Carotenoids and other pigments as natural colorants*

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Abstract: Food colorants may be classified into synthetic, nature-identical, inorganic, and natural colorants. Natural colorants for food are made from renewable sources. Most often, the colorants are extracted from plant material, but other sources such as insects, algae, cyanobacteria, and fungi are used as well. Natural colorants are usually extracted and concentrated using either water or lower alcohols for water-soluble pigments and organic solvents for lipophilic pigments. Legislation restricts which colorants are allowed, what sources may be used for that particular colorant, what solvents may be used to extract it, and the purity of the pigment. Colorants are formulated to make them more suitable for a variety of foods and drinks (e.g., lipophilic pigments for use in beverages) and to increase their stability. The natural colorants allowed in the EU and the USA are presented. Many of these colorants are also allowed in most parts of the world. A few colorants that are not allowed in the EU and the USA, but are important food colorants in other parts of the world, are also presented.

Keywords: food colorants; carotenoids; anthocyanins; betalains; carmine; caramel; legislation; formulation; extraction; stability.

INTRODUCTION

Color is an important characteristic of food. Based on the color of the food, first impressions are made: Is the fruit immature, ripe, or overripe? Is the toast burnt? Is the food fresh? Based on these first impressions, a judgment is made whether the food is safe to eat or not and whether it can be expected to taste good or not. Since color is closely associated with expectations, the addition of color to food is a way to fulfill these expectations. Color is added to food for one or more of the following reasons: (1) to replace color lost during processing, (2) to enhance color already present, (3) to minimize batch-to-batch variations, and (4) to color otherwise uncolored food.

Food colors can be divided into four categories: (1) natural colors, (2) nature-identical colors, (3) synthetic colors, and (4) inorganic colors. Natural colors are pigments made by living organisms. Usually, pigments made by modification of materials from living organisms, such as caramel, vegetable carbon, and Cu-chlorophyllin (vide infra), are also considered natural though they are in fact (except for carbon) not found in nature. Nature-identical colors are man-made pigments which are also found in nature. Examples are β-carotene, canthaxanthin, and riboflavin. Synthetic colors are man-made colors which are not found in nature—these are often azo-dyes. Examples of inorganic colors are titanium dioxide, gold, and silver.

Although structurally very diversified and from a variety of sources, natural food colorants can be grouped into a few classes, the three most important of which are: tetrapyrrols, tetraterpenoids, and flavonoids. The most important member of the tetrapyrrols is chlorophyll, which is found in all higher plants. Carotenoids are tetraterpenoids that are as ubiquitous as chlorophyll, since they too are part of the photosynthetic apparatus. They also give the yellow–orange–red color of many fruits. Anthocyanins are a group of flavonoids that provide the red–purple shade of many fruits, in particular berries (e.g., strawberries, elderberries, and black currants). Other important classes of colorants are the anthraquinones (carmine, lac, kermes, and madder) and the betalains (beetroot).

The terms "pigment" and "dye" are often used interchangeably. Strictly speaking, a pigment is insoluble in the given medium, whereas a dye is soluble. Thus, carotenoids are dyes in oil but pigments in water. This distinction may be difficult to maintain if nothing is assumed about the medium, and in the following the term "pigment" will be used for colored substances in general.

LEGISLATION

In most countries, the use of food additives (including colorants) is governed by strict regulation. The legislation specifies which colorants may be used, the source(s) of the colorant, the purity of the colorant, to which foods the colorant may be added, and at what level the colorant may be added to a specific food.

In the EU and the USA, the natural and nature-identical colorants shown in Table 1 are allowed (colorants only allowed in fish or chicken feed for pigmenting flesh and/or eggs are not included).

Table 1 Allowed natural and nature-identical colorants in the EU [1] and the USA [2].

