almost all plant parts are bound to be pigmented right from germination to their senescence (1-16, 18-22). Traits of the colours are thus a prerequisite for **breeding programmes** for developing coloured varieties (1-20). Colours are of prime importance for indicating diversity among genotypes for selecting elite parents during breeding programmes (16). Colour development is the most fascinating and complex phenomenon during ripening and is mainly governed by **pigments** (16). Colour is considered to be the first sensory perception of fruit, contributing to the commercial value of fruit because it attracts consumers (16). **Colour** is an important characteristic and selection criterion for food choice. Recent studies have highlighted this importance and have shown how selection of colours may change among certain populations over a period of time.

Natural Colorants or synthetic (Artificial) colorants are widely employed in the food industry as an essential ingredient in many products since color is one of the most valued attributes by consumers (1-22). Furthermore, the utilization of colorants is currently being extended to **the food packaging technologies** (24, 25).

***Corresponding Author: Ravindra B. Malabadi,**
1Department of Applied Botany, Mangalore University, Mangalagangotri-574199, Mangalore, Karnataka State, India.
1Miller Blvd, NW, Edmonton, Alberta, Canada.

Colour is a key component to enhance the ultimate appetizing value and consumer acceptance towards foods and beverages (1-23, 26-28). **Synthetic food colours** have been increasingly used than natural food colours by food manufacturers to attain certain properties such as low cost, improved appearance, high colour intensity, more colour stability, and uniformity. Varied **foods** and **beverages** available in the market may contain some **non-permitted synthetic colours** and overuse of **permitted synthetic colours**. It may lead to severe health problems such as mutations, **cancers**, reduced haemoglobin concentrations, and **allergic** reactions (1-22, 26-28). Therefore, there is a high tendency to use synthetic food colours in confectioneries and beverages and some confectioneries contain un-identified colours including a **textile dye** (1-22, 26-28). Hence, the implementation of regulations and awareness programs of food colours for consumers and food manufacturers are highly recommended (26-28).

Major plant pigments includes, Chlorophyll (green), Carotenoids (yellow, red, orange), Flavonoids: anthocyanins, anthoxanthins (red, blue, purple), and Betalains (red, yellow, purple) (1-23). Chlorophyll are green in color and Carotenoids (yellow, red, orange) plays pivotal role in photosynthesis (1-23). They occur in all green plants and are localized in plastids (1-22). Chlorophylls capture light energy and convert into chemical energy (1-25). On the other hand **carotenoids** protect chlorophylls from photo oxidation and are accessory light harvesting pigments and photoreceptors (1-23). Plant compounds such as **carotenoids, betalains, anthocyanins, anthoxanthin, chlorophylls** or **carminic acid** present in fruits and vegetables as natural colorants have been well characterized to possess bioactive properties (1-23). Nature provides a wide array of colors to fruits and vegetables, and thus is a pigment enriched food resource (1-20). These pigments occur mainly as secondary plant metabolites in fruits and vegetables and are usually coined as natural pigments (1-22).

Basically, **carotenoids** and **anthocyanin** pigments are responsible for the colours of fruit (1-23). Carotenoids and anthocyanins are secondary metabolites formed through biosynthetic pathways associated with the source-to-sink flux, involving substrate and enzymes contributing to the formation of the specific metabolites (1-5, 16-23). Anthocyanins impart the red, blue, and purple colours, whereas carotenoids are responsible for the yellow, red, and orange colours (1-16, 18-22). **Cyanidin, delphinidin, pelargonidin, peonidin, malvidin,** and **petunidin** are the most common anthocyanidins distributed in the plants (1-22).

Anthocyanins are commonly found in flowers and the fruits of many plants (18). Most of the red, purple, and blue-colored flowers contained anthocyanins (1-23). In addition to traditional usage, red, purple, and blue-colored fruits are commonly consumed for their beneficial effects (18). The colored pigments of **anthocyanin** from berries, blackcurrants, and other types of red to blue-colored fruits are **strong antioxidants** (18). Moreover, anthocyanin-rich black carrot, red cabbage, and purple potato are potential functional foods that have been consumed for prevention of diseases (1-18, 19-22). Anthocyanins are the value-added colorants that can be used for preventing several diseases, including CVDs, cancers, diabetes, some metabolic diseases, and microbial infection. These compounds also improve visual ability and have **neuroprotective** effect (1-22).

Carotenoids occur in all green tissues and independently of chlorophylls, in flowers where they serve to attract animals, storage organs and other plant parts (1-23**). Natural plant pigments** are a great source of powerful antioxidants and therapeutic properties that can have a profound effect on complete human health (1-23).

Flavonoids located in the cytoplasm and plastids include red or blue anthocynins (1-25). White or pale compounds such as **rutin, quercitin** and **kaempferol** (1-22). **Flavonoids** (5) in flowers and fruit provide visual cues for animal pollinators and seed dispersers to locate their target (1-5). **Betalians** also plays an important role in attracting animals (1-22).

PLANT NATURAL PIGMENT COLORS

Natural food colours or biological pigments originate from a wide range of sources like vegetables, fruits, plants, minerals and other edible natural sources (1-23). There are four families of plant pigments (5). Major plant pigments includes, **Chlorophyll (green), Carotenoids (yellow, red, orange), Flavonoids: anthocyanins, anthoxanthins (red, blue, purple), and Betalains (red, yellow, purple)** (1-22). Fruits and vegetables are colorful pigment-containing food sources (1-23). Owing to their nutritional benefits and phytochemicals, they are considered as 'functional food ingredients' (1-22). Following is the list of important natural plant pigment colors (5).

1) Green color: Chlorophyll: A water-insoluble plant pigment, primarily responsible for the green colour of all green vegetables and fruits like spinach, fenugreek leaves, coriander leaves, bell peppers, broccoli, green cabbage, celery, green beans, turnip greens, green chilies etc (1-22). There are two main types of chlorophyll namely; chlorophyll-a (intense blue-green) and chlorophyll b (dull yellow-green). In the food industry, **chlorophyll** is gaining importance as a natural food additive for its intense green colour and consumer's growing preference for natural foods (1-22).

2) **Red**, **Blue or Purple color: Anthocyanin:** Anthocyanin is a water-soluble vacuolar pigment, whereas **anthocyanidin** is the sugar-free counterpart of anthocyanin, responsible for the attractive red, purple and blue colours of many flowers, fruits (Berries, currants, grapes, and some tropical fruits have high anthocyanins content) and vegetables (1-25**). Anthocyanins** are synthesised in the vacuoles (1-22). Red to purplish blue-colored leafy vegetables, grains, roots, and tubers are the edible vegetables that contain a high level of anthocyanins (1-22). Red flowers sucha as red hibiscus, red rose, red pineapple sage, red clover, and pink blossom are rich in anthocyanins content (5, 18). These red flowers are edible. Blue (cornflower, blue chicory, and blue rosemary) and purple (purple mint, purple passion flower, purple sage, common violet, and lavender) flowers are the common edible flowers (5, 18). Some of these flowers have been traditionally used as **folk medicine**, as colorants, and as food (1-22).

The pigments are in **glycosylated forms** belonging to the phenolic group (1-23). Anthocyanins belong to a parent class of flavonoids synthesized via the phenylpropanoid pathway (1-22). Although they occur in all tissues of higher plants, they can be obtained from edible vegetables and fruits, such as blueberries, blackberries, strawberries, currants, raspberries, and grapes (1-22). They are sensitive to pH change, being reddest in strongly acidic conditions and become bluer as the pH rises (1-23). Therefore, color and stability of these pigments are influenced by pH, light, temperature, and structure (1-22). The colored anthocyanin pigments have been traditionally used as a **natural food colorant** (1-22).

Among the anthocyanin pigments**, Cyanidin-3-glucoside** is the major anthocyanin found in the most of the plants (1-23). **Delphinidin**, which is one of the derivatives of anthocyanidin is primarily responsible for the particular blue colouration in fruits and vegetables (1-22). **Malvidin** and **Petunidin**, both derivatives of anthocyanidin are responsible for the purple violet colouration (1-22). Blackberries, blueberries, purple grapes, plums, purple cabbage, eggplant are some of the richest sources of blue or purple pigmented fruits and vegetables (1-23).

High anthocyanin (5) contents are found in fruits such as red grape (30–750mg/100g fresh weight [FW]), blackberry (82.5–325.9mg/100g FW), bilberry (300–698mg/100g FW), cherry (2–450mg/100g FW), pomegranate (600–765mg/100g FW), raspberry (20–687mg/100g FW), red apple (1.3–12mg/100g FW),and strawberry (19–55mg/100g FW) (1-23). **Anthocyanins** play an important role in photosynthesis, pollination (by attracting pollinators), and seed dispersal (1-22).

