Food colours of plant origin

Article *in* CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources · December 2007

DOI: 10.1079/PAVSNNR20072087

KN Shiva
Indian Council of Agricultural Research

Parthasarathy Va
Bioversity International (CGIAR)

CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2007 **2**, No. 087

Review

Food colours of plant origin

A. Shamina, K.N. Shiva and V.A. Parthasarathy*

Address: Indian Institute of Spices Research, P.O. Marikunnu, Kozhikode-673 012, Kerala, India.

***Correspondence:** V.A. Parthasarathy. Email: parthasarathy@spices.res.in, parthasarathy_in@yahoo.com

Received: 17 September 2007
Accepted: 30 October 2007

doi: 10.1079/PAVSNNR20072087

The electronic version of this article is the definitive one. It is located here: http://www.cababstractsplus.org/cabreviews

Abstract

Pigments of plant origin are gaining importance globally as a potential source of natural food colours for their versatility and so as to avoid a variety of health hazards caused by synthetic colours. Colour has been added to our foods in one or another form for centuries. Earlier studies of food colours have focused mainly on synthetic colours and their chemistry, stability, metabolism and toxicity, but recent attention has been directed towards biochemical aspects of food colours of plant origin and their utilization studies. This review gives up-to-date information on various aspects of food colours such as history, types, sources, uses, chemistry, properties, colour additives and regulations for use.

Keywords: Natural food colours, Pigments, Chemistry of pigments, Anthocyanins, Carotenoids, Colour additives

Introduction

The palatability of food is enhanced by attractive colours. Colour, possibly more than any other factor, influences the acceptance of products by consumers. Colour is often seen as synonymous with quality and is used as a strong indicator of product safety and value. Foods with characteristic appeal and colour are generally preferred. Colour is an important element in enhancing foodstuffs, constituting one of the major dietary additives. The food industry has therefore resorted to enhancing or restoring the colour of foods to increase consumer acceptability. Consequently, over the centuries, colour has come to play a prominent role in things important to humans: food, medicine and physical appearance.

Food colour is contributed by various components in the food, some of which occur naturally, while others are produced by heating or processing and the rest are added to alter the colours. Food colours can be classified primarily into two types, viz., (i) natural colours and (ii) synthetic colours (permitted and non-permitted colours) [1]. Those available in the market are termed as nature-identical colours, where the colours are manufactured by chemical synthesis as that of synthetic colours, but do not require US Food and Drug Administration (FDA) certification, and chemically and functionally, they are undistinguishable from the natural colorant [2].

The safety of synthetic colorants has been a moot question for the past several decades, and with increased consumer awareness, the demand for and interest in natural colorants have increased significantly. This and legislative action have resulted in a reduction in the number of permitted colorants.

The limitations to the use of natural food colorants are their low stability in the food processing procedures, formulation and storage conditions and the fact that they may also impart undesirable odour or flavour to the food. The successful incorporation of red cabbage and radish colour components in food has been made possible only after the removal or substantial reduction of their strong aroma and flavour components [3].

The selection of a food colorant is usually made after it is deemed satisfactory according to the following criteria: target shade, the physical/chemical attributes of the food matrix, stability to processing and storage conditions and regulatory issues [3]. Interest in food colorants as shown by the number of patents has doubled in recent years, with natural pigments outnumbering synthetics by five to one.

History

Colour, in one form or another, has been added to our foods for centuries. It is known that the Egyptians coloured candy, and wine was coloured as long ago as 400 BC. The earliest record on the use of natural dyes was found in China, dated 2600 BC. Dyeing was known in the Indus Valley period as early as 2500 BC. Saffron is mentioned in the Bible and henna was used even before 2500 BC [4]. The ancient Britons were the first to use the blue dye, woad, which might have originated from Palestine in wild conditions. Even though dyes have been discovered accidentally, their use has become a part of human's customs in the modern world and the art of dyeing has spread widely with the advancement of civilization [5].

Some of the well-known dyes used in ancient times were: a red dye obtained from the roots of *Rubia tinctorium* L.; blue indigo dye from the leaves of *Indigofera tinctoria* L.; yellow dye from the stigma of saffron (*Crocus sativus* L.) as well as from the rhizomes of turmeric (*Curcuma longa* L.) [6].

The developing food industry had a vast array of synthetic colours in the late 1800s. This led to colours being added for decorative purposes and unfortunately to disguise low-quality foods. There was no control over this use of colour and so inevitably legislation came into force. In particular, this was as a result of health concerns over some of the toxic compounds used. An established list of permitted synthetic colours eventually came into force in most countries early in this century. In the last 20 years, however, consumers have become increasingly aware of the ingredients of their foods and as such they require foods to be as 'natural' as possible. This combined with technological developments has fuelled the increase in the usage of naturally derived colours. Today the food industry has an extensive palette of colours available, allowing selection of the most suitable colour for their application requirements. Legislation is also in place to protect the consumer. Colour suppliers are, however, constantly striving to improve the technical and physical properties of their colour portfolio, to make the use of colour easier, to improve the stability and to meet customer demands on the functional additives used within colour formulations [7].

Foods as Natural Antioxidants and Colorants

Oxidative stress is an important contributor to the risk of chronic diseases such as cancer, cardiovascular disease, osteoporosis and diabetes. Inclusion of fruits and vegetables in the diet, which are good sources of antioxidant phytochemicals that mitigate the damaging effect of oxidative stress, is highly recommended to combat the incidence of human diseases associated with such stress.

Consumption of fruits and vegetables, olive oil, red wine and tea is inversely correlated with heart disease rates. These foods are particularly rich in natural antioxidant nutrients, including ascorbate (vitamin C), the tocopherols (vitamin E) and carotenoids. More than 600 naturally occurring carotenoids have been identified. These compounds are plant pigments that provide the bright colour of various fruits and vegetables; lycopene, which gives tomatoes their red colour, is under active research. Flavonoids are >4000 naturally occurring substances that provide colour, texture and taste for plant foods. As free-radical scavengers, flavonoids inhibit lipid peroxidation, promote vascular relaxation and help prevent atherosclerosis. A sufficient supply with antioxidants from the diet might help prevent or delay the occurrence of pathological changes associated with oxidative stress. When the diet fails to meet the antioxidant requirement, dietary supplements might be indicated. The recently coined term 'nutraceuticals' describes a variety of non-prescription products that are used to enhance health. The best known are vitamin E, vitamin C, carotenoids, coenzyme Q10, flavonoids and the amino acid L-arginine [8].

Natural Food Colours

There is no universally accepted definition so far of a natural food colour, but it can be defined as any dye, pigment or any other substance obtained from plant, animal, insect, algae, mineral or other sources capable of colouring food drug, cosmetics or any part of the human body [9]. The colours are extracted from a variety of sources such as seeds, fruits, bark, leaf, root, stem, wood flower, rhizome and whole plant by conventional methods. Being biological in origin, they are often called as 'bio colours' [1]. The natural colour of foods may be the result of the presence of natural pigments such as carotenoids, chlorophylls, myoglobins and anthocyanins and chemical modification during the processing of natural constituents of foods, e.g. caramelization and colour additives [10]. A suitable natural colour can be developed by manipulation of certain factors such as pH, heat, light, storage and other ingredients. Food elements are used in the range of 10–500 ppm in food and beverages [11]. According to the FDA, colour pigments of natural biotic origin are exempt from certification (in 21 CFR, Part 73 for food, drug and cosmetics). There are 26 colours permitted for use in food and 28 for use in cosmetics and pharmaceuticals.

The natural colorant area can be subdivided into: anthocyanins, betalains, chlorophylls, carotenoids, flavonoids, polyphenols, Monascus, haems, quinones, biliproteins, safflower, turmeric and miscellaneous. All involve different groups of chemical compounds, which may be used directly as colorants, or may be chemically modified to produce different hues or increased stability. All usually involve a method of collection, extraction, purification, possibly stabilization and formulation. A variety of hues can be obtained ranging from green to yellow, orange, red, blue and violet, depending on the source of colorant. Similarly, water- or oil-soluble formulations can be prepared, depending on the type of colorant [12].

In a study on different fruit wines made from black mulberry, blackberry, quince, apple, apricot, melon, red raspberry, bilberry, sour cherry and strawberry, the highest antioxidant activities and total phenolic contents were determined in bilberry, blackberry and black mulberry wines. The results demonstrated the importance of bilberry, blackberry and black mulberry wines as natural antioxidants and colorants [13].

The yellow pigment from the flowers of *Leucosceptrum canum* is a traditional food pigment in some districts of China. Although its structure and characteristics are unclear, the pigment was stable in the presence of acid, light, heat, some metal ions, salt and sugar, and its stability was not affected by hydrogen peroxide without heat treatment, but was affected by alkali and reducing agents such as sodium sulphite, and also hydrogen peroxide under heat treatment. The pigment is an amine and it will be an ideal natural pigment for the food and pharmaceutical industries [14].

Plants Yielding Natural Dye/colour

Today's food, pharmaceutical and cosmetic manufacturers can draw from an array of dyes and pigments, synthetically or naturally derived, to colour their products. Although synthetic colours have been favoured over the past 100 years, processors have recently turned to naturally derived colours as a viable alternative, responding to an ever-increasing consumer demand for natural products.

Among the biological sources, many natural dyes/colours are obtained mainly from plants, producing various colours such as red, yellow, blue, black, brown and a combination of these. Green is the dominant pigment in plants, while the carotenoids are a large group of pigments associated with chlorophyll and responsible for autumn leaf pigmentation. Many of the intense colours in flowers and fruits are contributed by the flavonoid pigments and closely related compounds with a diverse range of colours, which are the result of structural differences between the compound and the relative concentration of specific pigments within the cells. Betalains, a restricted group of pigments, get their name from the red–violet pigment isolated for the first time in crystalline form from the root of the beet *Beta vulgaris*, L. [15].

Natural colorants and pigments from plants have use in colouring food, beverages, soft drinks, confectionery, bakery products, etc. (Table 1). Colours of plant origin are environmentally friendly and non-toxic and hence preferred over synthetic colours. Over 2000 pigments have been reported to be produced by various parts of plant, of which only little more than 150 have been exploited commercially [6]. In India alone, about 450 plants are known to yield colours [18], of which 50 are rated as important (ten from roots, four from barks, five from leaves, seven from flowers, seven from fruits, three from seeds, eight from wood and three from gums and resins) [6]. Plant pigments that provide natural colours to food can be grouped into four primary classes based on their chemistry, viz., chlorophylls, carotenoids, flavonoids and betalains (Table 2). Some of the important natural food colour/pigments-yielding plants and their origin and distribution are summarized in Table 3.

Types of Colours

The palette of colours available to manufacturers is of three types:

- **Synthetic colours:** These do not occur in Nature and are produced by chemical synthesis. These colours, especially in the USA, must be tested for purity and certified, batch by batch, prior to sale to manufacturers, and are thus termed 'certified colours'.

Seven synthetic colorants, belonging to four distinct chemical classes, are permitted by the FDA for use in foods and in orally ingested drugs and cosmetics: FD&C Red No. 40, FD&C Red No. 3, FD&C Yellow No. 5, FD&C Yellow No. 6, FD&C Blue No. 1, FD&C Blue No. 2 and FD&C Green No. 3. Citrus Red No. 2 is permitted only for colouring skins of oranges and Orange B for surfaces of casings of frankfurters and sausages. They share the common property of water solubility, being conferred by the presence of one or more sulphonic or carboxylic acid groups [10]. A brief description of the four classes is given below:

- **Azo dyes** are mono-, di- and tri-sulphonated compounds containing a naphthalene or pyrazolone ring linked by an azo bond to a second naphthalene or benzene ring. Highly sulphonated, intact azo dyes are poorly absorbed in the intestine, its reductive cleavage products are rapidly absorbed by intestinal bacteria, further modified by the liver and excreted in the bile and urine [19]. The toxicology of amaranth (FD&C Red No. 2) has been reviewed thoroughly, and despite its long history of apparent safe use, its safety has not been categorically established [20].
- **Triphenylmethane dyes** (FD&C Blue No. 1 and FD&C Green No. 3) are not widely used as such in foods, but as blends to produce purple and green hues. It is absorbed in the intestine to less than 10% and rapidly excreted in the bile and therefore low levels of toxicity have been reported [20].
- **Xanthene dyes** have poor light stability and therefore limited application in coatings and beverages. It is used in confections, dessert powders and baked goods. Erythrosine is the only xanthine derivative; it is poorly absorbed in the gastrointestinal tract and is excreted in the bile. Erythrosine has been reported to inhibit neurotransmitter uptake [21].