EU name	E-number	U.S. name	U.S. CFR number
Curcumin	E 100	Turmeric	73.600, 73.615
Riboflavin	E 101	Riboflavin	73.450
Cochineal, carminic acid, carmines	E 120	Cochineal extract, carmine	73.100
Chlorophyll(in)s	E 140	Not allowed	
Copper complexes of chlorophyll(in)s	E 141	Sodium copper chlorophyllin	73.125
Caramel	E 150	Caramel	73.85
Vegetable carbon	E 153	Not allowed	
Mixed carotenes	E 160a (i)	Carrot oil	73.300
β-Carotene	E 160a (ii)	β-Carotene	73.95
Annatto, bixin, norbixin	E 160b	Annatto extract	73.30
Paprika extract	E 160c	Paprika	73.340, 73.345
Lycopene	E 160d	Not allowed	
β-Apo-8'-carotenal	E 160e	β-Apo-8'-carotenal	73.90
Ethyl ester of β-apo-8'-carotenoic acid	E 160f	Not allowed	
Lutein	E 161b	Not allowed	
Canthaxanthin	E 161g	Canthaxanthin	73.75
Beetroot red	E 162	Dehydrated beets	73.40
Anthocyanins	E 163	Grape color/skin extract	73.169, 73.170
	Not allowed	Cottonseed flour	73.140
	*	Fruit juice	73.250
	*	Vegetable juice	73.260
	*	Saffron	73.500

*Allowed as a food ingredient (not an additive) that does not require an E-number.

Though there are many similarities between the EU and U.S. legislation as to which colorants are allowed, there are major differences when it comes to which sources are allowed and which foods may be colored. For instance, in the USA, sodium copper chlorophyllin may only be made from alfalfa (*Medicago sativa*) and only be used in citrus-based dry beverage mixes [2], whereas in the EU, allowed sources are alfalfa, grass, nettle, and edible plant material [1], and a long list of foods may be colored. On the other hand, canthaxanthin is only allowed for coloring *Saucisses de Strasbourg* (a sausage) in the EU, whereas it is generally allowed in the USA. It is beyond the scope of this review to give a detailed account of the various legislations.

Legislation is often based on local, traditional usage of coloring matter. Thus, lac, monascus, gardenia, and spirulina are important colorants in some parts of Asia, but none of them are allowed in the EU or in the USA, where there is no traditional use of the raw materials.

NATURAL COLORANTS

Sources of natural colorants

Pigments are found widespread in nature from the simplest procaryotic organisms like cyanobacteria, and throughout the kingdoms of fungi, plants, and animals. Most natural food colorants come from the division *Magnoliophyta* (flowering plants) of the plant kingdom. However, natural colorants from other sources such as scale insects (cochineal and lac), fungi (*Blakeslea trispora* and *Monascus* spp.), and cyanobacteria (*Arthrospira* spp.) are used today for coloring food.

Extraction of pigments

One way of coloring a food would be to add a strongly colored food (e.g., elderberry or black currant) to the food item (e.g., raspberry jam) that is to be colored. This is the approach used in home-cooking where spices, besides flavor, may impart color: turmeric and paprika are examples of this. However, for industrial food production this approach presents a series of problems: (1) low concentration of pigments in most foods means that a large amount would have to be added to give the desired shade, (2) unwanted flavor (elderberry flavor in a raspberry jam), and (3) insoluble matter (e.g., peel and seeds), which may not be a problem in some applications (jam) but is unacceptable in others (e.g., beverages). Therefore, pigments are extracted to overcome the problems of low concentration, flavor, and insoluble material. Lipid-soluble pigments like chlorophyll and carotenoids are usually extracted with organic solvents, which are subsequently removed, yielding an oleoresin rich in pigments, but also containing other material such as triglycerides, sterols, wax, and other lipid-soluble compounds. Water-soluble pigments such as carminic acid and anthocyanins are in general extracted with water or lower alcohols.

Pigments

Carotenoids
Carotenoids are lipid-soluble, yellow–orange–red pigments found in all higher plants and some animals. Animals cannot synthesize carotenoids, so their presence is due to dietary intake, e.g., the pink salmon flesh and many birds' plumage owe their color to carotenoids. Plant, algae, fungal, and synthetic (nature-identical) carotenoids are allowed as colorants, but not animal carotenoids. Carotenoids can be divided into carotenes containing only carbon and hydrogen, and xanthophylls made up of carbon, hydrogen, and oxygen. Carotenoids owe their name to carrots (*Daucus carota*), and xanthophyll is derived from the Greek words for yellow and leaf. Together with anthocyanins (vide infra), carotenoids are the most complex class of natural food colorants with around 750 different structures identified [3]. The xanthophylls β-apo-8'-carotenal, ethyl ester of β-apo-8'-carotenoic acid, and canthaxanthin (Table 1) will not be treated here as their only commercial source is via synthesis.