Anthocyanins are the most prominent pigments responsible for red, blue, and black hues in fruits (1-20). Available literature suggests that, in higher plants, only six types of anthocyanins are commonly found: cyanidin, pelargonidin, delphinidin, peonidin, petunidin, and malvidin (1-22).

Cyanidin-3-glucoside is the most widespread anthocyanidin of the cyanidin type (1-22). Furthermore, they impart aroma and provide protection against biotic and abiotic stresses (1-22). They possess **antioxidant** properties, contributing to their therapeutic value in the prevention of neuronal diseases, cardiovascular diseases, cancer, and diabetes in human beings (1-20). Also, they serve as an alternative to natural colourants (1-22). Cyanidin-3-O-rutinoside was the dominant anthocyanin along with two minor compounds, cyanidin-3-Oglucoside and peonidin-3-O-rutinoside (1-5).

The most important **anthocyanin source** in the food industry is the by-products of **red wine** (5). Among the anthocyanins extracted can be highlighted as Cyanidin ($C_{15}H_{11}O_6Cl$), Peonidin ($C_{16}H_{13}O_6Cl$), Malvidin ($C_{17}H_{15}O_6Cl$), Delphinidin ($C_{15}H_{11}O_7Cl$) and petunidin ($C_{16}H_{13}O_7Cl$) (1-22).

3) Red color: The betalains- The betalains consist of two sub-groups, red-violet (betacyanin) and yellow to orange (betaxanthin) pigments (1-20). Carotenoids (lycopene, canthaxanthin, andastaxanthin), anthocyanins, and betacyanins are natural red pigments found in fruits and vegetables like **tomatoes**, guava, red grapefruit, papaya, rosehips, and watermelon indicate the presence of lycopene (1-5). Cranberry, beet, watermelon, tomato, strawberry, pomegranate are some of the most commonly available red plant foods (1-22). **Red wine** is produced from red grapes contains anthocyanins primarily **Resveratrol**, a heart healthy antioxidant (1-22). Red potatoes (Solanum tuberosum and S. stenotomum) were evaluated as potential sources for **natural red colorants** (1-22).

4) Cochineal, Carminic Acid or Carmines: Cochineal, Carmines and Carminic acid are obtained from aqueous alcoholic or alcoholic extracts of cochineal, and consist of the dried bodies of the **female insect** *Dactylopius coccus* Costa (1-20). The **insects** of the Coccidae family are parasites of some species of cacti (1-5). **Carminic acid** is a **natural food colour**, with a purple or **red colour**, which can be widely used in preserved red fruits, fruit syrups, ice creams, meat products (such as sausages, chorizo and salami, pâtés, breakfast sausages) (1-23). **Carminic acid** is used in the meat preparation defined by lactic acid products (such as yogurt and fresh flavoured and other processed cheeses) (1-12). Further **Carminic acid** is also used in a wide variety of food products such as ripened cheese, desserts, edible cheese rinds, flavoured drinks, seasoning, marmalades and jams, pastries and fine bakery, confectionery (including breath refreshing and chewing gum), breakfast cereals flavoured with fruits, fish paste and crustacean paste, precooked crustaceans, smoked fish, some alcoholic beverages, and wine-based snacks (1-20).

5) Yellow/ Orange color: Carotene: Carotenoids are the lipid-soluble pigment compounds that impart yellow and orange colours to fruits and vegetables (1-20). These are the most widely distributed pigments in the plant kingdom (1-20). Carotenoids are basically classified into two types: **Carotenes,** which contain **no oxygen**, and **Xanthophylls**, which **contain oxygen** (1-22). Carotene absorbs blue and indigo hues, that provides rich yellows and oranges to different foods (1-20). Carotenoids are localised in chloroplasts and synthesised in the chromoplast (1-20). The most important and predominant carotenes are **α-carotene** and **β-carotene**, and common **xanthophylls** are lutein, zeaxanthin, and cryptoxanthin (1-20). These compounds vary in different crops. For example, **lycopene** is the dominant carotenoid in **red tomato** fruits, and **β-carotenes in papaya** and **mango** (1-20). **β-carotene** and **α-carotene** have the capacity to be converted into essential vitamin A and, hence, to help in the fulfilment of a balanced diet (1-20). **Vitamin A deficiency** is a severe disease observed worldwide in almost 122 countries (1-20). Fruits such as mango, papaya, passion fruit, and peaches are the most important source of pro-carotenoids, which help in the provision of vitamin A (1-20). **Vitamin A** also plays a vital role in improving vision and immunity and lowering the incidence of cancers (1-20). Fruits are the richest known source of carotenoids, particularly β-carotene, **the precursor of vitamin A** (5). This is known to improve vision and immunity and **to reduce cancers** (1-20).

Carotenes are hydrocarbons with a skeleton of 40 carbon atoms; like xanthophylls, they belong to the family of carotenoids (1-20). This pigment is important in our diet, as the human body breaks down eachcarotene molecule to produce two vitamin A molecules (1-22). **Apricots, mangoes, nectarines**, peaches, papaya, oranges, grapefruit, pineapple, passion fruit, carrots, sweet potatoes, butternut squash, pumpkin, yellow and orange peppers, sweet corn are some of the many yellow/orange foods rich in carotenes commonly found in the world (1-22). The Carotene is used as **natural colours** in a large amount of food products, namely orange, yellow or whitish coloured cheese, edible cheese rind, unflavoured melted cheese, processed cheese, butter, fat and oil emulsions, spreadable fats, sausages, salami and pâtés, preserves of fruits, jams and jellies, marmalades, confectionery including breath refreshing and chewing gum, pastry and fine bakery products, desserts, breakfast cereals flavoured with fruits, flavoured dairy products, edible ices, dried potato granules and flakes, sauces and seasonings, seasonings, fish paste and crustacean paste, precooked crustaceans, soups, smoked fish, and alcoholic beverages (1-20).

6) Curcumin: Curcumin also named as natural yellow or **Diferoyl methane** is obtained from the rhizome of *Curcuma longa* (5). Curcumin furnishes a yellow or orange-yellow colour to food products (1-20). This food colour consists essentially of curcumin (1-5). Curcumin is marketed as a spice under the name of "Indian saffron", and can be used as a partially purified food colour in the form of powdered preparations of the plant rhizome and oleoresin (1-20).

7) Riboflavin or Vitamin B2: Riboflavin, also identified as "**Lactoflavin" or "Vitamin B2"** ($C_{17}H_{20}N_4O_6$), and "riboflavin- 5'-phosphate" ($C_{17}H_{20}N_4NaO_9P.2H_2O$) are water-soluble colourants synthesized by plants and several microorganisms (1-20). They are essential micronutrients in the human diet, acting as precursors to flavine adenine dinucleotide and flavin mononucleotide, which function as hydrogen carriers in biological redox processes (1-5). These food colours are yellow or orange yellow crystalline powders, with a slight odour (1-20). **Milk** is the main source of **riboflavin**, but green vegetables also have appreciable quantities (5). As a food colour, riboflavin gives **yellow colour to whey** and thermally stable (1-14). However, it is sensitive to sunlight and fluorescent light, leading to decomposition reactions that alter the aroma and taste of food products (1-20). This effect can be important in sterilized milk bottled in glass containers (5). The acceptable daily intake of riboflavin is 0.5 mg/kgbw (1-22). Although **vitamin B2** or **riboflavin** is essential to the human body, but its lack does not produce any specific disease (1-20). Besides this, no adverse toxic, genotoxic, cytotoxic, or allergic effects have been related to **riboflavin or vitamin B2** (1-22).

8) Lycopene ($C_{40}H_{56}$): Lycopene (5) a bright red carotenoid, is a symmetrical tetraterpene (i.e., assembled from eight isoprene units) (1-10). Lycopene ($C_{40}H_{56}$) can be isolated from **red tomatoes**, and from Blakeslea trispora (1-20). **Lycopene,** the main colouring product of **red tomatoes** (which also have small amounts of other carotenoid pigments and oils, fats, waxes and naturally occurring aromas) (1-5).

9) Caramels: Caramels are often used in the food industry to impart or intensify the yellow or brown colour (5). Caramels being miscible with water (in liquid or powdered forms), but can also disperse in an oil system (producing pastes or emulsions) (1-10).