Table 1 Sources of natural food colours, pigments and their applications [9, 16, 17]

Colour	Source	Applications/uses
Chlorophylls/chlorophyllin		
Natural green and green	Nettles, grass, algae, alfalfa, celery, collard greens, sea vegetables, green beans, peas, green olives, parsley, spinach, green turnips, asparagus, bell peppers, broccoli, Brussels sprouts,green cabbage, barley and other herbs	Pastas, confectionery, medicines, processed food, vegetable oils, delicatessen, spice preparations, ice cream and colouring materials
Carotenoids and xanthophylls		
Natural orange	Mushrooms	Tanning pills, fruit-spreads, candies, syrups, sauces and carbonated drinks
Yellow and red	Gardenia	Confectionery, agricultural products and processed marine products
Yellowish orange	Turmeric, curcumin/CU-Chloro	Beverages, sauces, confectionery, desserts and ice cream
Yellow–orange to red–orange	Paprika extracts/capsanthin (paprika pod) and paprika leaf extract	Beverages, processed foods and tomato products
Yellow–orange to red–orange	Paprika oleoresin (paprika pod)	Beverages, processed foods such as sausage, dressings, dry soluble seasonings, food coatings, snack food seasonings and tomato products
Yellowish orange	Carrot	Sorbets, beverages, confectionery and sauces
Yellowish orange	Saffron	Confectionery, agricultural products, and processed marine products
Yellow–orange	Vegetables	Baby foods, cereals, sauces, processed cheese and fruit drinks
Yellow	Ginger	Food products and beverages
Red, yellow and orange	Carrots, sweet potatoes, spinach, collard greens and tomatoes	Food products, sauces and chutneys
Yellow	Paprika	Beverages, processed food and tomato products
Bright yellow	Marigold	Food colorants
Orange	Parsley and sweet pepper	Food products
Orange–yellow	Annatto	Ice cream and dairy products, bakery and snack foods, desserts, custard powder, cereal products, confectionery, colouring butter, citrus juices, concentrates, drinks, candies, etc.
Orange–yellow	Turmeric, saffron and parsley	Food products, cosmetic and pharmaceutical products
Orange to red	Citrus fruit skin and vegetable pulp	Food products, cosmetic and pharmaceutical products
Red to reddish orange	Paprika, red pepper, saffron and mustard	Beverages, processed food and tomato products
Red, dark red and purple red	Paprika and red pepper	Beverages, processed food and tomato products
Red to dark red	Red palm oil	Food products
Dark red	Paprika and parsley	Beverages, processed food and tomato products
Pink	Pink edible mushroom	Food products
Greenish yellow to yellow	Stinging nettle	Ice cream, delicatessen, baked goods, fruit preparations, and foods such as nettle soup and nettle cheese
Brown to reddish brown	Onion	Confectionery, stock farm and processed marine products, colouring of seasonings and sauce
Brown	Cacao	Colouring incorporated in candy or cake, chocolate, biscuits and other products
Brown	Shea nut	Colouring foods
Brown	Cinnamon	Preserving the food articles

Betalains		
Yellow to orange	Beetroot	Beverages, frozen foods, fruit fillings, candies and baked goods
Bluish-red	Beet juice colours (beet root) and opuntia	Fruit preparations, condiments sauces, fillings and candies, condiments, gelatine products, certain powdered beverage products and dairy products
Red–violet and yellow	Red beet root and opuntia	Beverages, frozen foods, food stuffs, fruit fillings, chewing gums, candies, baked goods, medicinal products, colouring red to soybean products
Flavonoids/anthocyanins		
Red, purple and blue	Strawberries, grape skin, black grapes blueberries, raspberries and red perilla	Confectionery, food products and dessert products
Red	Red cabbage	Colouring chewing gum and vegetable juice, making drinks free from nasty smell, and sedimentation
Red	Red-fleshed potato	Colorant used as an additive to foodstuffs, beverages, pharmaceuticals, toiletries, etc.
Deep red	Elderberry	Beverages, fruit confectionery, sorbets and sauces, food colorant, desserts and soft drinks
Bright red–orange to strawberry red	Chokeberry or red fruit (Aronia)	Jelly making, candies, pie and cookie fillings, yogurt, sorbet and flavoured milk
Bright red and purple	Hibiscus	Soft drinks and alcoholic beverages
Reddish-purple	Grape	All beverages, fruit base, sorbets and sauces
Yellow and reddish-orange	Safflower	Colouring food items and soft drinks
Yellow–orange	Tea (flavone)	Beverages, medicines, health-care products and food products
Purple–black	Black currant	Beverages, confectionery, fruit preparations, soft drinks and preserves
Dark violet–blue	Indigo plant	Fruits, dairy products, cosmetics and medicines
Bluish red	Hibiscus	Bakery products and tea-based beverages to enhance the brown tint and colouring food
Purplish red	Bayam (*Amaranthus*)	Colouring beverage of grape juice, jelly and powder juice
Bluish-purple	Litmus moss	Colouring foods and beverages
Blue	Clerodendron	Colouring food

Table 2 Plant pigments providing natural food colours [100, 101]

		Presence in		
Colour	Pigment	Cell component	Source	Potential benefits
Green	Chlorophyll (a, b, c and d)	Chloroplast	Herbs and leafy vegetables	Neutralize free radicals
Red, orange, yellow to brown	Carotenoids	Chloroplast and chromoplast	Carrots, fruits and vegetables	Neutralize free radicals
Red	Lutein and lycopene	Chloroplast and chromoplast	Green vegetables, corn and tomato products	Reduce the risk of macular degeneration and prostate cancer
Yellow, red, blue and orange	Phenolics: anthocyanidins, catechins, flavonoids, flavones, lignans and tannins (proanthocyanidines)	Cytosol and vacuole	Fruits, vegetables, tea, citrus, cranberries, cranberry products, pomegranates, cocoa, chocolate, flax and rye	Neutralize free radicals; reduce risk of cancer; prevention of cancer and renal failure; improve urinary tract health; reduce risk of cardiovascular disease
Yellow, orange, red and violet	Betalains	Cytosol and vacuole	Flower, fruits and other parts (as in beet root)	

- **Indigoid dyes** (FD&C Blue No. 2) are unstable in aqueous solution and are readily oxidized. The dye is safe for use [22].
- **Quinoline dyes** (FD&C Yellow No. 10) are provisionally listed in orally ingested drugs and cosmetics and for use in foods and beverages in the EU. Little is known about its metabolic fate; it is not mutagenic [23].
- **Polymeric dyes** were synthesized in an attempt to minimize intestinal absorption, thus decreasing their potential toxicity, making them more chemically and biologically stable, while retaining the properties of the monomers. They were selected on the basis of colour, solubility, thermal stability, light stability and compatibility with other food ingredients. Poly R-481 and Poly Y-607 are found to be non-mutagenic [24].
- **Lakes:** FD&C aluminium lakes are insoluble pigments made from certified FD&C dyes by adsorbing them on an aluminium hydroxide substrate with a basic aluminium or calcium radical [25].
- **Nature-identical Colours:** These colours are also manufactured by chemical synthesis, but do not require FDA certification and are considered chemically and functionally indistinguishable from the same colorant found in Nature.
- **Natural Colours:** natural food colour is any dye, pigment or any other substance extracted using conventional methods, from vegetable, animal, mineral or other sources capable of colouring food drug, cosmetics or any part of the human body; colours come from a variety of sources such as seeds, fruits, vegetables, algae and insect. FDA lists the colours exempt from Certification in 21 CFR, Part 73 for food, drug and cosmetics; details on usage limitations are also available.

As these are from agricultural/biological materials, they do not require certification. The word ‘natural’ as it pertains to colours has never been defined and therefore has no universally accepted definition. During preparation of the US Color Additives Amendment of 1960, law makers were faced with a dilemma caused by the commercial introduction of synthetically produced but chemically identical β-carotene: Should the colour require certification (since it was synthesized from acetone) or was it natural (since analytically it was indistinguishable from the naturally extracted colorant)? In an attempt to find a solution, law makers utilized a new term, ‘color additives exempt from certification’, to encompass both ‘natural’ colours and ‘nature-identical’ colours. Because of this turn of events, the term ‘natural colours’ does not legally exist and is not recognized by the FDA. It was left up to each manufacturer to define ‘natural’ for itself.

Depending upon the application, a suitable natural colour can be achieved by keeping in mind factors such as pH, heat, light storage and the other ingredients of the formula or recipe. The storage conditions for natural colours depend on the particular need of the product. A tightly sealed container is best for cold storage, to preserve colour strength and quality, taking into account its degree of cooling point.

Chemistry of Natural Food Colours

Anthraquinones

Carmine

Carmine, also called Crimson Lake, Cochineal, Natural Red 4, C.I. 75470 or E120, is a pigment of a bright red colour obtained by aqueous extraction of cochineal, which is derived from the dried bodies of the gravid female insect *Coccus cacti*, and is used as a general term for a particularly deep red colour [26]. It is not of plant origin. Carmine is an aluminium or calcium–aluminium lake on an aluminium hydroxide substrate of carminic acid (Figure 1). Carminic acid is a hydroxyanthraquinone linked to a glucose unit and comprises approximately 10% of cochineal and 2–4% of its extract [27].

The quality of carmine is affected by the temperature and the degree of illumination during its preparation, sunlight being a requisite for the production of a brilliant hue. It differs also according to the amount of alumina present in it. Carmine lake is a pigment obtained by adding freshly precipitated alumina to decoction of cochineal.

Carmine is used as a food dye in many different products such as juice, ice cream, yogurt and candies, eye shadow and lipstick. Carmine is used in the manufacture of artificial flowers, paints, rouge, cosmetics, food additives and crimson ink, as a staining agent in microbiology, as a Best's carmine to stain glycogen, mucicarmine to stain acidic mucopolysaccharides and carmalum to stain cell nuclei.

Although principally a red dye, it is found in many foods that are shades of red, pink and purple. Since typically it is used in low quantities, the likelihood of sensitization to carmine ingestion is low and is more likely to occur through high levels of exposure through occupational or cosmetic exposure. But it has been known to cause severe allergic reactions and anaphylactic shock in some people [28], including irritable bowel syndrome, anaphylactic reaction including widespread urticaria, rhinitis, nausea, vomiting, bronchospasm, chills, diarrhoea, itching skin and burning eyes (from cosmetics) [29]. Food products containing a carmine-based food dye may prove to be a concern for people who are allergic to carmine.

Although concerns over hazards from allergic reactions have been asserted, the US FDA has not banned the use of carmine and states that it found no evidence of a 'significant hazard' to the general population. In the European Union (EU), the use of carmine in foodstuffs is regulated under the European Commission's directives and listed as additive E 120. This directive approves the use of carmine for certain groups of foodstuffs only and specifies a maximum amount that is permitted or restricts it to the *quantum satis* [26].

Anthocyanins

Anthocyanins are natural colorants belonging to the flavonoid family of secondary metabolites; they are a diverse group of intensely coloured pigments synthesized in plants and bacteria responsible for the often brilliant orange, red, purple and blue colours in fruits, vegetables, flowers, leaves, roots and other plant storage organs. They are found predominantly in outer cell layers such as the epidermis and peripheral mesophyll cells. They are water-soluble, facilitating their easy use in aqueous media as food colorants [30], and have been used without any adverse effects for centuries. In plants, anthocyanins are present together with other natural pigments such as the closely chemically related flavonoids, carotenoids, anthoxanthins and betacyanins. Not all land plants contain anthocyanin and in the Caryophyllales, Cactus and *Galium mollugo*, they are replaced by betacyanins.