Carotenes

The E-number E 160a actually comprises four different sources of carotenes: plants, *Dunaliella salina* (an algae), synthetic, and *Blakeslea trispora* (a fungus) [1c]. Under the EU legislation, plant carotenes may be derived from edible plants, carrots (mentioned specifically, though one could easily argue that it is an edible plant), vegetable oils, grass, alfalfa, and nettle [1], but only from carrots according to U.S. legislation [2] (Table 1). A good source of plant carotenes is oil palm (*Elaeis guineensis*) fruits, the mesocarp of which contains an oil rich in carotenes. After separation of the carotenes from the palm fruit oil, which is used for making detergents, the carotenes are suspended in vegetable oil at a concentration of 30 %. The predominant carotenes are α- and β-carotene (Fig. 1) in the ratio 2:3, and other carotenes such as phytoene, phytofluene, ζ-carotene, γ-carotene, and lycopene (which are all precursors in the biosynthesis of α- and β-carotene) are present in smaller amounts [4]. Due to heat treatment of the oil palm fruit used in obtaining the oil, a complex mixture of geometric isomers is formed with only 60 % of α- and β-carotene as the *trans*-forms [4]. Synthetic β-carotene is predominantly *trans*-β-carotene. The presence of α-carotene and *cis*-isomers of α- and β-carotene in palm fruit carotenes means that synthetic β-carotene is more orange than palm fruit carotenes, which is more yellow*. Carotene from *B. trispora* is also mainly *trans*-β-carotene with approximately 3 % other carotenoids [1c]. Carotene from *D. salina* also primarily consists of β-carotene with 5–6 % other carotenoids (α-carotene, lutein, zeaxanthin, and β-cryptoxanthin); according to legislation, the content of *trans*-isomers coming from this source should be in the range 50–71 % [1c]. This means that its color shade would be in-between that of oil palm carotenes and synthetic β-carotene. Besides being used as colorants, carotenes are also used for nutritional purposes as provitamin A agents (e.g., in margarine where they also provide color) or as dietary supplements.

Lycopene

Being a precursor in the biosynthesis of β-carotene, lycopene (Fig. 1) can be expected to be found in plants containing β-carotene, albeit usually at a very low (sometimes undetectable) concentration. The best-known sources of lycopene are tomatoes, watermelon, guava, and pink grapefruit. Lycopene may also be produced synthetically and by *B. trispora*. Lycopene is allowed in the EU as a food colorant and has recently (July 2005) become allowed in the USA as well. The only allowed source is tomatoes (*Lycopersicon esculentum*, lycopersicon meaning wolf peach). Besides lycopene, a tomato oleoresin also contains appreciable amounts of β-carotene, phytoene, and phytofluene. In solution, lycopene is orange (as anyone making a dish with tomatoes and oil would have noticed) and not bright red as in the tomato. Lycopene is hardly used as a colorant because it is a rather expensive pigment and is very prone to oxidative degradation (much more so than β-carotene). Besides, paprika is cheaper, more stable, and provides a similar color shade.

Lutein

Lutein (Fig. 1) is also a very common carotenoid. The name is derived from the Latin word for yellow (compare xanthophyll, vide supra). Commercially, the most interesting source is Aztec marigold (*Tagetes erecta*) in which lutein is primarily found esterified with saturated fatty acids (lauric, myristic, palmitic, and stearic acid [5]). Lutein made from Aztec marigold also contains some zeaxanthin (typically less than 10 %). Containing only 10 conjugated double bonds, lutein is more yellowish-green than oil palm carotenes. Lutein is not allowed as a food colorant in the USA except for chicken feed [2].