10) Annatto bixin and Annatto norbixin: Annatto bixin and Annatto norbixin can be obtained by extraction from the spiney seed pods of *Bixa orellana* L. (also known as "Annatto" or 'Achiote' in large parts of **South America**, and as 'Urucum' in **Brazil**). This coloring agent provide a yellow-to-red colour to food products (5). Bixin, which is a carotenoid, is extracted from the seed using hot vegetable oil (1-20).

11) Paprika extract : **Paprika** extract, also known as "capsanthin" or "capsorubin", is a natural food colouring in the form of a viscous, dark-red liquid (1-20). Capsanthin and capsorubin are the colourings that impart the yellow-to-orange hue characteristics of paprika (1-5). This red spice imparts flavour, and the **paprika color compounds** can be solvent extracted to synthesize paprika oleoresin, a purified form of the colouring compounds (1-20). Paprika and paprika oleoresin are stable to heat but sensitive to light and alkaline conditions, and are insoluble in water (5). Paprika is extracted from ground fruits, with or without seeds, from the natural strains of ***Capsicum annuum*** L. that contain capsanthin ($C_{40}H_{56}O_3$), capsorubin ($C_{40}H_{56}O_4$), and other lesser quantities of coloured compounds, namely xanthophyll, β-carotene and capsaicin (1-22).

12) Black: Anthocyanins: Black / deep purple coloured foods like black beans, black raspberries, and **black tea** are packed with anthocyanins, the pigment that lends these foods with their dark hues (5). Black foods have more antioxidants than light-coloured foods because of their high pigment content (1-20).

13) Vegetable carbon or Vegetable black: Vegetable carbon, also known as "**vegetable black**" (5) is produced **from green bamboo** refined through a high temperature carbonization processed with steam activation (1-5). The steam activation is achieved by charring the vegetable fibres of vegetable materials, such as wood, cellulose residues, peat, coconut husks and other fruits (1-20). The obtained residues are ground into small particles, with glycerin or glucose added for use in food products (1-20). Therefore, this additive, which takes the form of an odourless black powder, essentially consists of finely divided carbon, but can also contain small amounts of nitrogen, oxygen and hydrogen which can absorb other substances (1-5).

14) Lutein or Xanthophyll ($C_{40}H_{56}O_2$): Lutein (5) is a xanthophyll ($C_{40}H_{56}O_2$) of natural origin used as food colouring agent, which appears in the form of a dark, yellowish-brown liquid, and gives a reddish-yellow colour to food products (1-20). This food colour, which is a fat-soluble and a powerful **anti-oxidant** is obtained by the extraction with acetone, methyl ethyl ketone, ethanol, methanol, 2-propanol, hexane, dichloromethane or carbon dioxide from natural varieties of **edible fruits** and **plants**, grass, lucerne (alfalfa) and *Targets erecta* (1-5).

15) Canthaxanthin ($C_{40}H_{52}O_2$): Canthaxanthin ($C_{40}H_{52}O_2$) is a keto-carotenoid pigment which is widely distributed in nature (namely, in flamingo feathers, koi carp skin and crustacean shells) that was firstly isolated in edible mushrooms (1-20). This food colour also known as *β*-Carotene-4,40-dione and 4,40-dioxo-*β*-carotene, is a potent lipid soluble antioxidant (1-5). It is a natural product for food colour purposes (1-22). Food colour takes the form of **deep-violet** crystals, or crystalline powder, and is sensitive to oxygen and light. Therefore, it must be kept in a light-resistant container under inert gas (1-20).

16) Beetroot-Red or Betanin or Beet-red: **Beetroot-red or Betanin** or **Beet-red** is a natural food colour displaying red to dark-red colours (1-5). It is a red glycosidic food dye obtained from the roots of red beets (***Beta vulgaris*** L. var. rubra) by pressing the crushed beets or by the aqueous extraction of shredded beet roots and subsequent enrichment in the active principle (1-20). The colour principle consists of several pigments belonging to the betalain class (1-5). Betacyanins (red) with betanin being the main component (75–95%) are the main coloured components (1-20). Betaxanthin (yellow) and some degradation products (light brown) may also occur in smaller amounts (1-5). **Betanin** is a red or dark red color depending on pH—between 4 and 5 (1-20). Further, it is a bright bluish-red becoming blue-violet as the pH increases (1-20). At alkaline levels, its degradation by hydrolysis produces a yellow-brown color liquid, paste, powder or solid substance (1-20).

17) White: Anthoxanthin: A group of pigments called anthoxanthin is responsible for shades of white to yellow in many fruits and vegetables (5). **Anthoxanthin** is a phytochemical belonging to the family of antioxidant flavonoid (1-5). **Cauliflower, garlic**, ginger, mushrooms, onion, turnip, potato are commonly found white pigmented vegetables in the market (1-20).

18) Edible Chemical Food dyes

In chemistry, the chemical added to impart or change the color of a substance is called a **dye**, which forms a chemical bond with the substrate (12). **Dyes** are often considered in reference to clothes, wool, paper and leather (12). But digestible colors have always been employed in food or drink products and are known as '**food dyes'**, 'food additives' or '**edible dyes** (12).

Calcium carbonate ($CaCO_3$) is a stable **food colouring** that does not require any specific processing to preserve its colouring properties (1-5). Additionally, calcium carbonate is also used as an acidity regulator, an **anti-caking** agent (i.e., prevents food particles from sticking together) and a stabilizer (thus maintaining the uniform dispersal of substances in a food) (1-20). **Calcium carbonate** is a natural mineral derived from earth's **limestone**, marble, or the sedimentation of crushed marine shells (1-20). For the food industry, the additive calcium carbonate is obtained by processing and cleaning chalk deposits (1-5).

Calcium carbonate ($CaCO_3$) (5) can be used in most **food products,** namely in confectionery products including dragees and chewing gum, decorations and coverings, desserts, cocoa and chocolate products, ripened cheese, grated or sliced cheese, processed eggs, salt and salt substitutes, seasoning and condiments, fish paste and crustacean paste, fruit juices and fruit nectars, similar vegetable products, flavoured fermented milk products, edible ices, and baby foods (1-20).

19) Titanium dioxide (TiO_2): Titanium dioxide (TiO_2) (5) is a metal oxide extracted from ilmenite, rutile and anatase (1-5). **Titanium dioxide** is used as a **white food colouring** dye due to its brightness and very high refractive index (1-20). Like all other food colourants, its technological function is to make food more visually appealing, to give colour to food that would otherwise be colourless, or to restore the original appearance of a foodstuff (1-5). **Titanium dioxide** is also a nontoxic antimicrobial (i.e., it has potential bactericidal and fungicidal applications) (1-15). This additive can be used in most food products, namely in confectionery including breath refreshing and chewing gum, decorations and coatings, flavoured fermented milk products, edible ices, desserts, edible cheese rind, seasoning, fish paste and crustacean paste, and precooked crustaceans (1-6). Some **carcinogenic effects** have been attributed to **Titanium dioxide** (TiO_2) (1-10).

20) Iron oxides and hydroxides: Iron oxides and hydroxides (5) can naturally be found in rusts, or can be artificially produced from iron sulphate (1-20). These **colorants** can be applied to food products, appear in the form of black, brown, red or yellow powders, and are insoluble in water and organic solvents (1-5). **Iron oxides and hydroxides** can be used in most food products, namely in confectionery including breath refreshing and chewing gum, desserts, decorations and coatings, flavoured fermented milk products, edible ices, edible cheese rinds, seasoning, fish paste and crustacean paste (1-20).

21) Aluminum: Aluminum can be used as a food colouring agent (1-20). **Aluminum**, being a silvery-grey powder or tiny sheets are insoluble in water and in organic solvents (1-5). **Aluminum powder** consists of finely divided particles that eventually can be carried out in the presence of edible vegetable oils or fatty acids, and are used as food colours (1-20). This **dye** which has a very limited use, can be applied in the external coating of sugar-based confectionery products for the decoration of cakes and pastries (1-5).

22) Silver: **Silver food colour** is present in its elemental form, and is chemically obtained from the electrolysis of silver ore (1-20). This coloured powder, or straw, is also a bactericide and a water-insoluble substance, and is only of use in coatings and decorations for cakes and sweets, liqueurs, and drinking water disinfection (1-5). At a physiological level, the elimination of **silver** through the **kidneys is slow**, which can induce side effects of deposits at the tissue level (1-20). For instance, a high intake (a few grams) leads to poisoning (1-10). However, there is a lack of data regarding the **toxicity and carcinogenic potential of the use of silver as a food additive** (5).