Anthocyanins are allowed as a food additive (designated E 163). Anthocyanins from tart cherries (*Prunus cerasus* L.) have been stabilized with phosphoric acid, maltodextrin and α- and β-cyclodextrins by Chandra *et al.* [31] and it was found that these pigments, when stored as powders with dextrins, were stable at room temperature even after 12 weeks; also powdered samples were less prone to degradation compared with the one in solution.

Chemistry and stability: The anthocyanins are a diverse group of glycosidic derivatives of the 2-phenylbenzophyryllum (flavylium) structure, intensely coloured ampholytes. At pH 1.0 and below, anthocyanins exist as red flavylium salts; the colour gradually fades as the pH rises. At pH 4–5, anthocyanins exist as colourless pseudobases, and as the pH goes above 5, they exist successively as purple quinoidal anhydro bases (pH <7), deep blue ionized anhydro bases (pH <8), and finally as yellow/brown chalcones (pH >12). High temperature and molecular oxygen lead to browning, precipitation and colour loss; also presence of certain sugars, particularly fructose or sulphur dioxide, can lead to loss of anthocyanin [32]. This chemical instability, coupled with high cost and low tinctorial strength, has limited the commercial application of anthocyanin pigments. The general structure of anthocyanins is given in Figure 2. The substitution patterns of some common compounds are given in Table 4.

The difference in chemical structure that occurs in response to changes in pH is the reason why anthocyanins are often used as a pH indicator, as they change from red in acids to blue in bases. The colour of non- and mono-acylated anthocyanins, under acidic conditions, is determined largely by substitution on the B-ring of the aglycon [33]. Increased hydroxyl substitution on the B-ring results in a shift of the λ_{max} to longer wavelengths (bathochromic shift) to yield a bluer hue. The sugar residues commonly acylated with aromatic acids include *p*-coumaric, caffeic,

Table 3 Important natural food colour/pigments-yielding plants [6, 16, 17]

Botanical name	Family	English name	Parts used	Colour obtained	Responsible pigment	Origin and distribution	Remarks
Curcuma longa L.	Zingiberaceae	Turmeric	Rhizome	Yellow	Curcumin	Originated in the Indo-Malayan region but widely distributed in the tropics of Asia to Africa and Australia. Grown generally as an annual crop, cultivable from sea level up to 1200 m.	Percentage of curcumin varies from 4.0 to 9.0.
Capsicum annuum L.	Solanaceae	Paprika, red pepper and sweet pepper	Fruit	Red, dark red, purple red, reddish orange and yellow	Cryptoxanthin lutein, zeaxanthin, capsanthin, capsorbin and violaxanthin	Native to New World tropics, now widely cultivated in the temperate zones as well as the tropics. Shrubby perennial, usually grown as herbaceous annuals.	
Crocus sativus L.	Iridaceae	Saffron	Flower	Yellow and orange	Crocin and crocetin	Autumn-flowering perennial plant of the eastern Mediterranean. A corm survives for only one season; upon flowering, it averages less than 30 cm in height, having crimson stigma. The plant can tolerate cold winters, surviving frosts as cold as $-10°C$ and short periods of snow cover.	
Bixa orellena L.	Bixaceae	Annatto	Seeds	Yellow/orange and red	Bixin, norbixin and carotenoids	Indigenous to tropical America and the West Indies, now cultivated in the rest of the tropics, particularly Mexico, Brazil, Guiana and the Antilles; naturalized in the hotter parts of India; small evergreen tree (about 5 m); thrives at elevations of 600–900 m.	Dye content varies from 5 to 6% by weight of seeds. However, bixin occupies 70–80% in each seed.
Daucus carota L.	Apiaceae	Carrot	Root	Orange	β-Carotene	Native to Europe and southwestern Asia; grown all over the world; biennial plant; cool season crop; stout taproot stores large amounts of sugars for the plant to flower in the second year; prefers moist, loose, well-drained light loamy soils.	Contains about 16,700 IUs of β-carotene.
Beta vulgaris L., Beta *vulgaris* var. *conditiva* L.	Chenopodiaceae	Beet root, red beet	Root	Red, red–violet and yellow	Betanins (betanidin, betacyanin and betaxanthin)	Native to the Mediterranean but spread eastwards into West Asia. An erect herb with thick, fleshy root.	

Vitis vinifera L. and *Vitis romanetii* Romanet. ex Foex	Vitaceae	Grape (European) and black grapes	Fruit	Red, purple, yellow and blue	Anthocyanins (cyanidin, pelargonidin, peonidin, delphinidin, petunidin and malvidin)	Believed to have originated near the shores of Caspian sea and spread to other parts of the world (*V. romanetii* from E. Asia – China); grown under semi-arid and subtropical climatic conditions; perennial vine; woody climbing by coiled tendrils; ellipsoidal fruits with a solid flesh and relatively thin skin (epicarp).
Fragaria virginiana, *Fragaria chiloensis* (L.) Duchesne, *Fragaria daltoniana* J. Gay	Rosaceae	Strawberries	Fruit	Red	Anthocyanin	Native to Europe and the Americas; grown extensively in most temperate and in some subtropical countries (*F. daltoniana* native to Asia – temperate (China) and tropics (Indian subcontinent and Indo-China)); perennial herbs with short, woody stems or stocks with rosette leaves.
Rubus idaecus L.	Rosaceae	Raspberries	Fruit	Red and purple	Anthocyanin	Native to Europe, widely grown in all temperate regions of the world; traditionally, a mid-summer crop, but now obtained year-round; requires ample sun and water for optimal development.
Vaccinium corymbosum L.	Ericaceae	Blueberries	Fruit	Blue	Anthocyanin	Native to North America, eastern Asia and Northern Europe and spread to Germany, Poland, Italy and other countries of Europe; grow in semi-shade (light woodland) or no shade; shrubs varying in size (10 cm to 4 m tall); initially, fruits are pale greenish, but turn to reddish-purple, and finally dark purple on ripening; the crop requires about 50 000 beehives for pollination.

Table 3 (Continued)

Botanical name	Family	English name	Parts used	Colour obtained	Responsible pigment	Origin and distribution	Remarks
Aronia arbutifolia (L.) Pers.	Rosaceae	Chokeberry or red fruit (Aronia)	Fruit	Bright red, red and orange	Anthocyanin and carotenes	Native to eastern North America and most commonly found in wet woods and swamps; deciduous shrubs grow usually up to 2–4 m tall, red fruits persisting into winter.	Anthocyanins combat oxidative stress and urinary tract infections, stimulate circulation, lower cholesterol levels and are beneficial to cardiac health.
Ribes nigrum L.	Grossulariaceae	Black currant	Fruit	Purple–black	Anthocyanin	Native to Eurasia; distributed mainly in temperate regions of Europe, Asia and North and South America; small shrub growing to 1–2 m tall; fruit very dark purple in colour edible berry, with a glossy skin and a persistent calyx at the apex.	
Sambucus spp.	Adoxaceae	Elderberry	Fruit	Dark red	Cyanidin	Native to temperate to subtropical regions of both the Northern Hemisphere and the Southern Hemisphere; more widespread in the Northern Hemisphere, with Southern Hemisphere occurrence restricted to parts of Australasia and South America; shrubs or small trees.	
Solanum lycopersicum L. (syn. *Lycopersicon esculateum* L.)	Solanaceae	Tomato	Fruit	Red	Lycopene	Originated from Peru–Ecuador–Bolivia area of South America but domesticated from central Mexico and spread to other warm temperate and tropical countries of the world. Short-lived perennial, but cultivated as annual.	
Allium cepa L.	Alliaceae	Onion	Bulb	Brown	Quercetin	Probably, originated from central Asia and Near East; spread to other parts of the world. Bulbous perennial, grown as annual.	

Medicago sativa L.	Fabaceae	Alfalfa grass and Lucerne	Leaf	Green	Chlorophyll/ chlorophyllin	Native to Iran; spread to Central Asia, Greece, Chile to the United States; widely grown throughout the world as forage for cattle, and most often harvested as hay; wide range of adaptation from the very cold Northern Plains to high mountain valleys, and from rich temperate agricultural regions to Mediterranean climates and searing hot deserts; perennial plant cultivated as an important forage crop.
Brassica oleracea L. var. *capitata* L.	Brassicaceae	Green/red cabbage	Leaf	Green, reddish-purple/ purplish red	Chlorophyll and anthocyanin	Native of the Mediterranean region as well as southern England, Wales and northern France. Widely grown in temperate regions and of great importance in Europe; well adopted to cool, moist climate of the temperate zones; biennial; grown as annual.
Urtica dioica L.	Urticaceae	Nettle and stinging nettle	Leaf	Natural green, yellow and orange	Chlorophyll/ chlorophyllin, xanthophyll and carotene	Native to North America; widespread throughout the eastern USA and in most counties in Ohio; grows in moist, shady spots, in flood plains, woodlands and along streams and river banks; spread in temperate and tropical wasteland areas around the world; naturalized in Brazil and other parts of South America; perennial robust herb (2–4 m) with pointed leaves.
Apium graveolens L.	Apiaceae	Celery	Leaf	Green	Chlorophyll/ chlorophyllin	Native of temperate Europe, from England to Asia Minor; widely grown in temperate countries; strong-smelling glabrous biennial herb; requires a cool climate with moist sandy loam soils.

Table 3 (Continued)

Botanical name	Family	English name	Parts used	Colour obtained	Responsible pigment	Origin and distribution	Remarks
Petroselinum crispum (Mill.) Nym. Ex Hill	Apiaceae	Parsley	Leaf	Green	Chlorophyll/ chlorophyllin	Native to the Mediterranean area; a bright green, biennial herb but grown as annual; very common in Middle Eastern, European and American countries; flat leaves valued for their strong flavour; requires an ordinary, good well-worked soil, but a moist one and a partially shaded position.	
Spinacia oleracea L.	Chenopodiaceae	Spinach	Leaf	Green	Chlorophyll/ chlorophyllin	Originally from Persia, dispersed to various parts of Asia, Spain, spread to the rest of Europe; herbaceous biennial; grown as annual of variable habitat with arrow-shaped or more rounded thick leaves fused into a rosette.	
Hibiscus sabdariffa L.	Malvaceae	Roselle, Jamaican sorrel and Hibiscus	Calyx	Bluish red and purple (pink)	Anthocyanins	Native to the Old World tropics, probably in the East Indies; now cultivated throughout the tropics; suitable for tropical climates from sea level to about 600 m altitude; annual or perennial herb or woody-based sub-shrub (up to 3 m tall); fruit consists of the large reddish calyces surrounding the small seed pods.	Calyces contain 6.7% proteins by fresh weight and 7.9% by dry weight.
Tagetes erecta L.	Asteraceae	Marigold	Petal	Yellow/ orange	Xanthophyll (lutein)	Native to Mexico; adopted to tropical, subtropical and temperate regions; herbaceous annual; grown in gardens for its bright attractive flowers.	
Gardenia augusta (L.) Merr. (syn. *Gardenia jasminoides*)	Rubiaceae	Gardenia and cape jasmine	Fruit	Yellow and dark reddish violet	Carotenoids and xanthophyll?	Native to southern China, Taiwan, Japan and nearby regions of the subtropical; mostly evergreen shrubs or small trees; grown as an ornamental plant in cold and warm climates (sunny or partly shaded position); glossy evergreen foliage and fragrant flowers.	

Carthamus tinctorius L.	Asteraceae	Safflower	Flower	Scarlet red and yellow	Carthamin and 'safflower yellow'	Native to southern Asia (probably India), cultivated in India (Madhya Pradesh, Andhra Pradesh, Karnataka and Maharastra), the Middle East and East Africa; now primarily cultivated as oilseed crop; erect, herbaceous annual bearing yellow to orange–red flowers.	Carthamin constitutes 0.3–0.6% and 'safflower yellow', about 30–35%.
Indigofera tinctoria L.	Fabaceae	Indian indigo and common indigo	Leaf	Blue and blue–black	Indigotine (in the form of glycoside, indican)	Native to India; cultivated and distributed commonly in the tropical region (India, China and other eastern countries); a common, small shrub with pinnate compound leaf; usually grown as cover and green manure crop.	Indigotine content varies from 70–90%
Perilla fruitescens (L.) Britton	Lamiaceae	Red perilla	Leaves	Red	Sisonin and peranin	Native to South East and East Asia, west to the Himalayas; cultivated in India, China and Japan; a herbaceous plant; naturalized in Eastern USA.	