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*Here, as well as anywhere else, it should be emphasized that color hue is dependent on pigment concentration. Thus, palm fruit carotenes are yellow at low concentration, but gradually turn orange and even red at higher concentrations. Palm oil is used as a cooking oil either bleached or containing around 800 ppm carotenes, in which case the oil is deep red.
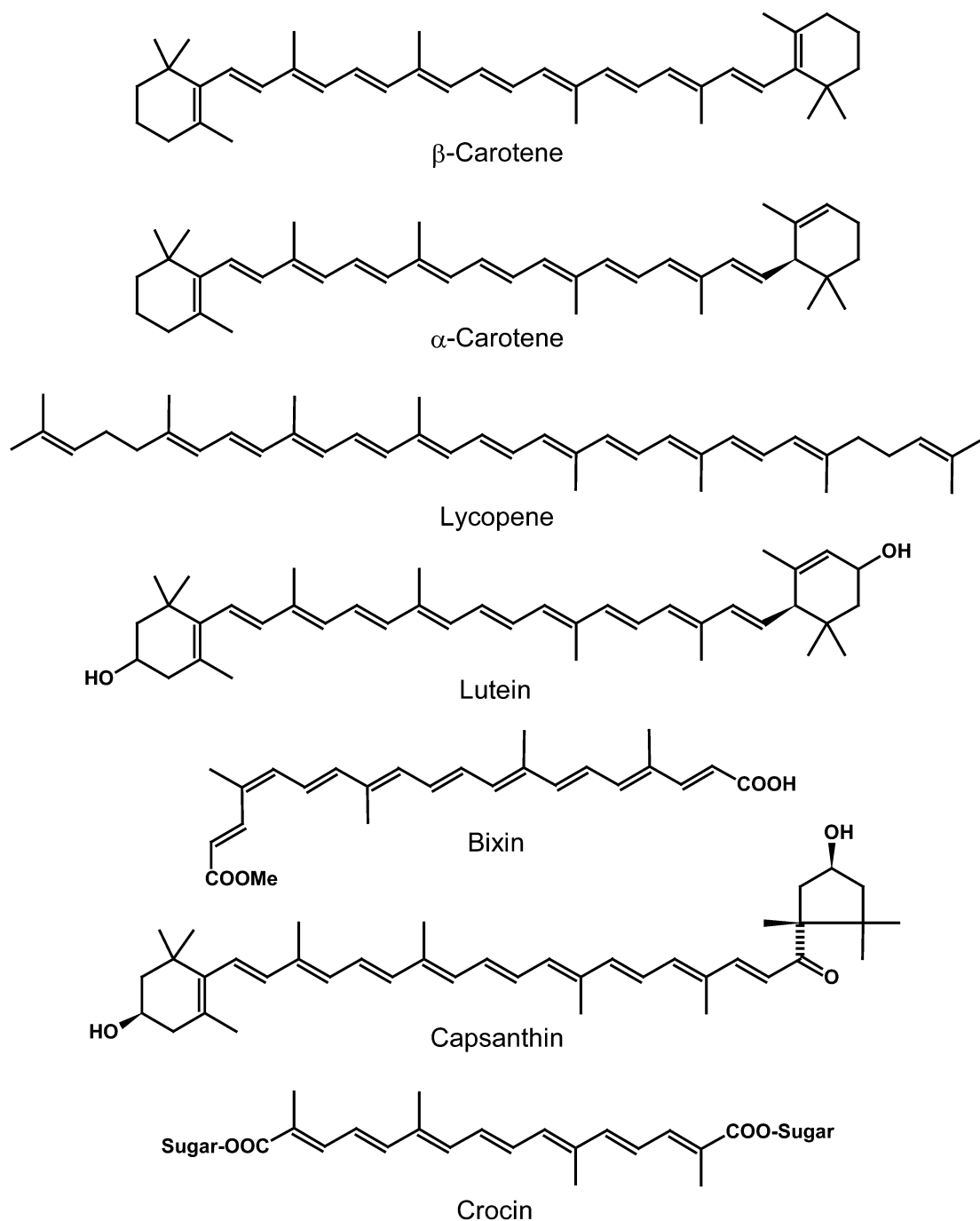
Fig. 1 Structure of some carotenoids.