23) Gold: Gold can be applied as a **food colour** giving rise to a very unreactive metallic surface colour (1-5). It occurs in the form of golden-coloured powders, or straws (1-10). **Gold** is used in liqueurs and in the outer coating of confectionery and chocolate decorations (1-20). At a physiological level, some side effects on health namely disorders of the blood formula are described (1-5). Nevertheless, although there are some studies with rats, the available data did not allow an evaluation of the genotoxic hazard associated to the use of **gold as a food colour** (1-5).

Fruits and vegetable wastes by-products to produce natural pigment colors

Natural pigments, including carotenoids, flavonoids and anthocyanidins, determine the attractive color of fruits (1 -20). These **natural pigments** are essential secondary metabolites, which play multiple roles in the whole life cycle of plants and are characterized by powerful antioxidant activity (1-20). These pigments play an essential roles in plant growth and development, photosynthesis, attracting pollinators and seed carriers, and resisting biotic and/or abiotic stresses (1 -5). **Edible pigments** are ubiquitously present in plant origin foods, especially in colored fruits and vegetables (1-20). These pigments occur mainly as secondary plant metabolites in fruits and vegetables and are usually coined as natural pigments (1 -22). Among all of these, pigments are considered to be natural, safe, and possess potential antioxidant activities, as well as can be a potential coloring material source in food applications (1-20).

Valorization of vegetal wastes (fruits and vegetables) and their by-products (e.g. peels, seeds or pomace) can meet the demands of **natural pigment** production at the industrial levels for potential food, pharmaceuticals, and cosmeceuticals applications (1). These wastes/by-products are a rich source of natural pigments such as: anthocyanins, betalains, carotenoids, and chlorophylls (1-20). It is envisaged that these natural pigments can contribute significantly to the development of functional foods as well as impart rich bio-therapeutic potential (1-20). The natural pigments in fruits are not only powerful antioxidants, but they also have a host of other health benefits (1).

Natural pigments of fruits are nontoxic, renewable, and easily available, which endow wide application prospects in the food, medicine, and cosmetics industries (1-19). Many studies have reported the biological and pharmacological activities of carotenoids, flavonoids and anthocyanidins, including antioxidant, anticancer, antiviral, antibacterial, anti-inflammatory, antiallergic, antithrombotic, cardioprotective, hepatoprotective, neuroprotective, antimalarial, antileishmanial, antitrypanosomal, and anti-amebial properties (1-20). These are excellent resources for ingredients such as protein, peptides, polysaccharides, dietary fibers, and others along with bioactive-functional ingredients such as polyphenols, antioxidants and antimicrobial compounds including natural pigments (1). These **natural pigments**, with coloring and pharmacological properties, have wide applications in the food industry and can be utilized in product development (functional and nutraceutical foods) because of their health-related beneficial effects (1). The wastes contain significant amounts of phyto-pigments which can help in overcoming **cardiovascular, cerebrovascular**, and certain types of **cancers** (1). These natural pigments can be isolated from wastes and by-products and can possess potential bioactivities such as antimicrobial, antioxidant, antiproliferative, and anti-inflammatory properties, etc (19).

The **food processing** industry produces enormous amount of waste and by-products, and these form the second-largest generator of wastes after household sewage wastes (1). Fruit and vegetable wastes and/or by-products obtained from the processing industries mainly contains the skin portion or the peel, seeds, and pomace (1). Due to significant nutritional and beneficial health properties, as well as phyto-pigment-antioxidant nature, these plants secondary metabolites can be considered as ‘functional food ingredients’ (1).

For the extraction of natural colorants, the first step is to obtain the crude pigment from the plant resources (1). Usually, conventional methods such as **Soxhlet extraction** techniques using organic/inorganic solvents, maceration or hydro-distillation have been widely used for natural pigment extraction (1). For conventional extraction, generally water or diluted alcohol are employed for water-soluble pigments, while non-polar solvents are used for the extraction of lipophilic pigments (1). The non-polar solvents used for the extraction of pigments are mainly of petrochemical origin e.g. hexane (carotenoids from Gac fruit peel), acetone (lycopene from tomato pulp waste, methanol (anthocyanins from eggplant peel), trifluroacetic acid (betalains from papaya fruits peel) (1).

Greener extraction techniques involve solvents such as: ionic liquids (ILs), water, ethanol, esters of fatty acid or oils of fruits and vegetables (soybean, rapeseed oil, cocoa oils etc.), glycerol etc. which are all gaining importance for the extraction methods for natural pigments (1) The use of **greener extraction techniques** is now considered to be an emerging, re-discovered and innovative method gaining much importance aimed toward avoiding the adverse effects of synthetic solvents (1). Nevertheless, these green extraction modes can also improve the extraction efficiency of natural pigments from vegetal sources (1). The greener extraction techniques that assist with various innovative techniques such as: Ultra-sound-assisted extraction (UAE), Microwave assisted extraction (MAE), Enzyme-assisted extraction (EAE), Supercritical fluid extraction (SFE), Pressurized liquid extraction (PLE), and Pulse-electric field-assisted extraction (PEF) employed for the extraction of natural pigments from vegetal wastes and by-products (1).

Efforts need to be made to develop greener technologies to extract natural pigments from vegetal wastes, micro/ nanoencapsulation techniques to enrich the bioavailability and stability that can simultaneously provide health benefits (1). Thus, the selection of greener technologies, suitable encapsulating material, nature of the encapsulant, encapsulation methods, nature of the core (natural pigment) material, processing conditions suitable for natural pigments still remains a challengeable constraint (1).

PLANT NATURAL PIGMENTS: HEALTH BENEFITS

A study has been reported that chlorophyll-rich plant extracts like wheat grass and other green vegetables can inhibit the **cancer-causing effects** of two mutagens (benzopyrene and methylcholanthrene) (1-20). The structural resemblance of hemoglobin with chlorophyll makes to restore the RBCs and enhances their ability to carry more oxygen as well as eliminate the factors responsible for anaemia (1-10). Furthermore dietary **chlorophyll** and its derivatives prevalent in both fresh and processed foods have antioxidant and **antimutagenic** activities (1-5). In addition to this chlorophyll stops bacterial growth in wounds, eliminates bad breath and body odour (1-5). Chlorophyll also helps to remove heavy metals from the body that have accumulated due to the ingestion of contaminated food products (5).

Blue and **purplish pigmented foods** possess important anti-angiogenic properties which are helpful for the prevention and treatment of **cancer**, protection against liver injuries and improvement of eyesight (1-20). **Delphinidin** has various pharmacological activities such as antioxidant, antimutagenesis, anti-inflammatory and antiangiogenic (1-20). Delphindin can also suppress the growth of **human tumor** cells. It has been observed that berry anthocyanins can trigger genetic signaling in promoting human health and disease prevention (1-5).

It has been found that **anthocyanins** are powerful antioxidants and help to boost the immune system, maintain good health and prevent diseases (1-22). **Resveratrol**, a phytoalexin antioxidant found in red grapes have both chemopreventive and therapeutic effects against many diseases (1-20). It has also been reported that **Resveratrol** has several neuroprotective roles in various neurodegenerative impairments, such as **Alzheimer’s,** Huntington’s, Parkinson’s diseases, amyotrophic lateral sclerosis and alcohol-induced neurodegenerative disorders (1-20). Certain laboratory studies have shown **red coloured apples** containing a variety of **phytochemicals**, including quercetin, catechin, phloridzin and chlorogenic acidhave strong antioxidant activity, inhibited cancer cell proliferation, decreased lipid oxidation, and lowered cholesterol (1-20). Studies have shown that **lycopene** in the diet can help to increase the breakdown of low-density lipoproteins in the body (1-20). **Anthocyanins** are now being studied for DNA protection, fighting estrogen-dependent diseases, anti-inflammatory response stimulation and their role in immune system regulation (1-15). **Red beet products** used regularly in the diet may protect against certain oxidative stress-related disorders in humans (1-20).

Three of the most common carotenoids **– alphacarotene, beta carotene and beta-cryptoxanthin** – can be converted from foods into vitamin A in the body (1-15). **Vitamin A** is needed for good vision in dim light, normal growth and development, a strong immune system and to keep the skin and cells that line the airways, digestive tract and urinary tract healthy (1-15). There's also evidence to suggest that carotenoids – and especially **beta carotene**, might help to reduce the risk of heart disease and certain cancers, especially **lung cancer** (1-20). Citrus fruits and their juices are also packed with the phytochemical **hesperidin**, (protect against heart disease) and tangeritin (may prevent cancer of the head and neck) (1-20). It has been found that the zest of yellow citrus fruits is also a good source of limonene, a phytochemical that helps keep **lungs** healthy (1-15).