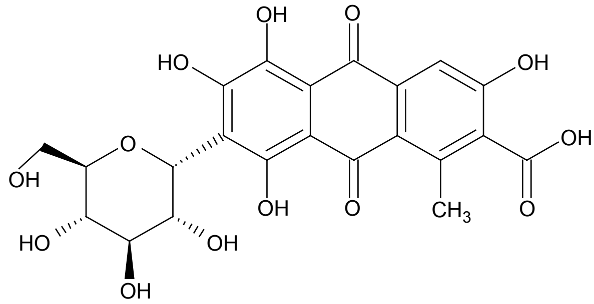


Figure 1 Carminic acid

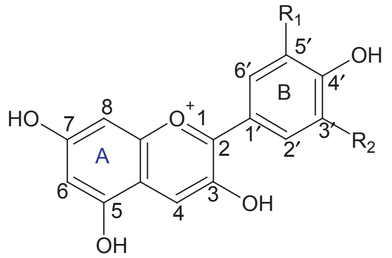


Figure 2 General structure of anthocyanins

ferulic, sinapic, gallic or *p*-hydroxybenzoic acid and/or aliphatic acids such as malonic, acetic, malic, succinic or oxalic acid.

The pigments present in the kernel of four native maize varieties related to the races Arrocillo, Conico, Peruano and Purepecha were characterized to determine their possible use as natural dyes. The maize samples contained the anthocyanins cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-(6″malonylglucoside) and cyanidin-3-(3″,6″-dimalonylglucoside), in both the pericarp and aleurone layer [34].

Anthocyanins from black, brown (containing tannins) and red sorghums were characterized by Awika *et al.* [35] for antioxidant activity and pH stability. Sorghum brans had 3–4 times higher anthocyanin contents than the whole grains. Black sorghum had the highest anthocyanin content. Only 3-deoxyanthocyanidins were detected in sorghum, which are more stable to pH-induced colour change than the common anthocyanidins and their glycosides. Also, crude sorghum anthocyanin extracts were more stable than the pure 3-deoxyanthocyanidins. The antioxidant properties of the 3-deoxyanthocyanidins were similar to those of the anthocyanins. Pigmented sorghum bran has high levels of unique 3-deoxyanthocyanidins, which are stable to change in pH and have a good potential as natural food pigments.

The major anthocyanins in the berries of *Laurus nobilis* L., a perennial tree or shrub typical of the Mediterranean region, were characterized as cyanidin 3-*O*-glucoside (41%) and cyanidin 3-*O*-rutinoside (53%); the two minor

Table 4 Substitution patterns of some common anthocyanins [33]

Aglycon	R1	R2	λ_{max} (nm) visible/colour
Pelargonidin	H	H	494 nm/orange
Cyanidin	OH	H	506 nm/orange–red
Peonidin	OMe	H	506 nm/orange–red
Delphinidin	OH	OH	508 nm/red
Petunidin	OMe	OH	508 nm/red
Malvidin	OMe	OMe	510 nm/bluish–red

anthocyanins were identified as 3-*O*-glucoside and 3-*O*-rutinoside derivatives of peonidin (5%) [36]. Longo and Vasapollo [37] have identified the pigments from the skin of the red berries of *Ruscus aculeatus* L., a wild shrub typical of Mediterranean Europe and Africa, and identified the major anthocyanins as pelargonidin 3-*O*-rutinoside (64%), pelargonidin 3-*O*-glucoside (16%) and pelargonidin 3-*O*-*trans*-*p*-coumarylglucoside (13%). The attractive colour of *R. aculeatus* berries and the great abundance of the plant in the south of Italy make these berries a new and promising source of natural colorants.

Mateus *et al*. [38] have recently found vinylpyranoanthocyanins to naturally occur in ageing red wine. This new family of anthocyanin-derived pigments may be obtained directly through the reaction between anthocyanin derivatives and other compounds. These newly formed pigments exhibit a bluish colour at acidic pH through reaction between anthocyanin–pyruvic-acid adducts and flavonols in the presence of acetaldehyde. The chromatic features of this kind of pigment are good news for the use of these naturally occurring blue pigments in the food industry.

Anthocyanin molecules with complex patterns of glycosylation and acylation that are remarkably stable to pH and temperature changes, and light exposure have been discovered [39]. The improved stabilization has been attributed to intra- and inter-molecular co-pigmentation, self-association, metal complexing and presence of inorganic salts [40]. A review by Giusti and Wrolstad [3] on the applications of acylated anthocyanins in food systems reveals that of the edible sources, radishes and red potatoes are especially potential alternatives to FD&C Red No. 40 (allura red); radish extract imparted a bright, attractive and stable red colour to Maraschino cherries; radish and purple sweet potato extracts imparted colour to juices similar to allura red; other potential natural sources of acylated anthocyanins are red cabbage and black carrots. The greater stability of these pigments could be taken advantage of in a variety of food applications including dairy products.

From the studies of Bordignon-Luiz *et al*. [41] on the colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.), it has been found that the half-life time of the pigment can be increased significantly by the addition of tannic acid and by the control of factors such as pH,

Table 5 Amount of anthocyanins in foodstuff

Foodstuff	Anthocyanin[1]	Reference
Blackcurrant	250	[33]
Redcurrant	12–19	[33]
Blackberry	83–326	[33]
Blueberry	25–495	[33]
Raspberry – black	214–428	[33]
Raspberry – red	20–60	[33]
Sweet cherry	350–450	[33]
Cranberry	78	[33]
Cranberry juice	18–87	[33]
Strawberry	7–30	[43]
Strawberry juice	21–333	[44]
Apple (Scugog)	10	[33]
Red cabbage	25	[45]
Red onion	9–21	[33]
Red grape	30–750	[33]
Red wine	100–1000	[45]

[1]All values expressed on fresh weight basis (mg/100 g); for juice and wine (mg/l).

temperature, presence of light and presence of nitrogen flow. The study also revealed that the pigment could be commercially used as a colorant in yoghurt, given its excellent stability in yoghurt.

Occurrence: The amounts are relatively large: 1 kg of blackberry, for example, contains approximately 1.15 g, and red and black legumes can contain 20 g/kg. Anthocyanins are less abundant in banana, asparagus, pea, fennel, pear and potato. Roughly 2% of all hydrocarbons fixated in photosynthesis are converted into flavonoids and their derivatives such as the anthocyanins, no less than 10^9 tonnes/year. The daily intake of anthocyanins in humans has been estimated to be as much as 180–215 mg/day in the USA [42]. The amount of anthocyanins in foodstuff is given in Table 5.

There are a number of highly pigmented fruits that are rich in anthocyanins and are potential commercial sources of food colours, the limitation being pigment stability, raw material availability and economic feasibility. Potential plant sources include extracts of red grapes and their by-products, cranberry press cake, blueberries, blackchokeberries, elderberries, *Hibiscus calyces*, blackcurrants and purple maize. The anthocyanins in these sources are mainly in mono- and di-glucosides with limited stability against hydration and pH changes [3].

The anthocyanin-rich waste (ARW) in purple corncobs has been characterized by Jing and Giusti [46], to find a suitable application in a food matrix. ARW provided milk with an attractive purple hue. ARW shows potential as a natural colorant for a pH range unusual for anthocyanin applications. A protective effect of matrix constituents on the stability of anthocyanins was evident. Anthocyanins may interact with different compounds in biological systems when the pH values are close to neutral.

Clinical importance: Anthocyanins are also popular for their possible health benefits such as enhancement of sight acuteness [44], antioxidant capacity [47–51], treatment of various blood circulation disorders resulting from capillary fragility [47], vaso-protective and anti-inflammatory properties [52], inhibition of platelet aggregation [53], maintenance of normal vascular permeation [47], control of diabetes, antineoplastic and chemoprotective action [54], radiation-protective action [55] and other effects associated with their action on various enzymes and metabolic processes [47]. Berry anthocyanins were shown to turn off genes involved in proliferation, apoptosis, inflammation and angiogenesis. In 2007, black raspberry studies entered the next pivotal level of research (the human clinical trial) to examine the anticancer effects of black raspberries and cranberries on tumours in the oesophagus, prostate and colon [56].

Pronounced inhibition, by the natural anthocyanins from pigments of purple corn [57], purple sweet potato and red cabbage [58], of colorectal carcinogenesis has been reported. The incorporation of these vegetables in the diet has been advised.

The antiradical capacity (radical scavenger capacity (RSC)) of anthocyanin-based fruit extracts prepared in the laboratory (black chokeberry, blackthorn and strawberry) was compared with that of natural α-tocopherol, or synthetic (butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)) antioxidants, as well as for a commercial elderberry concentrate and a synthetic colorant (Ponceau 4R). The RSC values in descending order were 1.87, 0.7, 0.42, 0.2, 0.05, 0.03 and 0.008 for α-tocopherol, black chokeberry, BHA, black-thorn, BHT, strawberry and elderberry, respectively. Ponceau 4R lacked RSC. Therefore, these natural colorants prove to be a combined source of colour and RSC for food material.

Anthocyanins have been shown to act as a 'sunscreen', protecting cells from photo-damage by absorbing blue–green light, thereby protecting the tissues from photoinhibition or high light stress. This has been shown to occur in red juvenile leaves, autumn leaves and broad-leaved evergreen leaves that turn red during the winter. It is also thought that red coloration of leaves may camouflage leaves from herbivores blind to red wavelengths, or signal unpalatability to herbivores, since anthocyanin synthesis often coincides with synthesis of unpalatable phenolic compounds. They also play an important role in attracting animals for pollination and seed dispersal; they also have an important role in plant resistance mechanisms in response to insect attack [59].

In addition to their role as light-attenuators, anthocyanins also act as powerful antioxidants, helping to protect the plant from radicals formed by ultraviolet (UV) light and during metabolic processes. This antioxidant property is conserved even after consumption by another organism, which is another reason why fruits and vegetables with red skins and tissues are a nutritious food source. Wang *et al*. [47] have observed that different patterns of hydroxylation and glycosylation in anthocyanins modulate their antioxidant properties: cyanidin-3-glucoside exhibited the highest oxygen radical absorbance capacity among 14 anthocyanins including the aglycons and the sugar derivatives.

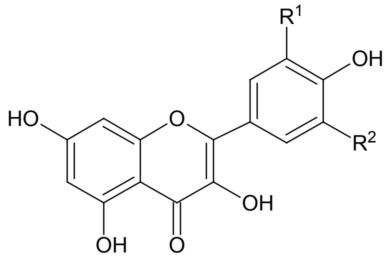
In vitro enzymatic and non-enzymatic polyunsaturated fatty acid peroxidation was significantly inhibited in a dose-dependent manner by purified anthocyanin, a deep-red colour pigment from carrot cell culture. The kinetics showed that anthocyanin is a non-competitive inhibitor of lipid peroxidation. Anthocyanin has been found to be a potent antioxidant compared with classical antioxidants such as BHA, BHT and α-tocopherol. This natural agent, in addition to imparting colour to the food, might prevent auto-oxidation of lipids as well as lipid peroxidation in biological systems [60].

Common sources of anthocyanins

- **Grape anthocyanins:** Grape colour extract or grape skin extract (enocianina) are purplish-red anthocyanin-containing colour additives approved for use in the USA. Grape colour extract is an aqueous extract of grape anthocyanin pigments made from Concord grape or a dehydrated water-soluble powder prepared from the aqueous solution. Water-soluble pigments such as 3-mono- and 3,5-di-glucosides of malvidin, delphinidin and cyanidin, and their acylated derivatives are responsible for the purple colour of grape colour extract. The properties of grape skin extract are similar to those of grape colour extract [27]. Although numerous allergic reactions have been reported following ingestion of grapes or grape products, none have thus far been reported for either grape skin extract or grape colour extract [26].