Annatto

Annatto is both the name of the colorant and the tree providing the colorant. Other names of the tree (*Bixa orellana*) are lipstick tree and achiote. The colorant is extracted from the seeds, which are covered by a red, resinous coating containing the pigments. The main pigment is bixin (Fig. 1) which is a

cis-isomer. Smaller amounts of *trans*-bixin, norbixin (demethylated bixin), and *trans*-norbixin are also present. Annatto may be extracted using either of three methods: (1) extraction with (hot) vegetable oil, (2) extraction with organic solvents, or (3) extraction with alkali [1,2]. Extraction with vegetable oil or organic solvents gives a colorant containing bixin as the main coloring principle, whereas extraction with alkali leads to saponification of bixin yielding the water-soluble (at neutral and high pH) norbixin, which may be precipitated by acid to give a powder. Norbixin is used to color cheese (e.g., cheddar) because it binds to the proteins. It may also be used to color beverages with neutral pH, e.g., flavored milk drinks, but not with low pH because of precipitation. Annatto is slightly more reddish in application than β-carotene. Annatto is generally allowed in the USA as a food colorant, whereas its use in the EU is more restricted.

Paprika
Paprika is a well-known spice made from the fruit pods of *Capsicum annuum*. The name paprika comes from Serbian and Hungarian and can be traced back to the Latin word for pepper (piper). When making an oleoresin that is to be used as a colorant rather than as a flavor, *C. annuum* varieties with low pungency and little flavor are used as raw material. The main pigment in paprika is capsanthin (Fig. 1) which accounts for around half the carotenoids in paprika [6]. Paprika contains the orange–red pigments capsanthin and capsorubin and a number of yellow pigments, most notably violaxanthin, zeaxanthin, β-cryptoxanthin, and β-carotene [6]. A high concentration of the orange–red pigments is desirable, as a yellow colorant can be found elsewhere, e.g., lutein and carotenes. An ideal situation would be a very red paprika that could be mixed with the yellow carotenes or lutein to give a continuous range of yellow–orange–red colorants. Just as with lutein in Aztec marigold, the xanthophylls in paprika are esterified with fatty acids.

Saffron
Saffron (from Arabic meaning "yellow") is the dried stigma of *Crocus sativus*. The major pigment is the water-soluble crocin (Fig. 1), a digentiobioside of crocetin. In contrast to the other carotenoids, the pigment is not extracted from the raw material, rather the whole stigma (possibly finely divided) is added to the food. The associated saffron flavor and the high cost of saffron limit its use as colorant to special applications. In the EU, saffron is not regarded a colorant, but is considered an ingredient or a spice.

Chlorophyll
Chlorophyll is the green pigment utilized by all higher plants for photosynthesis. The name derives from the Greek words for green and leaf (compare to xanthophyll). Chlorophyll is a cyclic tetrapyrrole with coordinated magnesium in the center (Fig. 2). In plants, there are two forms of chlorophyll (*a* and *b*) which only differ in the substitution of the tetrapyrrole ring (Fig. 2). Being lipid-soluble, chlorophyll is extracted with the use of organic solvents. The oleoresin thus obtained typically contains 10–20 % chlorophyll and some carotenoids (mainly lutein and β-carotene), which are co-extracted by the organic solvents. Chlorophyll can be made water-soluble by saponification of the oleoresin, in which case it is called chlorophyllin. The coordinated magnesium is easily lost (during extraction and processing), especially under the action of acid, yielding a yellow–brown pigment (pheophytin). Chlorophyll is not allowed in the USA but is legal in the EU when extracted from edible plants, nettle, grass, or alfalfa; in the Far East, chlorophyll is also extracted from silkworm droppings and mulberry leaves (feed for silkworms), but neither are allowed sources in the EU. Because of the lability of the coordinated magnesium and the associated color change, chlorophyll(in) as such finds limited use as a colorant.