It has been observed that black garlic (an aged version of garlic) has twice the antioxidants of regular garlic (1-20). Squid ink gives black pasta its striking colour (1-15). **Squid ink** has been shown to stop the growth of new blood vessels which is believed to slow the growth of cancer (1-15). **Black sesame seeds** are high in **calcium** and make for a great appetizer (1-15). Different types of black **forest mushrooms** contain a compound that stimulates the immune system and can help prevent premature aging and cancer (1-20). **Figs** are a delicious summer fruit that are very high in fiber, potassium, manganese, and calcium (1-20). **Black rice** contains anthocyanin pigments with notable antioxidant and anti-inflammatory properties for potential use in **nutraceuticals** or functional food formulations (1-20).

Some white vegetables contain potent phytochemicals like **allicin** in garlic and onions that enhances the effectiveness of anthoxanthin and also helps in lowering blood cholesterol level (1-15). White foods like potatoes and bananas are good sources of **potassium** needed for nerve and muscle functioning (1-20). Regular consumption of flavonoid-rich white foods reduced the risk of several chronic diseases, including **cancer**, CVD and neurodegenerative disorders (1-15).

Flavonoids exhibit antiallergenic, antiviral, anti-inflammatory and vasodilating actions (1-20). Flavonoid compounds exhibit lipid-lowering effects and have anti-inflammatory and antiatherogenic properties (1-20). Eating high levels of **flavonoids** (anthocyanins) could offer protection from type 2 diabetes by lowering **insulin** resistance and improving blood glucose regulation (1-20). It has been found that cauliflower and turnips contain rich amounts of compounds known as glucosinolates, which may provide some protection against cancer (1-20). **White beans** are valuable sources of protein and fiber, as well as B-vitamins, potassium and iron (1-15).

Anthocyanins found in plants have a wide range of usage (1-15). **Blue, red,** and **purple colored pigments** extracted from flowers, fruits, and vegetables are traditionally used as dye and food colorant (1-20). Besides being used as natural colorants, some of the anthocyanin-rich flowers and fruits have been traditionally used as medicine to treat various diseases (1-20). On the other hand, plant anthocyanins have been widely studied for their medicinal values (1-10).

Anthocyanins possess **antidiabetic**, anticancer, anti-inflammatory, antimicrobial, and anti-obesity effects, as well as prevention of cardiovascular diseases (CVDs) (1-20). Therefore, anthocyanins extracted from edible plants are potential pharmaceutical ingredients (1-20).

Anthocyanin is one of the bioactive components as nutraceutical and traditional medicine. It has been traditionally used as a phytopharmaceutical, appetite stimulant, choleretic agent, and for treatment of many other diseases (1-20). These colored pigments are potent nutraceutical or pharmaceutical ingredients (1-20). As a nutraceutical, the bioavailability of anthocyanin is the key factor for maintaining good health and for prevention of diseases (1-15).

SYNTHETIC FOOD COLORANTS

Synthetic food colours have been increasingly used rather than natural food colours by food manufacturers, as they have several economically relevant traits, such as their low cost; resistance to light, oxygen, and pH changes; and high colour stability (1-17). In contrast to natural food colours, which are usually extracted from several natural sources and purified, synthetic food colours are produced by full **chemical synthesis** or the modification of several **precursor compounds** (1-20). Besides this, they can be used without further transformation, and do not degrade during food processing (1-17). On the other hand, synthetic colors, produced from coal and petroleum, **could be harmful**. So why use artificial colors? (12).

1. The foremost reason is cost.
2. Synthetic colors can be mass produced at a much lower cost than natural colors (12).
3. In addition, the availability of materials to produce natural colors is limited and season dependent (12).
4. The stability of the color, fastness, vibrancy, hues, and shades desired in the present world cannot be met with natural colors alone (12). Color additives are used in food for many reasons. The addition of color in the food increases its attractiveness, make it look fresh and mask its original color (1-20).
5. It also counterbalances the color loss that happens due to exposure to light, air, temperature, moisture, storage conditions, etc (12-25). Giving an identity to colorless food products can also be the reason for adding color to food (1-20).
6. Nevertheless, large number of inorganic chemicals, like mercury sulphide, red lead, white lead, yellow lead chromate, verdigris (chemical mixture of copper salts of acetate, carbonate, chloride, formate, hydroxide, and sulphate), copper sulphate (blue vitriol or bluestone) and Schee le's green (copper arsenite), etc. were also extensively used as colorants in ancient times (1-20).
7. Most **natural colorants** have some **disadvantages,** such as reactivity towards the other ingredients of foodstuffs or in the presence of aromas/ odours, as well as instability in water, or when exposed to light and heat (1-20).

In India, the use of **non-permitted colours** was found to be more in rural areas than in the urban areas (26). Also, more of the **rural market** samples contained permitted colours exceeding the maximum allowed prescribed levels as compared to **urban markets** (26). A study by Tripathi et al. (2007) was carried out to find the type and level of synthetic food colours added to various eatables in the urban and rural areas of Lucknow (26). In urban areas, samples of crushed ice which are preferentially consumed by children population, the presence of **Sunset Yellow FCF** and **Tartrazine** was found to exceed the permissible limit by 8 and 20 times while in rural areas Sunset Yellow FCF, Tartrazine and Carmoisine exceeded the permissible limit by 23, 16 and 15 times, respectively (26). Non-permitted colours such as Rhodamine B, Metanil Yellow, Orange II, Malachite Green, Auramine, Quinoline Yellow, Amaranth and Sudan dyes were identified in various food stuffs (26). The use of these dyes is more common in the rural markets than in the urban markets (26).

Extensive food quality monitoring and surveillance programmes are needed for exposure assessment and to safeguard the health of population at large (26).

Indian Prevention of Food Adulteration Act, 1954

In **India,** the **Prevention of Food Adulteration Act, 1954** (now called the Food Safety and Standards Act, 2006), has been implemented for the quality assurance of various types of foods and food products (12). Further, only **eight dyes** have been suggested edible, but within prescribed limits (12). This act recognizes eight coal tar dyes as safe for use in foods and cosmetics (12). As far as the usage of food dyes in other countries is concerned, authorities allow only 7 dyes in the United States of America, **8 in Canada**, and 15 in several European countries (12). According to the **Indian PFA/FSSA**, **eight synthetic dyes** which are allowed to be used as red, yellow, blue and green colors in food are – Brilliant Blue FCF (Blue 1), Indigo Carmine (Blue 2), Fast Green FCF (Green 3), Tartrazine (Yellow 5), Sunset Yellow FCF (Yellow 6), Erythrosine (Red 3), Carmoisine (Red 10) and Ponceau 4R (Red 18) (12). The maximum permissible level of all food colours that can be added either individually or in blend form to different food is **100 ppm**. It is mandatory to declare the addition of artificial colour on the label of the food item (26). In spite of the regulatory provisions, studies have shown the use of various non-permitted synthetic colours and revealed that colours are used in foods in which they are not permitted (26). The shift from synthetics dyes much simpler, cheaper, brighter, and versatile products to natural dyes is a big challenge for the food industry, and more serious efforts are required to suggest new cost-effective natural alternatives (12).

Synthetic colours are added to foods to replace natural colour lost during processing, to reduce batch-to-batch variation and to produce products with consumer appeal where no natural colour exists (26-28). Thus colouration of food allows the desired esthetic quality of a particular product, and for this reason it is of importance to food industries (26-28). Several sociological, technical and economic factors have influenced the food industry over the past two decades (26-28). The food market has changed rapidly with a much large proportion of foods being "processed" to meet the needs of new consumer segments. Hence food industry strives to provide visually appealing foods that have good taste and meet the consumers demand on quality and price (26-28).

Thus, a number of **non-food dyes** meant for textiles, paper, polishes and other purposes are **used in foods** either deliberately or through ignorance due to their low cost and easy accessibility (26). The use of these dyes is more common in the rural markets than in the urban markets presumably due to the fact that regulatory surveillance is comparatively less intensive and infrequent in rural areas (26). Majority of **non-permitted dyes** such as Metanil Yellow, Orange II, Rhodamine B, Malachite Green, Auramine, Amaranth and Sudan dyes are known to cause varied **toxic** manifestations in the experimental animals (26). It is a matter of serious concern that in spite of regulatory surveillance, the use of **non-permitted colours** in some loose/ non-branded products in both rural and urban markets is continuing (26). Use of **non-food grade colours** and excessive use of even **permitted food colours** needs to be checked by mass awareness and educational programmes, including those specially organised for street level vendors/retailers (26).

Following is the **list synthetic or Artificial colorants** used in the beverage and food industries.