  Over the past few years, it has been accepted that a moderate red wine consumption is a factor beneficial to human health. Indeed, people of France and Italy, the two major wine-producing European countries, eat a lot of fatty foods but suffer less from fatal heart strokes than people in North America or in the northern regions of Europe, where wine is not consumed on a regular basis. For a time, ethanol was thought to be the chemical factor responsible for the 'French paradox'. It is now generally accepted that polyphenols, which are only found in plants and are abundant in grapes and which are active radical scavengers and antioxidants (which can protect LDL cholesterol from oxidation, a process thought to be at the origin of many fatal heart attacks), are the principal bioactive compounds. Also, structural changes occur during wine conservation, and one of the most studied of those changes concerns red wine colour evolution, called 'wine ageing'. As a wine ages, it has been demonstrated that the initially present grape pigments slowly turn into new more stable red pigments. Since grape and wine polyphenols are chemically distinct, their antioxidant activities cannot be the same. So, eating grapes might well lead to beneficial effects on human health, owing to the variety and sometimes high levels of their polyphenolic content [61].

- **Red cabbage:** Red cabbage dye is an anthocyanin pigment used mainly as a food colour, both the colour and intensity of which are affected by the pH. The pK_a of the red cabbage solution at 612 nm was within the range of 6.8–7.2. Thus red cabbage dye could be used as a pH indicator in pharmaceutical formulations. In acidic conditions, it has its original red colour but at a basic pH its colour changes to deep blue. This colour is more stable at a low temperature and pH [62].
- ***Opuntia:*** The pigments extracted from *Opuntia stricta, Opuntia undulata* and *Opuntia ficus-indica* fruits were identified as betalains. In *O. undulata* and *Opuntia ficus-indica* fruits, both betacyanins and betaxanthins were identified, while in *O. stricta* fruits, only betacyanins (betanin and isobetanin) were detected. *O. stricta* fruits showed the highest betacyanin content. The thermal stability of the pigment extracts was dependent on the pH, with the maximum stability being at pH 5, as expected for betacyanins. At this pH value and a storage temperature of 4°C, a deactivation half-life of more than 1 year, with no added stabilizers, was determined. Therefore cactus pears from *O. stricta* may well be considered as a potential source of natural red colorants [63].
- **Rose:** The flavonol glycosides extracted from petals of *Rosa damascena* Mill., after industrial distillation for essential-oil recovery, were found to be a mixture of kaempferol, quercetin glycosides, quercetin 3-*O*-galactoside and quercetin 3-*O*-xyloside, several acylated quercetin and kaempferol glycosides, some of them being disaccharides (Figure 3). The high flavonol content of approximately 16 g/kg on a dry weight basis revealed that distilled rose petals are a promising source of phenolic compounds that might be used as functional food ingredients, as natural antioxidants or as colour enhancers [64].
- **Onions:** Unusual gold-coloured onions in a cross between the US-type yellow and Brazilian yellow onions contained a significantly reduced amount of quercetin, the most abundant flavonoid in onions, indicating that an early step in the flavonoid biosynthesis pathway might be abnormal in these onions. A premature stop codon and a subsequent single base-pair addition causing a frameshift were identified in the coding region of flavonoid synthesis genes encoding enzymes involved in early steps of the pathway, chalcone isomerase [65].



Compound	R1	R2
Kaempferol	H	H
Quercetin	OH	H
Myricetin	OH	OH
Isorhamnetin	OCH_3	H

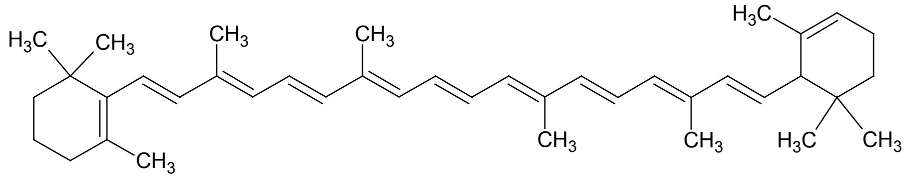
Figure 3 Chemical structures of some flavonols [99]

Carotenoids

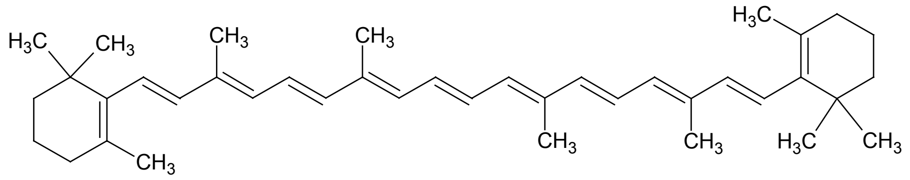
Carotenoids are a group of phytochemicals responsible for yellow, orange, red and violet colours of foods and having an important role in the prevention of human diseases and maintaining good health. In addition to being potent antioxidants some carotenoids also contribute to dietary vitamin A. Although the chemistry of carotenoids has been studied extensively, their bioavailability, metabolism and biological functions are only now beginning to be investigated [66].

All carotenoids are built from isoprene units; the most common ones possess eight such units and therefore 40 carbon atoms (Figure 4). These isoprene units which are located in the central part of the molecule are dehydrogenated and form part of the chromophore, whereas those near or at the ends of the molecule are hydrogenated. With the exception of bixin, astaxanthin and crocin, the carotenoids are insoluble in water and soluble in fats or fat solvents [67].

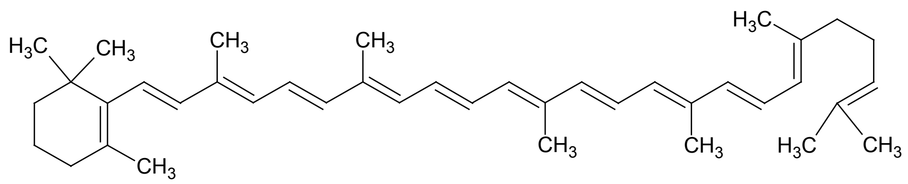
β-Carotene, β-apo-8′-carotenal and canthaxanthin: These are the most commercially important of carotenoids. β-Carotene is the isomer of the naturally occurring carotenoid, carotene. Carotene is the pigment largely responsible for the colour of butter, cheese, carrots, alfalfa and cereal grains. The colorant is synthetically extracted with acetone, which results in the all-*trans* form. Both the synthetic and natural sources of β-carotene are permitted colour additives [27]. Natural sources of carotenoids include annatto from *Bixa orellana*; saffron, which contains crocetin, β-carotene and zeaxanthin; paprika extract (*Capsicum annuum*) containing capsanthin and capsorubin; xanthophyll extract from leaves; carrot extracts with β-and α-carotene; canthaxanthin from the pink edible mushroom *Cantharellus cinnabarinus*; and red palm oil, which contains lycopene and lutein [10]. (Figure 5).



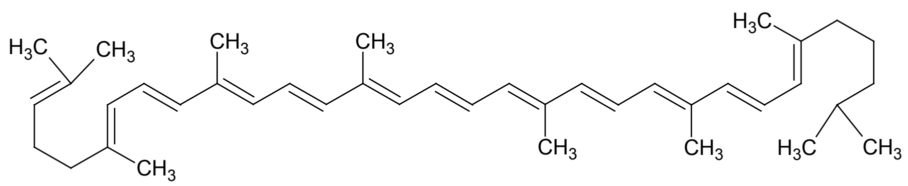
α-Carotene



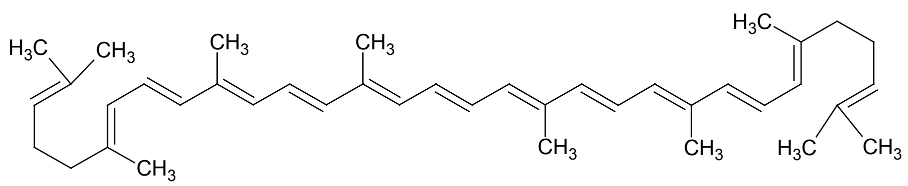
β-Carotene



γ-Carotene



Lycopene



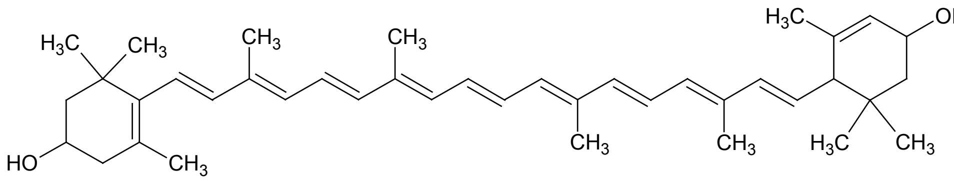
Phytoene

Figure 4 Structure of a few carotenoids

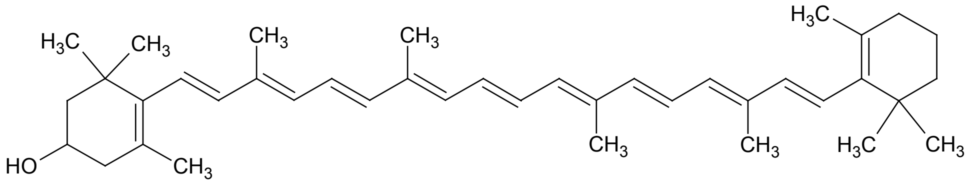
Canthaxanthin was first isolated in 1950 from an edible mushroom (*C. cinnabarinus*), and has since been isolated from sea trout, algae, daphnia, salmon, brine shrimp and several species of flamingo [27]. Reports of allergies to the carotenoid colour family are rare.

Clinical importance: Carotenoids are known photo-protectants. Excessive exposure to solar radiation, especially UV A (UVA; 320–400 nm) and UV B (UVB; 290–320 nm) radiation, may induce UV-carcinogenesis and erythema in the skin. Although the protective effects of carotenoids against skin lesions are still unclear, β-carotene has been proposed as an oral sun protectant. Studies by Lee *et al.* [68] suggest that supplementation with natural carotenoids may partially protect human skin from UVA- and UVB-induced erythema, although the magnitude of the protective effect is modest.

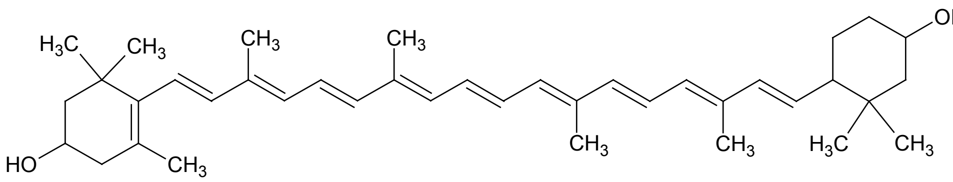
When photo-oxidative stress was imposed under natural solar radiation on exposed and shaded sections of detached fruit of immature green tomato mutants



Lutein (xanthophyll)



Cryptoxanthin



Zeaxanthin

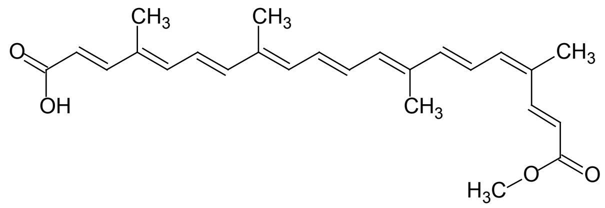
Figure 5 Structure of a few xanthophylls

(anthocyanin absent, β-carotene, delta and high pigment-1) and their nearly isogenic parents reproduced the symptoms observed on attached fruit. Both high temperature and solar irradiance caused fruit surface discoloration with faster degradation of chlorophyll (Chl) than carotenoids (Car), leading to an increase in the Car/Chl ratio [69].

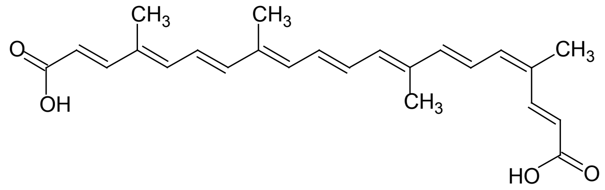
Neta-Sharir *et al.* [70] have reported that the tomato chloroplast small heat-shock protein (sHSP), *HSP21*, is induced by heat treatment in leaves, as also under normal growth conditions in developing fruits during the transition of chloroplasts to chromoplasts. The protein protects PSII from temperature-dependent oxidative stress, has a role in fruit reddening and the conversion of chloroplasts into chromoplasts. Furthermore, when detached mature green fruits were stored for 2 weeks at 2°C and then transferred to room temperature, the natural accumulation of carotenoids was blocked.