Cu-chlorophyll
Copper forms a complex with pheophytin. These complexes are often referred to as copper chlorophylls or copper complexes of chlorophyll [1], though they are in fact copper pheophytins. Cu-chlorophyll behaves very much like chlorophyll except that it is more brightly green and much more stable, i.e., copper is not easily displaced.
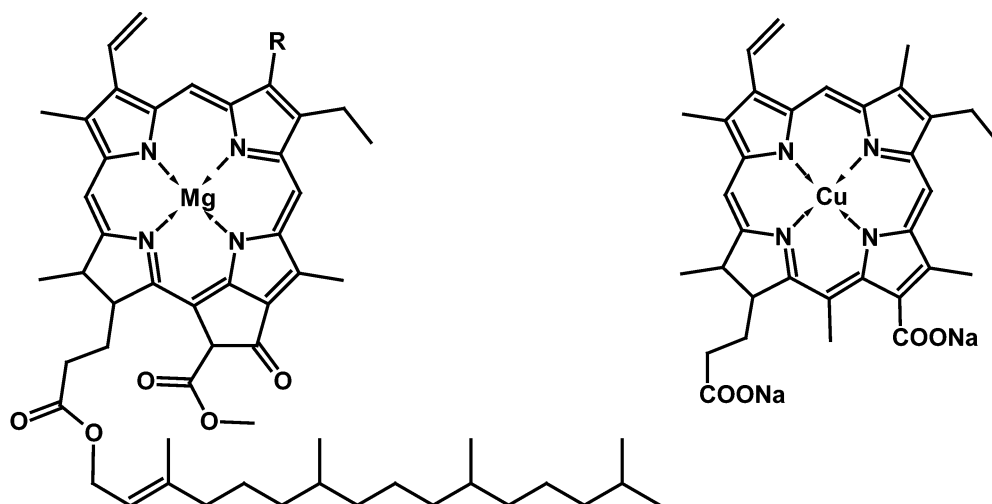
Fig. 2 Structure of chlorophyll *a* (R = CH$_3$) and *b* (R = CHO) and Cu chlorin *e*$_4$ (as sodium salt).

Cu-chlorophyllin

Saponification of chlorophyll makes it water-soluble. However, it is not simply a de-esterification of the phytyl and methyl esters (Fig. 2) taking place. Being a β-ketoester, the isocyclic ring of chlorophyll is labile toward alkali and will to a large extent be destroyed. Thus, the major component of commercial Cu-chlorophyllin was found to be Cu chlorin *e*$_4$ (Fig. 2) [7]. Cu-chlorophyllin can be made very pure (purity exceeding 100 % when using the official assay [1]), and being water-soluble it of course contains no carotenoids. Cu-chlorophyllin is sold as the sodium or potassium salt which is readily soluble in water. However, at low pH Cu-chlorophyllin precipitates as the insoluble protonated acids.

Curcumin

Turmeric is the yellow component of curry powder. It is the dried, ground rhizomes of *Curcuma longa*. The name *Curcuma* is derived from the Arabic word kurkum, which originally meant saffron, but is now used exclusively for turmeric. Turmeric contains three pigments; the major one is called curcumin and the two others are derivatives of this (Fig. 3). Turmeric oleoresin is made by extraction with organic solvents and may contain up to 58 % pigment [8], though commercially available oleoresins are more typically 25–40 %. Besides the pigments, the oleoresin also contains the flavor components of turmeric. The pigments may be separated from the oleoresin, yielding virtually flavor-free curcumin. In the USA, both turmeric oleoresin and curcumin are allowed colorants [2], whereas only curcumin is recognized as a colorant in the EU [1] (turmeric oleoresin may be used as a spice, though). Curcumin is insoluble in water and only slightly soluble in vegetable oil. Curcumin is greenish-yellow, very much like lutein. It is preferred over lutein because it is cheaper, but where light stability is essential, lutein is preferred because curcumin is very prone to photobleaching.
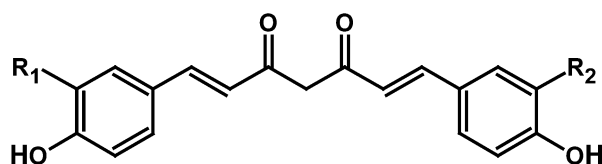
Fig. 3 Structure of curcumin (R$_1$,R$_2$ = OCH$_3$), demethoxycurcumin (R$_1$ = H, R$_2$ = OCH$_3$), and bisdemethoxycurcumin (R$_1$,R$_2$ = H).