1) Tartrazine

Tartrazine is a synthetic lemon-yellow **Azo dye** used as a food colour (1-20). **Azo food colours** are synthetic, and are prepared from aromatic amines, which contain an Azo group of two nitrogen atoms linked together (-N = N-) and linked to aromatic rings (1-20). The **azo food** colours usually have a yellow, red or brown colour (1-15). In this context, tartrazine ($C_{16}H_9N_4Na_3O_9S_2$), with the chemical name 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazol-3-carboxylate trisodium, usually takes the form of granules or light orange-coloured powders, being soluble in water and sparingly soluble in ethanol (1-20). This additive is usually described in the form of the sodium salt, but potassium and calcium salts are also authorized (1-5). This colourant gives a lemon-yellow colour, but is also used together with blue dyes to bring about a green colour (5).

Tartrazine is used as a food colouring in most food products, namely flavoured drinks, alcoholic beverages, seasonings, flavoured fermented milk products, desserts, edible ices, flavoured processed cheese, edible cheese rinds, appetizers, fish roe, fish paste, crustacean paste, precooked crustaceans, smoked fish, pastry, fine bakery products, confectionery including breath refreshing and chewing gum, soft drinks, fruit preserves, processed mushy and garden peas (canned), and other foodstuffs (5).

2) Quinoline Yellow

The food colouring quinoline yellow belongs to the class of **quinophthalone dyes** (1-17). This colourant consist of a mixture of colours of synthetic origin derived from the quinolone (5**). Quinoline yellow** is a yellow powder or granule, owing to the presence of sulfonate groups, which is soluble in water and sparingly soluble in ethanol. It is a mixture mostly of disulfonates, but it also has monosulfonates and trisulfonates of 2-(2-quinolyl)indan-1,3-dione (1-20). This additive is usually a sodium salt, but potassium and calcium salts are also permitted (5).

Quinoline yellow has been used in marmalades, jams and jellies, decorations and coatings, pastry and fine bakery products, confectionery including breath refreshing and chewing gum, flavoured fermented milk products, edible cheese rind, flavoured drinks, seasoning, desserts, ice cream, smoked fish, fish roe, fish paste and crustacean paste, shells, cheeses, edible casings, and some alcoholic beverages (1-20). This food colouring has not been associated with any significant long-term toxicity and is not genotoxic or carcinogenic. There is no evidence of adverse effects on reproduction or development (5).

3) Sunset Yellow FCF

The food colouring **Sunset Yellow FCF** is a petroleum-derived orange **Azo dye** ($C_{16}H_{10}N_2Na_2O_7S_2$) that gives a yellow-orange colour to food products (5). Sunset Yellow FCF is an orange-red powder or granule, which is soluble in water and sparingly soluble in ethanol (1-16). Like other **Azo food colours**, Sunset yellow-orange is usually present as a sodium salt, but potassium and calcium salts are also allowed (5).

Food colouring **Sunset Yellow FCF** comes in the form of orange-to red-granules, or powders, and can be used in a large number of foodstuffs, including flavoured drinks, flavoured fermented milk products, edible ices, edible cheese rind, seasoning, confectionery including breath refreshing and chewing gum, marmalades, jams and jellies, desserts, processed cheeses, flavoured cheeses, cheese rinds, soups, fish roe, fish paste and crustacean paste, precooked crustaceans, smoked fish, and alcoholic beverages (1-15).

4) Azorubine

The food colouring azorubine is an **Azo dye** consisting of two naphthalene subunits ($C_{20}H_{12}N_2Na_2O_7S_2$) (5). This synthetic food colour gives a red colour to foodstuffs (1-17). This colorant is soluble in water slightly soluble in ethanol solution, but insoluble in vegetable oil (1-15). Due to the general stability **of Azo dyes, Azorubine** is pH and heat stable, and it does not fade away when exposed to light and oxygen (1-10). It is mainly used in foods that are heat-treated after fermentation, occurs as red to maroon powder or granules, and is soluble in water and sparingly soluble in ethanol (1-20).

Azurobine (5) has been used in a large number of foodstuffs, namely flavoured drinks, fruit syrups, canned red fruits, ice creams, flavoured fermented milk products, edible ices, desserts, pastry and fine bakery products, confectionery including breath refreshing and chewing gum, soups, sauces, seasoning, seafood, fish roe, fish paste and crustacean paste, precooked crustaceans, appetizers and alcoholic beverages (1-20).

5) Amaranth

The **amaranth** food colouring is a modified red Azo dye obtained synthetically ($C_{20}H_{11}N_2Na_3O_{10}S_3$) (5). This anionic dye is a reddish-brown powder or granule (soluble in water and sparingly soluble in ethanol) that decomposes at 120 °C without melting (5). Amaranth is used in the form of a tri-sodium salt, but potassium and calcium salts can also be applied (5).

The use of **amaranth** is very restricted, being limited to drinks and some alcoholic drinks, including spirits, aperitif wines, and fish roe (1-10). An acceptable daily intake of 0.15 mg/kgbw was established for amaranth (5). **Amarnath dye** can present health problems, namely allergic reactions, asthma (as it is an histamine liberator), or hives (1-20). It is also suspected of hyperkinesia, may have residues of potentially carcinogenic substances, and can induce the appearance of calcareous deposits in the kidneys (5). Immunological studies using amaranth as a food colouring agent in albino rats reported that no alteration of humoral immunity was observed (5). Amaranth was also associated with **cancer, birth defects, stillbirths, sterility,** and **early foetal deaths** when fed to laboratory rats (5).

6) Ponceau 4R

Ponceau 4R synthetic **Azo food colouring** that gives a red colour to foodstuffs (5). This food colour (1-(4-sulfo-1-napthylazo)-2-napthol-6,8-disulfonic acid, trisodium salt) is a reddish powder or granule which is soluble in water and sparingly soluble in ethanol (1-15). This **Ponceau 4R** colourant is manufactured by coupling diazotized naphthionic acid to G acid (2-naphthol- 6,8-disulfonic acid) followed by the conversion of the coupling product to the trisodium salt (5). Although it is usually described as a **sodium salt** ($C_{20}H_{14}N_2Na_3O_{10}S_3$), and used as a potassium or calcium salts (5).

Ponceau 4 (5) is used as food coloring agent to intensify red colour commonly used in some sausages, flavoured drinks, flavoured fermented milk products, edible ices, desserts, edible cheese rind, fruit syrups, red fruit preserves, jellies, jams and marmalades, pastries and fine baked goods, confectionery including breath refreshing and chewing gum, soups, appetizers, seasonings, sauces, seafood, fish roe, fish paste and crustacean paste, precooked crustaceans, smoked fish, and alcoholic beverages (5). The acceptable daily intake for ponceau 4R is 0.7 mg/kgbw (5).

There is no evidence of carcinogenicity, genotoxicity, neurotoxicity, or reproductive and developmental toxicity at the allowed dietary exposures, but surpassing this limit—as with other **Azo food colours**—is known to induce allergic reactions, especially when paralleling intolerance to acetylsalicylic acid, which is suspected of hyperkinesia (5). In this context, it seems that it may further contain residues of potentially carcinogenic substances, namely unsulfonated aromatic amines (1-20). Inetianbor et al. further reported the occurrence of **DNA damage**, tumours in animals, and effects in asthmatics (5).

7) Erythrosine

Erythrosine ($C_{20}H_6I_4Na_2O_5H_2O$) is a xanthenedye (thus, it is not an Azo substance) (1-20). It is a red food colouring of synthetic origin (5). **Erythrosine** is a disodium salt of 2,4,5,7-etraiodofluorescein is a red powder or granule which is soluble in water and slightly soluble in ethanol (5).

8) Allura Red AC

Allura red AC, is a synthetic red **Azo food colouring** with the chemical formula $C_{18}H_{14}N_2Na_2O_8S_2$ (5). Although it is usually supplied as a sodium salt, it can also be used in the form of calcium and potassium salts (5). This food colour which takes the form of a dark-red powder or granule, is soluble in water but insoluble in ethanol (5).

This additive is used in decorations and coatings for pastry products, confectionery including breath refreshing and chewing gum, pastry and fine bakery products, flavoured fermented milk products, edible cheese rinds, desserts, preserves of fruits, ice cream, flavoured drinks, baked crustaceans, seafood, breakfast sausages, appetizers, sauces, seasonings, soups, luncheon meat, replacements, and alcoholic beverages (5).