Higuera-Ciapara *et al.* [71] have published a review on astaxanthins, a carotenoid widely used in salmonid and crustacean aquaculture to provide the pink colour characteristic of that species. Astaxanthin plays a key role as an intermediary in reproductive processes. Synthetic astaxanthin dominates the world market but recent interest in natural sources of the pigment has increased substantially. Common sources of natural astaxanthin are the green algae *Haematococcus pluvialis*, the red yeast *Phaffia rhodozyma*, as well as crustacean by-products. Astaxanthin possesses an unusual antioxidant activity that has caused a surge in the nutraceutical market for the encapsulated product. Additional health benefits include cardiovascular disease prevention, immune system boosting, bioactivity against *Helicobacter pylori*, and cataract prevention. Research on the health benefits of astaxanthin is very recent and has mostly been performed *in vitro* or at the preclinical level with humans.

Annatto: Annatto is a natural food colorant extracted from the seeds of the *B. orellana* L. plant. Annatto is used in Latin American cuisine to add a deep red colour as well as distinctive flavour notes to fish, meat and rice dishes. In the United States, annatto extracts are primarily used to impart orange/yellow hues to cheese and other dairy foods. The annatto tree, *B. orellana*, is a large, fast-growing shrub of the tropics. The large clusters of brown or crimson capsular fruit-containing seeds coated with a thin, highly coloured resinous coating serves as the raw material for the preparation of the colorant annatto, which is prepared by leaching the annatto seeds with an extractant prepared from one or more food-grade materials including various solvents, edible vegetable oils and fats and alkaline aqueous and alcoholic solutions.



Bixin



Norbixin

Figure 6 Bixin and norbixin from annatto

The chief colouring principle found in the oil-soluble extracts of annatto is the carotenoid bixin; the major colorant in the alkaline aqueous extracts is norbixin [27] (Figure 6). Of the 107 volatile compounds detected in water- and oil-soluble annatto extracts, 56 compounds were tentatively identified and 51 were positively identified. Volatile profile differences exist between water- and oil-soluble extracts, and annatto extracts contain odorants with the potential to influence food aroma [72].

Clinical implications: Allergic reactions to annatto extract are rare; symptoms of anaphylactic shock including generalized pruritis, generalized urticaria, angiooedema of eyes and lips, undetectable blood pressure and loss of consciousness were reported in one case [73] and asthma in another [74].

Hagiwara *et al.* [75] have reported on the modifying potential of annatto extract (norbixin) on liver carcinogenesis in rats initially treated with *N*-nitrosodiethylamine (NDEA). There were no deaths related to annatto extract ingestion, and the treatment had no effects on body weights or food and water consumption. Statistically significant increases of absolute and relative liver weights were apparent; however, annatto extract did not significantly increase the quantitative values for glutathione transferase. The results thus demonstrate that annatto extract at a dietary level of 0.3% lacks modifying potential for liver carcinogenesis.

Hagiwara *et al.* [76] have also reported that when rats were fed with annatto extract (norbixin), no treatment-related adverse effects were found on body weight, food and water consumption, ophthalmology and haematology data (in this study as well). Blood biochemical analysis revealed increased alkaline phosphatase, phospholipid, total protein, albumin and albumin/globulin ratio. Marked elevation in absolute and relative liver weights was also found at higher doses; hepatocyte hypertrophy was evident and this was linked to abundant mitochondria. Thus, the No-Observed-Adverse-Effect-Level (NOAEL) was judged to be a dietary level of 0.1% of annatto extract (norbixin).

Annatto in different concentrations did not present mutagenic or antimutagenic activities on the mice bone marrow cells. However, an increased frequency of micronucleated cells was observed at the highest concentration when administered simultaneously with cyclophosphamide. Annatto colour, it is concluded from this study, is neither mutagenic nor an inhibitor of induced mutations although it should be used carefully since high doses may increase the effect of a mutagen [77].

A study by Mikkelsen *et al.* [78] indicates that orally administered annatto extract provoked chornic urticaria and/or angioneurotic oedema in 56 of 61 patients. Twenty-six percent of the patients reacted to this colour 4 h after intake. Similar challenges with synthetic dyes showed the following results: Tartrazine, 11%; Sunset Yellow FCF, 17%; Food Red 17, 16%; Amaranth, 9%; Ponceau 4 R, 15%; Erythrosine 12%; and Brillant Blue FCF, 14%. The present study indicates that natural food colours may induce hypersensitivity reactions as frequently as synthetic dyes.

Lycopene: Lycopene is the pigment principally responsible for the characteristic deep red colour of ripe tomato fruits and tomato products; it is also found in watermelon, papaya, pink grapefruit and pink guava. Processed tomato products are more available dietary sources of lycopene than fresh tomatoes. Lycopene is a member of the carotenoid family; it is a natural fat-soluble pigment found in certain plants and microorganisms, where it serves as an accessory light-gathering pigment and to protect these organisms against the toxic effects of oxygen and light [79]. The average daily intake of lycopene is approximately 25 mg; 50% of this is in the form of processed tomato products.

Lycopene is a lipophilic compound and is insoluble in water. Lycopene is also known as ψ-carotene. Its molecular formula is $C_{40}H_{56}$ and its molecular weight is 536.88 Da. In contrast to β-carotene, lycopene has no vitamin A activity and thus is a non-provitamin A carotenoid. Lycopene is an acyclic isomer of β-carotene. β-Carotene, which contains β-ionone rings at each end of the molecule, is formed in plants, including tomatoes, via the action of the enzyme lycopene β-cyclase. Lycopene is a 40-carbon-atom, open chain polyisoprenoid with 11 conjugated double bonds. The structural formula of lycopene is presented in Figure 7 [80].

all-*trans* lycopene is the predominant geometric isomer found in plants. *cis*-isomers of lycopene are also found in Nature, including 5-*cis*-, 9-*cis*-, 13-*cis*- and 15-*cis*-isomers. Lycopene found in human plasma is a mixture of approximately 50% *cis* lycopene and 50% all-*trans* lycopene.

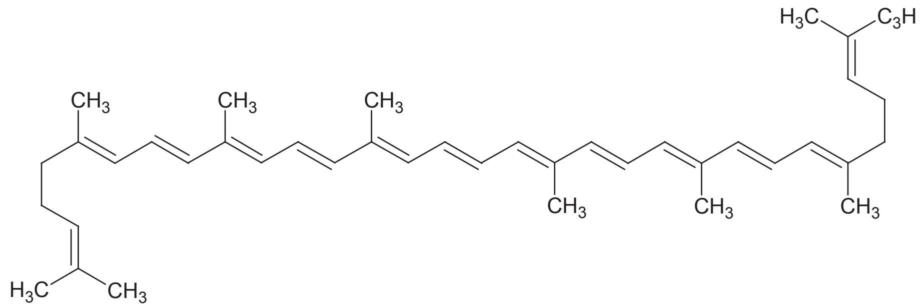


Figure 7 Lycopene

Lycopene in processed foods is mainly in the form of the *cis*-isomer [81].

Lycopene has attracted attention because of its biological and physicochemical properties, especially related to its effects as a natural antioxidant [82]. Although it has no provitamin A activity, lycopene does exhibit a physical quenching rate constant with singlet oxygen almost twice that of β-carotene. This makes its presence in the diet of considerable interest. Increasing clinical evidence supports the role of lycopene as a micronutrient with important health benefits, because it appears to provide protection against a broad range of epithelial cancers. Tomatoes and related tomato products are the major source of lycopene compounds, and are also considered an important source of carotenoids in the human diet [83].

Undesirable degradation of lycopene not only affects the sensory quality of the final products, but also the health benefit of tomato-based foods for the human body. Lycopene in fresh tomato fruits occurs essentially in the all-*trans* configuration. The main causes of tomato lycopene degradation during processing are isomerization and oxidation. Isomerization converts all-*trans* isomers into *cis*-isomers, because of additional energy input, and results in an unstable, energy-rich station. Determination of the degree of lycopene isomerization during processing would provide a measure of the potential health benefits of tomato-based foods. Thermal processing (bleaching, retorting and freezing processes) generally causes some loss of lycopene in tomato-based foods. Heat induces isomerization of the all-*trans* to *cis* forms. The *cis*-isomers increase with temperature and processing time. In general, dehydrated and powdered tomatoes have poor lycopene stability unless carefully processed and promptly placed in a hermetically sealed and inert atmosphere for storage. A significant increase in the *cis*-isomers with a simultaneous decrease in the all-*trans* isomers can be observed in the dehydrated tomato samples using the different dehydration methods. Frozen foods and heat-sterilized foods exhibit excellent lycopene stability throughout their normal temperature storage shelf life. Lycopene bioavailability (absorption) can be influenced by many factors. The bioavailability of *cis*-isomers in food is higher than that of all-*trans*-isomers. Lycopene bioavailability in processed tomato products is higher than in unprocessed fresh tomatoes. The composition and structure of the food also have an impact on the bioavailability of lycopene and may affect the release of lycopene from the tomato tissue matrix. Food processing may improve lycopene bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and tissue matrix, thus making lycopene more accessible and enhancing the *cis*-isomerization. Information on lycopene bioavailability and pharmacokinetic properties of lycopene remains poorly understood. Consumer demand for healthy food products provides an opportunity to develop lycopene-rich food as new functional foods, as well as food-grade and pharmaceutical-grade lycopene as new nutraceutical products. An industrial-scale, environmentally friendly lycopene extraction and purification procedure with minimal loss of bioactivities is highly desirable for the food, feed, cosmetic and pharmaceutical industries. High-quality lycopene products that meet food safety regulations will offer potential benefits to the food industry [84].

Prostate cancer has the third highest incidence of all cancers in men worldwide and is the most common neoplasm diagnosed among men beyond middle age in many developed countries. Mounting evidence surrounding the consumption of tomato products has shown promise for the prevention of prostate cancer. This protective effect has more recently been linked to lycopene, the most abundant carotenoid in tomatoes. Lycopene is a natural pigment that gives the red colour to many foods. In Western countries, 85% of dietary lycopene can be attributed to the consumption of tomato-based products. Recent human clinical trials and animal studies have provided additional support [85].

It has been reported that eggs of female quail fed with diets supplemented with natural carotenoid (alfalfa (or lucerne) concentrate, tomato powder and marigold extract) showed an increase in the yolk concentrations of lutein, zeaxanthin, lycopene and β-carotene [86].

Osterlie [87] found that the quality of tomato sausage depends on the stability of lycopene during process and storage, among other factors. Lycopene, being lipophilic, is extracted together with the polar and neutral fat in food. At the end of the storage, loss of pigment in the sausage without nitrite is 26% when stored at 4°C and 19% at 8°C. Corresponding results for the sausage with nitrite added as well as tomato paste show that the loss of pigment is 20 and 45%.

Haila *et al*. [88] followed the effects of lycopene, lutein, annatto and γ-tocopherol on auto-oxidized triglycerides; oxidation was followed by determination of the peroxide value. Lutein and lycopene were pro-oxidants, while annatto and γ-tocopherol effectively inhibited hydroperoxide formation. γ-Tocopherol also inhibited the pro-oxidant effect of carotenoids and loss of yellow carotenoid colour was retarded. A combination of lutein and γ-tocopherol was more efficient than γ-tocopherol in inhibiting hydroperoxide formation of triglycerides. Therefore it would be judicious to remember the pro-oxidant effects of carotenoids when used as food colorants in lipid-containing foods.

Water-soluble (cacao pigment, cochineal pigment, corn pigment, betanin, carthamus yellow and monascus pigment) and water-insoluble (gardenia yellow, laccaic acid, bixin and curcumin) natural colourings inhibited IgE production by rat spleen lymphocytes, although many of these colourings only inhibited the production of IgG and IgM at high concentrations, the water-insoluble colourings enhanced IgM production even at lower concentrations [89].

Turmeric/curcumin

The yellow colour additive turmeric is the ground powder of the rhizomes of *C. longa* L. Turmeric contains 3–5% volatile oils and 2.5–6% curcuminoids. Turmeric oleoresin contains 15–40% curcuminoids, apart from volatile oils and other extractable plant constituents [27]. Turmeric has been attributed a number of medicinal properties. No convincing evidence of allergies to the spice has been so far reported.