Vegetable carbon

This colorant is made by heating plant material at a high temperature, leading to carbonization. Vegetable carbon is a very fine powder that is neither lipid- nor water-soluble. The color in application is gray or black depending on dosage. Vegetable carbon is not allowed in the USA, but is allowed in the EU [1].

Caramel

In terms of volume, caramel is the largest "natural" colorant. Caramel is made by heating carbo-hydrates, which produces polymeric, brown, water-soluble pigments. Four different types of caramels are recognized [1,2]: plain caramel, sulfite caramel, ammonia caramel, and sulfite ammonia caramel. The sulfite and ammonia caramels are made by the addition of ammonia/ammonium salts and/or sul-fites. The caramels have different isoelectric points, making them suitable for various applications. Thus, plain caramel is used for beverages with high alcohol strength (e.g., whiskey) and so is sulfite caramel. Ammonia caramel is used in beer and bakery goods, whereas ammonia sulfite caramel is pri-marily used for soft drinks (e.g., cola), the largest application of caramel.

Anthocyanins

Anthocyanins give rise to the blue–purple–red–orange color of flowers and fruits, in particular, of many plants. The name comes from two Greek words meaning flower and dark blue (and not the blue–green color we usually associate with cyan). Anthocyanins are glycosides of anthocyanidins (also called agly-cones, Fig. 4) and sugars. Anthocyanidins are almost always glycosylated in the 3-position, though gly-cosylation in other positions and in more than one position at a time is also encountered. Furthermore, the sugar moiety may be acylated with aliphatic or aromatic acids. Thus, though the number of known anthocyanidins is quite limited (around 25), anthocyanins number several hundreds because of the great diversity offered by glycosylation and acylation. The six anthocyanidins shown in Fig. 4 are the most abundant. Anthocyanins, carrying a net-charge and being glycosylated, are highly water-soluble. Therefore, anthocyanins are usually extracted with water, though the use of lower alcohols is also per-mitted [1]. The most important source of anthocyanins is grape pomace from wine production. Other important sources are red cabbage, elderberry, black currant, purple carrot, sweet potato, and red radish. In the EU, vegetables and edible fruits are recognized as sources [1], whereas in the USA only grapes are allowed. However, in the USA, vegetable and fruit juices are also allowed colorants, meaning that other sources of anthocyanins may find use.
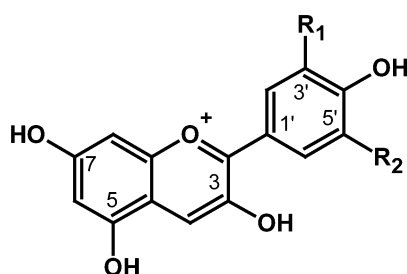

Fig. 4 Structure of the six most common anthocyanidins: pelargonidin ($R_1, R_2 = H$), cyanidin ($R_1 = OH, R_2 = H$), delphinidin ($R_1, R_2 = OH$), peonidin ($R_1 = OCH_3, R_2 = H$), petunidin ($R_1 = OCH_3, R_2 = OH$), and malvidin ($R_1, R_2 = OCH_3$).

Anthocyanins span quite a range of color hues. This is caused by a number of factors. First of all, the substitution pattern of the anthocyanin has a great impact on the color: increasing number of hy-droxy groups yields a more bluish color, whereas methoxy groups give a more red color. Thus, pelargonidin is orange, whereas delphinidin and malvidin are purple. Secondly, the color changes with

pH. At low pH (around 3), the anthocyanins are most strongly colored, exhibiting their well-known purple–red color. Around pH 5, anthocyanins turn almost colorless, and at neutral and alkaline pH the color goes from blue to green. Acidity also affects the stability of anthocyanins, which are rather unstable at weakly acidic to alkaline pH. Another factor contributing to color shade is copigmentation. Copigmentation is the phenomenon that some compounds may cause a red-shift of the anthocyanin absorption, and hence give a more bluish color, and a concomitant increase in absorption. Aromatic compounds like flavonoids and cinnamic acids are particularly effective at this. Copigmentation is believed to occur by sandwiching of the anthocyanin between two copigments or simply by interaction with just one. Copigmentation may be intermolecular or intramolecular if the sugar residue(s) is/are acylated with one or more aromatic acid. Besides giving rise to color change and increased absorption, copigmentation also confers higher stability to the anthocyanins.