9) Patent blue V

This **Azo** food colouring **Patent blue is a sky-blue synthetic** tri-phenyl-methane dye ([4-(α-(4-di-ethyl-amino-phenyl)-5-hydroxy-2,4-di-sulfophenylmethylidene)-2,5-cyclohexadien-1-ylidene] di-ethyl-ammonium hydroxide), that consists of sodium $C_{27}H_{31}N_2O_2S_2Na$ or calcium $C_{27}H_{31}N_2O_2S_2Ca_{1/2}$ salts (5). The use of the potassium salt is also authorized (1-15). This food colour usually takes the form of a blue powder or granule, and is soluble in water and slightly soluble in ethanol (5). It is redox-sensitive, changing from reduced yellow to oxidized red forms in solution (1-20). This **synthetic colourant** has been used in flavoured drinks, decorations and coatings for pastry products, pastry and fine bakery products, confectionery including breath refreshing and chewing gum, flavoured fermented milk products, edible cheese rinds, desserts, edible ices, several types of preserves of red fruits, seasonings, snacks, and alcoholic beverages (5).

10) Indigotine

Indigotine, or “indigo carmine” is a food colouring of synthetic origin, occurring in the form of 5, 5-indigodisulfonic acid sodium ($C_{16}H_8Na_2O_8S_2$), or as calcium and potassium salts (5). **Indigotine** is a 0.2% aqueous solution, and is blue at pH 11.4 and yellow at 13.0 (1-20). It is very sensitive to light and oxidizing agents such as bleach (1-15). This food colour is present in the form of dark blue powders or granules which are sparingly soluble in ethanol (5).

11) Brilliant Blue FCF

Brilliant blue FCF is a triarylmethane dye ($C_{16}H_8N_2Na_2O_8S_2$) (5). It is a blue-coloured synthetic dye which is very sensitive to oxidizing agents (bleach) and light (1-20). **Brilliant blue FCF** is soluble in water and glycerol, but slightly soluble in ethanol (5). It is a sodium, calcium or potassium salt (1-13). **Brilliant blue FCF**, which can be presented in the form of reddish-blue powders or granules, is used in flavoured drinks, flavoured fermented milk products, edible cheese rinds, seasonings, desserts, edible ices, confectionery including breath refreshing and chewing gum, preserves of red fruits, processed mushy and garden peas (canned), snacks, and alcoholic beverages (5).

Only about 5% of the ingested **brilliant blue FCF** (5) is absorbed from the gastrointestinal tract, and the remainder occurs in the feces (5). However, it can be absorbed directly into the blood stream through the tongue or shaved skin (1-10). In this context, an acceptable daily intake of 6 mg/kgbw has been reported (5). Concerning the side effects on human health, it is reported that, in high doses, it could cause deposits in the kidneys and lymphatic vessels, and that residues of potentially carcinogenic substances can also occur (5). Inetianbor et al. further highlighted, as possible negative health effects, hyperactivity and skin rashes, **DNA damage** and **tumours** (found in animals) (5).

12) Green S

Green S or Brilliant green BS is a green synthetic coal tar tri-arylmethane food colouring agent in the form of sodium ($C_{27}H_{25}N_2NaO_7S_2$), potassium or calcium salts (5). This dye is soluble in water but only slightly soluble in ethanol, and comes in the form of powders or granules, in a dark-green to dark-blue colour (1-15). **Green S** is used in syrups of candied fruits, fruits and vegetables, marmalades, jams and jellies, flavoured drinks, confectionery including breath refreshing and chewing gum, pastry and fine bakery products, decorations and coatings, desserts, flavoured fermented milk products, edible cheese rinds, ice creams, soups, sauces, precooked crustaceans, fish paste and crustacean paste, processed mushy and garden peas (canned), seasonings and alcoholic beverages (5).

An acceptable daily intake of **Green S** is 5 mg/kgbw has been indicated (5). Harmful side effects on human health have been described when consumed in very high doses, and there is a possibility that there may be residues of potentially carcinogenic substances (5). However, the data on the absorption, distribution, metabolism and excretion of **Green S** showed that it is poorly absorbed, and is mainly excreted unchanged in faeces (5).

13) Brilliant Black PN

Brilliant black PN, is an artificial **black Di-Azo food colouring** agent in the form of sodium ($C_{28}H_{17}N_5Na_4O_{14}S_4$), potassium or calcium salts (5). It occurs in the form of black powder or granules, being soluble in water but sparingly soluble in ethanol, and giving a black colour to foodstuffs (1-20). This food colouring is used in confectionery including breath refreshing and chewing gum, pastry and fine bakery products, flavoured fermented milk products, flavoured drinks, desserts, edible ices, edible cheese rinds, fish roe, seasonings, soups, cheese rinds, edible casings, fish paste and crustacean paste, precooked crustaceans, smoked fish, appetizers and alcoholic beverages (5). An acceptable daily intake of 5 mg/kgbw has been indicated (5). Harmful side effects on human health have been described when consumed in very high doses, and there is a possibility that there may be residues of potentially **carcinogenic** substances (5).

14) Brown HT

Giving a reddish-brown colour, the food colouring Brown HT, also known as is an Azo food dye in the form of sodium ($C_{27}H_{18}N_4Na_2O_9S_2$), calcium or potassium salts (5). **Brown HT**, being a brown powder or granules which are soluble in water and insoluble in ethanol (5). **Brown HT** can be used in most food products, namely in pastry and fine bakery products, confectionery including breath refreshing and chewing gum, flavoured fermented milk products, desserts, edible cheese rind, ice creams, smoked fish, soups, appetizers, unprocessed meat, precooked crustaceans, seasonings, sauces, flavoured drinks, and alcoholic beverages (5).

Allergic reactions mainly in **Asthamatic** patients and people with an intolerance to acetylsalicylic acid, or those who suffer from skin allergies has been reported (1-15). This additive is suspected of hyperkinesia, and may have residues of potentially carcinogenic substances (5). When consumed in high doses, experiments with rats revealed deposits in the kidneys and lymph nodes (5). However, carcinogenic effects were not detected in long-term toxicity tests with rats and mice (5).

15) Litholrubine BK

Litholrubine BK is an Azo food colouring agent of synthetic origin which is only used to give a red colour to edible cheese rinds (5). It is a red powder which is slightly soluble in hot water but insoluble in cold water and in ethanol (1-20). Some side effects on human health, arising from the consumption of this dye are similar to those of other **Azo** dyes, (*i.e*, it is an allergizing substance) (5). Additionally, it is suspected of hyperkinesia, and there is the possibility of having residues of potentially carcinogenic substances (5).

SYNTHETIC FOOD COLORANTS; TOXIC EFFECTS

Food colour is also one of the most impressive attributes of foodstuffs, which directly determines the preference, selection and eating desires of the consumers (1-23). The utilization of colouring additives in food is confronted with debate (5). On a global scale, the use of colours in food has faced challenges with disagreement, particularly when added at high doses (i.e., when exceeding the recommended doses) (1-20). The demand for natural colours for food processing is increasing, mostly due to concerns about **health risks** triggered by some synthetic additives that can be toxic at metabolic, physiological and toxicological levels (1-20).

Major plant pigments includes, Chlorophyll (green), Carotenoids (yellow, red, orange), Flavonoids: anthocyanins, anthoxanthins (red, blue, purple), and Betalains (red, yellow, purple (1-20). Natural colors include β-carotene, chlorophyll, caramel, annatto and saffron. Synthetic colorants include the permitted organic synthetic dyes, Brilliant blue, tartrazine, erythrosine, ponceau 4R, allura red, sunset yellow, fast red E, orange GGN and scarlet GN (1-22).

Synthetic dyes are widely used due to their coloring properties, uniformity, stability and low cost (1-20). However, many of them become **toxic** after prolonged use, causing health problems such as indigestion, anemia and allergic reactions as asthma and urticaria, pathological lesions in the brain, kidney, spleen and liver, tumors and cancer paralysis, mental retardation, abnormalities in offsprings, growth retardation and eye defects resulting in blindness (1-22). Considering, among others, genotoxic and carcinogenic risks, urticaria, asthma, nausea, eczema, bronchitis, bronchitis, bronchospasm, headache, reduced blood coagulation and hyperactivity are some of the health issues (1-22). **Most natural colorants** have some disadvantages, such as reactivity towards the other ingredients of foodstuffs or in the presence of aromas/ odours, as well as instability in water, or when exposed to light and heat (1-20). Moreover, contrarily to synthetic dyes, the consumption of natural food colourants can have significant benefits; they are in demand for their reliability, functionality, biological potential and health benefits

(1-22). **Natural colours** are obtained from nature, and can be isolated by more or less complicated extraction processes, whereas synthetic dyes are chemically synthesised. However, sometimes it is not easy to classify them into synthetic or natural colours (1-22).