Curcumin (diferuloylmethane) exhibits growth inhibitory effects in a broad range of tumours as well as in TPA-induced skin tumours in mice. Chendil *et al*. [90] studied the radiosensitizing effects of curcumin on p53 mutant prostate cancer cell line PC-3. Treatment of cells with curcumin in combination with radiation showed significant enhancement of radiation-induced clonogenic inhibition and apoptosis. Thus, curcumin is a potent radiosensitizer, and it acts by overcoming the effects of radiation-induced prosurvival gene expression in prostate cancer.

Saffron

Saffron colour comes from the crocus plant, each blossom of which contains one pistil, consisting of three stigma, a style and an ovary. The saffron spice consists of the dried stigmas and style of the crocus bulb, the colouring matter of which is crocin. Crocin is readily hydrolysed to crocetin and D-glucose *in vivo* [91]. Anaphylactic reaction to saffron rice, including asthma, hives, angiooedema and itching has been reported by Wüthrich *et al*. [92], which was confirmed with skin prick and RAST tests.

Table 6 Colour additives exempt from certification in the USA (CFR citation) [94]

Annatto (21 CFR 73.30)
Dehydrated beets (21 CFR 73.40)
Ultramarine Blue (21 CFR 73.50)
Canthaxanthin (21 CFR 73.75)
Caramel (21 CFR 73.85)
β-Apo-8′-carotenal (21 CFR 73.90)
β-Carotene (21 CFR 73.90)
Cochineal extract: carmine (21 CFR 73.100)
Toasted partially defatted cooked cottonseed flour (21 CFR 73.140)
Ferrous gluconate (21 CFR 73.160)
Grape colour extract (21 CFR 73.69)
Grape skin extract (enocianina) (21 CFR 73.170)
Synthetic iron oxide (21 CFR 73.200)
Fruit juice (21 CFR 73.250)
Vegetable juice (21 CFR 73.260)
Dried algae meal (21 CFR 73.275)
Tagetes (Aztec marigold) meal and extract (21 CFR 73.295)
Carrot oil (21 CFR 73.300)
Corn endosperm oil (21 CFR 73.315)
Paprika (21 CFR 73.340)
Paprika oleoresin (21 CFR 73.345)
Riboflavin (21 CFR 73.450)
Saffron (21 CFR 73.500)
Titanium dioxide (21 CFR 73.575)
Turmeric (21 CFR 73.600)
Turmeric oleoresin (21 CFR 73.615)

Natural Colour Additives and Regulations Regarding Their Use

The Codex Alimentarius Commission of the World Health Organization considers eight foods or food groups to be the major causes of food allergy [93]. Natural colour additives are (as mentioned earlier) not included in the foods and food groups identified by Codex. 'Natural' colour additives are generally considered to be colour additives derived from plant or animal sources by extraction or other physical processing. Examples include carmine/cochineal, annatto extract, grape skin extract, turmeric, saffron and β-carotene. Synthetic colour additives include chemically synthesized substances and include tartrazine (FD&C Yellow No. 5), erythrosine (FD&C Red No. 3) and indigo carmine (FD&C Blue No. 2). Although both natural and synthetic colour additives are subject to the same safety standards under the regulatory scheme delineated in the US Food and Drug, and Cosmetic Act, they are regulated in two classes by the US FDA [94]. Generally, synthetic colour additives are subject to a certificate requirement to assure that each batch of material manufactured meets the mandated specifications, while the natural colours (listed in Table 3) is 'exempt from certification' and may be manufactured and marketed without certification from the FDA. In the USA, the terms 'certified' colour additives (synthetic) and colour additives 'exempt from certification' (natural) apply.

Currently, 43 colorants are authorized as food additives by the Council of the European Union, and have been assigned an E number. Sixteen of these are of plant origin. Juices or extracts from some fruits and vegetables are also used for colouring purposes. In the USA, two categories of colorants are permitted: 'certified color additives' and 'colorants exempt from certification'. Considering the regulatory policy, both in the EU and in the USA, and recent court decisions in Germany, this situation is not likely to change in the near future. Table 6 lists the colour additives exempt from certification in the USA.

The current consumer preference for naturally derived colorants is associated with their image of being healthy and of good quality. Natural colorants have become increasingly popular with consumers because synthetic colorants tend to be perceived as undesirable and harmful; some are considered to be responsible for allergenic and intolerance reactions. Despite centuries of interest in natural pigments, our knowledge of their distribution, availability and properties is limited. Worldwide, ~70% of all plants have not been investigated at all, and the chemical composition of only 0.5% has been exhaustively studied. On the other hand, as many as 95% of all plants growing on the North American and European continents are known and have been catalogued. Therefore, novel plant pigments must be searched for in unprospected land or in the sea. Being confronted with the various drawbacks of commercial natural food colorants (instability to light, heat or adverse pH), workers in the area of food product development frequently ask for alternative sources of food colouring materials [95].

A survey by Tripathi *et al.* [96] on the use of synthetic colours in India, in urban and rural areas of Lucknow, revealed that there were more types and varieties of coloured eatables in the urban than in the rural areas. Of the samples analysed, 69% of eatables contained permitted colours, while 39% contained non-permitted colours, the use of the latter being more in the rural than urban areas. Also, the rural areas contained the permitted colours in amounts exceeding the prescribed levels. In the urban areas, in crushed ice, preferred by children, the use of Sunset Yellow FCF, Tartrazine and Carmoisine exceeded the permitted levels by 8–20 times, while the same in rural areas exceeded by 15–23 times. Amaranth and Sudan dyes were also detected.

Food allergies and food intolerance can be distinguished by the mechanisms involved, the severity of the symptoms and the degree of tolerance to the offending food. True food allergies are abnormal reactions of the immune system to the allergen. The allergens are typically naturally occurring proteins in the food. The allergies could be an immediate hypersensitivity reaction, developing within a few minutes to a few hours of ingestion of the offending food, or a delayed hypersensitivity reaction, where the symptoms do not appear in 24 h or more. Food intolerances occur through a number of different non-immunological mechanisms like metabolic disorders, anaphylactoid reactions and idiosyncratic illnesses [97].

The colouring compounds in natural colour additives are low-molecular-weight, non-protein chemicals that stimulate the immune system by binding to proteins and acting as haptens. Reactions to natural colour additives are reported only occasionally and are attributed to the presence of protein residues in colours such as carmine and annatto [98].

References

1. Indu Rani C, Arumuganatha T, Muthuvel I. Food colors. Agro India 2004;7(8):15.
2. [cited 23 March 2007] http://colormaker.com/CM/AboutNC/
3. Giusti MM, Wrolstad RE. Acylated anthocyanins from edible sources and their applications in food systems. Biochemical Engineering Journal 2003;14:217–25.
4. Gulrajani ML. Present status of natural dyes. Indian Journal of Fibre and Textile Research 2001;26:191–201.
5. Krishnamurthy KV, Siva R, Senthil Kumar T. Natural dye-yielding plants of Shervaroy Hills of Eastern Ghats. In: Proceedings of the National Seminar on Conservation of the Eastern Ghats. Environment Protection Training and Research Institute, Hyderabad, India; 2002. p. 151–3.
6. Siva M. Status of natural dyes and dye-yielding plants in India. Current Science 2007;92(7):916–25.
7. Downham A, Collins P. Colouring our foods in the last and next millennium. International Journal of Food Science and Technology 2000;35(1):5–22.
8. Giugliano D. Dietary antioxidants for cardiovascular prevention. Nutrition, Metabolism and Cardiovascular Diseases 2000;10(1):38–44.
9. Food Additives: Natural Food Colors [cited 23 March 2007]. Available from: URL: http://www.foodadditivesworld.com/natural-food-colors.html
10. Parkinson TM, Brown JP. Metabolic fate of food colorants. Annual Review of Nutrition 1981;1:175–205.
11. Coulson J. Synthetic organic colors for food. In: Walford J, editor. Developments in Food Colors – I. Applied Science Publishers, London; 1980. p. 47–94.
12. Francis FJ. Food colorants: anthocyanins. Critical Reviews in Food Science and Nutrition 1989;28(4):273–314.
13. Kalkan YH. Evaluation of colour parameters and antioxidant activities of fruit wines. International Journal of Food Science and Nutrition 2006;57(1–2):47–63.
14. Caihuan H, Siyi O, Liang F, Yong W, Ning Z, Zhijie Q, *et al.* Study on extraction and stability of pigment from flowers of *Leucosceptrum canum*. Zhong Yao Cai 2004;27(4):243–6.
15. Kays SJ. Postharvest Physiology of Perishable Plant Products. CBS Publishers and Distributors, New Delhi, India; 1998. p. 187–207.

16. Hirasa K, Takemasa M (editors). Spices and herbs: basic concepts. In: Spice Science and Technology. Marcel Dekker, New York; 1998. p. 1–27.

17. Mittal R, Sharma A, Singh G. Food colors from plants: patenting scenario. Indian Food Industry 2007;26(3):52–8.

18. Chandramouli KV. Sources of Natural Dyes in India – A Compendium with Regional Names. PPST Foundation, Chennai, India; 1995.

19. Walker R. The metabolism of azo compounds: a review of the literature. Food and Cosmetics Toxicology 1970;8:659–76.

20. Drake JJP. Food colours – harmless aesthetics or epicurean luxuries? Toxicology 1975;5:3–42.

21. Lafferman JA, Silbergeld EK. Erythrosine B inhibits dopamine transport in rat caudate synaptosomes. Science 1979;205:410–2.

22. Brown JP, Roehm GW, Brown RJ. Mutagenicity testing of certified food colors and related azo, xanthene and triphenylmethane dyes with the *Salmonella*/microsome systems. Mutation Research 1978;56:249–71.

23. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives. Some Food Colours, Thickening Agents and Certain Other Substances. WHO Food Additive Series, No. 8. WHO, Geneva; 1975. p. 28–31.

24. Brown JP, Roehm GW, Brown RJ. The application of short-term microbial mutagenicity tests in the identification and development of non-toxic, non-absorbable food additives. In: Scott D, Bridges BA, Sobels FH, editors. Progress in Genetic Toxicology. Elsevier/North-Holland; 1977. p. 185–90.

25. Dunn MJ, Steinbach SP. Food, drug, and cosmetic colors. In: Patton TC, editor. Pigment Handbook. Wiley, New York; 1973. p. 953–76.

26. Lucas CD, Hallagan JB, Taylor SL. The role of natural color additives in food allergy. Advances in Food and Nutrition Research 2001;43:195–216.

27. Marmion DM. Handbook of US Colorants: Foods, Drugs, Cosmetics and Medical Devices, 3rd ed. John Wiley and Sons, New York; 1991.

28. Greenfield AB. A Perfect Red: Empire, Espionage, and the Quest for the Color of Desire. Harper Collins, New York; 2005.

29. Kagi MK, Wüthrich B, Johansson SG. Campari-Orange anaphylaxis due to carmine allergy. Lancet 1994;344:60–1.

30. Markakis P. Stability of anthocyanins in foods. In: Markakis P, editor. Anthocyanins as Food Colors. Chapter 6. Academic Press, New York; 1992.

31. Chandra A, Nair MG, Iezzoni AF. Isolation and stabilization of anthocyanins from tart cherries (*Prunus cerasus* L.). Journal of Agricultural and Food Chemistry 1993;41:1062–5.

32. Engel C. Natural Colors. Their Stability and Application in Food. The British Food Manufacturing Industries Research Association Science and Technology Survey. No. 117. The British Food Manufacturing Industries Research Association, Leatherhead, UK; 1979. p. 87.

33. Jackman RL, Smith JL. Anthocyanins and betalains. In: Hendry GAF, Houghton JD, editors. Natural Food Colorants, 2nd ed. Chapter 8. Blackie Academic and Professional, London; 1996.

34. Moreno YS, Sanchez GS, Hernandez DR, Lobato NR. Characterization of anthocyanin extracts from maize kernels. Journal of Chromatographic Science 2005;43(9):483–7.

35. Awika JM, Rooney LW and Waniska RD. Properties of 3-deoxyanthocyanins from sorghum. Journal of Agricultural and Food Chemistry 2004;52(14):4388–94.

36. Longo L, Vasapollo G. Anthocyanins from bay (*Laurus nobilis* L.) berries. Journal of Agricultural and Food Chemistry 2005;53(20):8063–7.

37. Longo L, Vasapollo G. Determination of anthocyanins in *Ruscus aculeatus* L. berries. Journal of Agricultural and Food Chemistry 2005;53(2):475–9.

38. Mateus N, Oliveira J, Haettich-Motta M, de Freitas V. New family of bluish pyranoanthocyanins. Journal of Biomedicine and Biotechnology 2004;2004(5):299–305.

39. Francis FJ. A new group of food colorants. Trends in Food Science and Technology 1992;3:27–31.

40. Mailen-Aubert C, Dangles O, Amiot MJ. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intramolecular and intermolecular copigmentation. Journal of Agricultural and Food Chemistry 2001;49:170–6.

41. Bordignon-Luiz MT, Gauche C, Gris EF, Falcao LD. Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems. Lebensmittel-Wissenschaft und-Technologie – Food Science and Technology 2007;40:594–9.

42. Kühnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Review of Nutrition and Dietetics 1976;24:117–91.

43. Bakker J, Bridle P, Bellworthy SJ. Strawberry juice color: a study of quantitative and qualitative pigment composition of juices from 39 genotypes. Journal of Science of Food and Agriculture 1994;64:31–7.

44. Timberlake CF, Henry BS. Anthocyanins as natural food colorants. Progress in Clinical and Biological Research 1988;280:107–21.

45. Glories Y. Anthocyanins and tannins from wine: organoleptic properties. Progress in Clinical and Biological Research 1988;280:123–34.

46. Jing P, Giusti MM. Characterization of anthocyanin-rich waste from purple corncobs (*Zea mays* L.) and its application to color milk. Journal of Agriculture and Food Chemistry 2005;53(22):8775–81.

47. Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. Journal of Agricultural and Food Chemistry 1997;45(2):304–9.

48. Tamura H, Yamagami A. Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. Journal of Agricultural and Food Chemistry 1994;42(8):1612–5.

49. Rice-Evans CA, Miller NJ. Antioxidant activities of flavonoids as bioactive components of food. Transactions 1996;24(3):790–5.

50. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, *et al.* Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium* species. Journal of Agricultural and Food Chemistry 1998;46(7):2686–93.

51. Degenhardt A, Knapp H, Winterhalter P. Separation and purification of anthocyanins by high-speed countercurrent chromatography and screening for antioxidant activity. Journal of Agricultural and Food Chemistry 2004;8(2):338–43.
52. Lieti A, Cristoni A, Picci M. Studies on *Vaccinium myrtillus* anthocyanosides. I. Vasoprotective and anti-inflammatory activity. Arzneimittel-Forschung 1976;26(5):829–32.
53. Morazzoni P, Magistretti MJ. Effects of *Vaccinium myrtillus* anthocyanosides on prostacyclins-like activity in rat arterial tissue. Fitoterpia 1986;57(1):11–14.
54. Kamei H, Kojima T, Hasegawa M, Koide T, Umeda T, Yukawa T, *et al.* Suppression of tumor cell growth by anthocyanins *in vitro*. Cancer Investigations 1995;13(6):590–4.
55. Akhmadieva AK, Zaichkina SI, Ruzieva RK, Ganassi EE. The protective action of a natural preparation of anthocyanin (pelargonidin-3,5-diglucoside). Radiobiologiia 1993;33(3):433–5.
56. Stoner GD, Wang LS, Zikri N, Chen T, Hecht SS, Huang C, *et al.* Cancer prevention with freeze-dried berries and berry components. Seminars in Cancer Biology 2007;17(5):403–410.
57. Hagiwara A, Miyashita K, Nakanishi T, Sano M, Tamano S, Kadota T, *et al.* Pronounced inhibition by a natural anthocyanin, purple corn color, of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-associated colorectal carcinogenesis in male F344 rats pretreated with 1,2-dimethylhydrazine. Cancer Letters 2001;171(1):17–25.
58. Hagiwara A, Yoshino H, Ichihara T, Kawabe M, Tamano S, Aoki H, *et al.* Prevention by natural food anthocyanins, purple sweet potato color and red cabbage color, of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-associated colorectal carcinogenesis in rats initiated with 1,2-dimethylhydrazine. Journal of Toxicological Science 2002;27(1):57–68.
59. Strack D, Wray V. The anthocyanins. In: Harborne JB, editor. The Flavonoids: Advances in Research Since 1986. Chapman and Hall, London; 1993. p. 1–22.
60. Narayan MS, Naidu KA, Ravishankar GA, Srinivas L, Venkataraman LV. Antioxidant effect of anthocyanin on enzymatic and non-enzymatic lipid peroxidation. Prostaglandins, Leukotrienes and Essential Fatty Acids 1999;60(1):1–4.
61. Brouillard R, George F, Fougerousse A. Polyphenols produced during red wine ageing. Biofactors 1997;6(4):403–10.
62. Chigurupati N, Saiki L, Gayser C, Dash AK. Evaluation of red cabbage dye as a potential natural color for pharmaceutical use. International Journal of Pharmaceutics 2002;241(2):293–9.
63. Castellar R, Obon JM, Alacid M, Fernandez-Lopez JA. Color properties and stability of betacyanins from *Opuntia* fruits. Journal of Agricultural and Food Chemistry 2003;51(9):2772–6.
64. Schiber A, Mihalev K, Berardini N, Mollov P, Carle R. Flavonol glycosides from distilled petals of *Rosa damascena* Mill. Zeitschrift für Naturforschung [C] 2005;60(5–6):379–84.
65. Kim S, Jones R, Yoo KS, Pike LM. Gold color in onions (*Allium cepa*): a natural mutation of the chalcone isomerase gene resulting in a premature stop codon. Molecular Genetics and Genomics 2004;72(4):411–9.
66. Rao AV, Rao LG. Carotenoids and human health. Pharmacological Research 2007;55:207–216.
67. Zechmeister L. *cis–trans* isomerization and sterochemistry of carotenoids and dipenyl polyenes. Chemical Reviews 1944;34:267–344.
68. Lee J, Jiang S, Levine N, Watson RR. Carotenoid supplementation reduces erythema in human skin after simulated solar radiation exposure. Proceedings of the Society for Experimental Biology and Medicine 2000;223(2):170–4.
69. Torres CA, Andrews PK, Davies NM. Physiological and biochemical responses of fruit exocarp of tomato (*Lycopersicon esculentum* Mill.) mutants to natural photo-oxidative conditions. Journal of Experimental Botany 2006;57(9):1933–47.
70. Neta-Sharir I, Isaacson T, Lurie S, Weiss D. Dual role for tomato heat shock protein 21: protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. Plant Cell 2005;17(6):1829–38.
71. Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea FM. Astaxanthin: a review of its chemistry and applications. Critical Reviews on Food Science and Nutrition 2006;46(2):185–96.
72. Galindo-Cuspinera V, Lubran MB, Rankin SA. Comparison of volatile compounds in water- and oil-soluble annatto (*Bixa orellana* L.) extracts. Journal of Agricultural and Food Chemistry 2002;50(7):2010–5.
73. Nish W, Whisman B, Goetz D, Ramirez D. Anaphylaxis to annatto dye: a case report. Annals of Allergy 1991;66:129–31.
74. van Assendelft AHW. Bronchospasm induced by vanilla and lactose. European Journal of Respiratory Diseases 1984;65:468–72.
75. Hagiwara A, Imai N, Doi Y, Nabae K, Hirota T, Yoshino H, *et al.* Absence of liver tumor promoting effects of annatto extract (norbixin), a natural carotenoid food color, in a medium-term liver carcinogenesis bioassay using male F344 rats. Cancer Letters 2003;199(1):9–17.
76. Hagiwara A, Imai N, Ichihara T, Sano M, Tamano S, Aoki H, *et al.* A thirteen-week oral toxicity study of annatto extract (norbixin), a natural food color extracted from the seed coat of annatto (*Bixa orellana* L.), in Sprague–Dawley rats. Food Chemistry and Toxicology 2003;41(8):1157–64.
77. Alves de Lima RO, Azevedo L, Ribeiro LR, Salvadori DM. Study on the mutagenicity and antimutagenicity of a natural food colour (annatto) in mouse bone marrow cells. Food Chemistry and Toxicology 2003;41(2):189–92.
78. Mikkelsen H, Larsen JC, Tarding F. Hypersensitivity reactions to food colours with special reference to the natural colour annatto extract (butter colour). Archives of Toxicology 1978;1(Suppl.):141–3.
79. Rao AV, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutrition Research 1999;19:305–23.
80. Clinton SK. Lycopene: chemistry, biology, and implications for human health and disease. Nutrition Review 1998;56:35–51.

81. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, *et al.* *cis–trans* lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiology Biomarkers and Prevention 1996;5:823–33.

82. Sies H, Stahl W. Lycopene: antioxidant and biological effects and its bioavailability in the human. Proceedings of the Society for Experimental Biology and Medicine 1998;218:121–4.

83. Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. CMAJ 2000;163:739–44.

84. Shi J, Le Maguer M. Lycopene in tomatoes: chemical and physical properties affected by food processing. Critical Reviews in Biotechnology 2000;20(4):293–334.

85. Fraser ML, Lee AH, Binns CW. Lycopene and prostate cancer: emerging evidence. Expert Reviews in Anticancer Therapy 2005;5(5):847–54.

86. Karadas F, Grammenidis E, Surai PF, Acamovic T, Sparks NH. Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. British Poultry Science 2006;47(5):561–6.

87. Osterlie M. Chromatographic determination of the effect of storage on lycopene. Journal of Chromatographic Science 2005;43(9):473–7.

88. Haila KM, Lievonen SM, Heinonen MI. Effect of lutein, lycopene, annatto and γ-tocopherol on autoxidation of triglycerides. Journal of Agricultural and Food Chemistry 1996;44:2096–100.

89. Kuramoto Y, Yamada K, Tsuruta O, Sugano M. Effect of natural food colorings on immunoglobulin production *in vitro* by rat spleen lymphocytes. Bioscience, Biotechnology and Biochemistry 1996;60(10):1712–3.

90. Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MM. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. Oncogene 2004;23(8):1599–607.

91. Farrell KT. Spices, Condiments and Seasoning. Van Nostrand Reinhold Company, New York; 1985.

92. Wüthrich B, Schmid-Grendelmeye P, Lundberg M. Anaphylaxis to saffron. Allergy 1997;52:476–7.

93. Codex Alimentarius Commission. ALINORM 99/22. Draft Recommendations for the Labeling of Foods that can Cause Hypersensitivity (Draft Amendment to the General Standard for the Labeling of Prepackaged Foods) at Step 8 of the Procedure. 1998; Appendix III.

94. Hallagan JB, Allen DC, Borzelleca JF. The safety and regulatory status of food, drug and cosmetics colour additives exempt from certification. Food Chemistry and Toxicology 1995;33:515–28.

95. Wissgott U, Bortlik K. Prospects for new natural food colorants. Trends in Food Science and Technology 1996;7(9):298–302.

96. Tripathi M, Khanna SK, Das M. Surveillance on use of synthetic colours in eatables *vis-a-vis* prevention of food adulteration act of India. Food Control 2007;18:211–9.

97. Lemke PJ, Taylor SL. Allergic reactions and food intolerances. In: Kotsonis FN, Mackey M, Hjelle J, editors. Nutritional Toxicology. Raven Press, New York; 1994. p. 117–37.

98. Taylor SL, Dormedy ES. Flavorings and colorings. Allergy 1998;53(Suppl. 46):80–2.

99. Grotewold E. The Science of Flavonoids. In: Grotewold E, editor. Plant pigments, Springer, New York; 2006. p. 216.

100. Stanley JK. Post-harvest physiology of perishable plant products. CBS Publishers and Distributors, New Delhi, India; 1998. p. 187–207.

101. Hirasa K, Takemasa M. Spices and Herbs: Basic Concepts. In: Spice Science and Technology. Marcel Dekker Inc., New York; 1998. p. 1–27.