Artificial food colorings have been in the controversy for many years and scrutinized for being possibly linked to cancer, allergies and hyperactivity (1-22). Globally, natural as well as artificial dyes are being cautiously researched and regulated by the food safety authorities. **In India, the Prevention of Food Adulteration Act, 1954** (now called the Food Safety and Standards Act, 2006), has been implemented for the quality assurance of various types of foods and food products. According to Indian PFA act **only eight synthetic dyes** which are allowed to be used as red, yellow, blue and green colors in food are – Brilliant Blue FCF (Blue 1), Indigo Carmine (Blue 2), Fast Green FCF (Green 3), Tartrazine (Yellow 5), Sunset Yellow FCF (Yellow 6), Erythrosine (Red 3), Carmoisine (Red 10) and Ponceau 4R (Red 18) (12) have been suggested edible, but within prescribed limits (12).

The **synthetic food colours** do not occur in nature due to chemical structures, but have the advantage of prevailing in the form of powders, pastes or granulates, and are soluble in water (1-20). Nevertheless, some synthetic colour additives may present health problems, namely allergenic problems, which in children can cause hyperactivity and even mutagenic and/or carcinogenic pathologies (1-22). Therefore, some food safety studies have led to the banning of some synthetic food additives (1-20). Similarly, the food colouring (Ethyl-β-apo-8'-carotenate (ethyl ester of β -apo-8'-carotenic acid)) is no longer included in the European Union additives list (1-20).

The average daily intake of tartrazine was set as 7.5 mg/kgbw (5). Tartrazine seems to induce the most **allergic** and intolerance reactions among all of the **Azo** dyes (particularly among asthmatics and those with an aspirin intolerance) (5). It is further suspected of childhood hyperactivity, and is has been mentioned that it may contain residues of carcinogens (1-20). However, studies to determine the eventual carcinogenic effects of tartrazine on rats showed no carcinogenic changes in the gastric area (5). Additionally, t**artrazine** is also suspected of triggering urticaria and angioedema (the swelling of the lips, tongue, throat, and neck caused by the release of **histamine** in an allergic reaction) (1-20).

The use of **Sunset Yellow FCF** colourant can trigger health problems, namely allergic reactions, especially in people with an intolerance to acetylsalicylic acid (1-20). It is further suspected of hyperkinesia, and may have residues of potentially carcinogenic substances (1-22).

Unhealthy processed foods have become a part of our daily diet (1-20). Such foods are often added with illegal food dyes specifically synthetic ones as coloring agents having **genotoxic** or **carcinogenic properties** and thus is a matter of public health concern (1-20). Consumption of artificial food dye has increased many folds in the last 50 years, and children are the biggest consumers (1-20). Some dyes may contain **cancer-causing contaminants (**5). **Food dyes** such as Erythrosine, Carmoisine, and Tartrazine are the leading causes of liver carcinogenesis (1-20). **Azo dyes** and triphenylmethanes are the most common classes of illegally added food dyes (1-20). The **toxic activity of Azo** dyes, which are widely used in industry, is a result of their metabolism (1-20). The enzyme-mediated Azo reduction leads to the formation of active aromatic amines attacking DNA (1-20). Intake of these agents in amounts higher than the advised daily intake (ADI) has been shown to have adverse effects in mammalian models (1-20). Thus, a large number of food products incorporate colours in order to obtain a pleasing appearance or dye feature (1-20). For instance, in order to enhance consumers preferences, food colours are usually applied to edible ices, desserts, pastry and fine bakery products, decorations and the coatings of pastry, confectionary products, sauces, fruit juices, snacks and soft drinks, and alcoholic beverages (5-20). A number of chemical food color and flavour additives are routinely added during processing to improve the aesthetic appearance of the dietary items. However, many of them are toxic after prolonged use (1-20).

However, as food colouring pigments are generally unstable and become modified during processing, in order to maintain or restore product colour uniformity, colorants are added to food products around the world (1-20). In this context, although they are still widely used, synthetic food colorants, due to their potential hazards, are being replaced by those obtained from natural origins (1-22). Indeed, numerous side effects and toxicities, at both the medium and long-terms—namely allergic reactions, and behavioural and **neurocognitive effects**—have been related to the use of synthetic colorants, whereas their naturally-derived counterparts seem to provide a somewhat high-quality and effective contribution as a health promoter (1-20). The flavouring material is widely used in the food industry to impart sensory pleasures to food and beverage (1-20). **Natural flavours**, which are vegetables or animal products, are used either as such or as processed and acceptable for human consumption, for example fruit juices, herbs and spices (1-20). **Synthetic flavours**, which are substances not yet been identified in natural products intended for human consumption, are either processed or not, for example **ethylvanillin** and **2-methyl pentonic acid** which has strawberry flavour (1-20).

How to detect synthetic colorants in Food or Beverages.

In food industry, **food colorants** of several types are chemical substances that are added to food matrices to enhance or sustain the sensory characteristics of the food product, which may be affected or lost during processing or storage, and in order to retain the desired color appearance (27). Several matrix-dependent methods have been developed and applied to determine food colorants by employing different analytical techniques along with appropriate sample preparation protocols (27). Major techniques applied for their determination are chromatography with spectophotometric detectors and spectrophotometry while sample preparation procedures greatly depend on the food matrix (27).

HPLC is the most frequently used followed by capillary electrophoresis (27). In terms of detection methods, the simple UV-Vis/DAD is the predominant one followed by tandem MS (27). Sample preparation is of great importance and must be carefully developed, in order to avoid or eliminate existing matrix interferences aiming to the development of simple, selective, and precise methods of extraction (27). In the case of simple liquid samples, dilution and injection are preferred, though in other cases such as high protein content foods, specific steps need to be followed for sufficient sample clean-up (27).

Komissarchik and Nyanikova (2014) (28) has developed a **new test systems** and expression method were developed for the rapid detection of synthetic food dyes in drinks including alcoholic drinks, fruit juices, soft drinks and other non-alcoholic drinks (28). The detection is based on selective adsorption of synthetic food dyes from a drink by the specific adsorbent contained in the developed test system (28). This method allows screening of samples for synthetic food dyes in the presence of natural colors by differential spectrophotometry following adsorption (28). The said adsorbent provides adsorption of synthetic food dyes and substantially does not interact with natural dyes of drinks (28). Therefore, when a drink contains synthetic dyes, the color of the sample changes, whereas the color of a drink having only natural dyes stays substantially the same (28). This test can be used as a first step analysis for screening. It was shown that the test can take several minutes. The detectable concentration limits are from 1.0 mg/100 ml to 50.0 mg/

100 ml dyes (28). So, only in a case when the test showed the presence of synthetic dyes in a drink, further laborious identification and quantitative determination of the revealed dyes could be performed (28). The use of such an approach reduces the time and costs required to perform the analysis (28).

CONCLUSION

Experimental evidences have confirmed that plant pigments not only protect plants from external damages, they also have a great impact on human health due to its curative and healing properties The very first pigment, chlorophyll is a water-insoluble plant pigment that has antioxidant, **antimutagenic**, anti-cancer and antimicrobial properties. Water-soluble vacuolar pigment anthocyanin is responsible for the blue and purple colouration of fruits and vegetables. It can cure liver injuries, improves eyesight and also can suppress tumor growth. Carotenoids, betalains and anthocyanins responsible for red colouration, possesses antioxidant, anticancer and CVD preventing properties. Yellow and orange pigmented carotenoids can be converted into vitamin A, which is essential for growth, immune functioning and eye health**. Anthocyanin** and anthoxanthin responsible for giving black and white colouration to foodstuffs.

The increasing variety of food production, which is associated with the diversification of technological developments and changes in consumers' nutrition habits, has increased the number of processed foods. In this context, the colouring additives applied to food products play an important role in consumers' preferences. However, their preferences for **naturally derived colourants** which are closely associated to the image of healthy, safe and good-quality products sharply increased, as a large number of synthetic dyes have recognized side effects on human health**. Synthetic colour additives** may present health problems, namely allergenic problems, which in children can cause hyperactivity and even mutagenic and/or carcinogenic pathologies. **Synthetic colorants become toxic** after prolonged use, causing health problems such as indigestion, anemia and allergic reactions as asthma and urticaria, pathological lesions in the **brain, kidney**, spleen and liver, tumors and cancer paralysis, mental retardation, abnormalities in offsprings, growth retardation and eye defects resulting in blindness. There is a high tendency to use synthetic food colours in confectioneries and beverages and some confectioneries contain unidentified colours including a **textile dye**. Therefore, implementation of regulations and awareness programs of food colours for consumers and food manufacturers are highly recommended.

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