Beetroot

Beet (*Beta vulgaris* subsp. *vulgaris*) has been bred to produce a number of varieties, some of which are used as fodder and some of which are used for human food consumption: chard (var. *cicla*), sugar beet (var. *altissima*), and beetroot (var. *rubra*). Beetroot is a variety with a strongly colored root. The purple root owes its color to the presence of betalains. Betalains are found in other plants than beetroot, but beetroot is the only allowed source of betalain colorant in the EU and the USA [1,2]. Betalains are actually comprised of two groups of pigments: the red–purple betacyanidins and the yellow betaxanthins (Fig. 5), both of which are water-soluble. Betacyanidins are conjugates of cyclo-DOPA and betalamic acid, and betaxanthins are conjugates of amino acids or amines and betalamic acid. Just as with anthocyanins, the betacyanidins (aglycones) are most often glycosylated, in which case they are called betacyanins, and the sugar residue may also be acylated. The red–purple betacyanins comprise the major part of pigments in beetroot, and of these a single one, betanin, comprises 75–95 % [1]. The pigments are not extracted, instead the beetroots are pressed and the water partially removed to give a product containing typically 0.5 % pigments. Compared to anthocyanins, beetroot color is more purple and brighter, and the color hue does not change with pH in the range 4–7. The major disadvantage of beetroot color is its low heat stability.
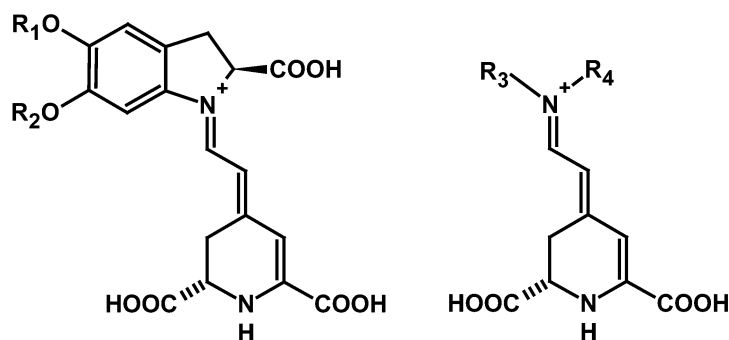

Fig. 5 Structures of betacyanins (left) and betaxanthins (right). Betanidin: R_1, R_2 = H; betanin: R_1 = glucose, R_2 = H.

Cochineal and lac

Scale insects (superfamily Coccoidea) have had great importance since ancient times as textile dyes. Various species have been used: *Kermes vermilio* (kermes), *Porphyrophora polonica* (Polish cochineal), *Porphyrophora hamelii* (Armenian cochineal), *Dactylopius coccus* (American cochineal or simply cochineal), and *Kerria lacca* (lac—often referred to by its old name: *Laccifer lacca*). Of these, cochineal and lac are used as food colorants. Cochineal, but not lac, is allowed in the EU and the USA [1,2], whereas lac is used in the Far East. The pigments, which are anthraquinones, are extracted from

the gravid female insect. They are water-soluble, meaning that they can be extracted with water or lower alcohols. The main pigment (>95 %) in cochineals is carminic acid (Fig. 6), which is special in that it is a *C*-glycoside. Lac contains several different pigments (Fig. 6) of which laccaic acids A, B, and C are dominating [9]. The color of carminic acid in solution changes with pH, since the phenolic groups are rather acidic. Thus, at low pH carminic acid is orange, changes to red at slightly acidic and neutral pH, and finally turns violet in alkaline solution. An interesting property of carminic acid is its ability to form chelates with metal ions—of particular interest are the chelates formed with aluminum and calcium, which are called carmine. The color of carmine ranges from pink through red to violet. The color shade is less dependent on pH than the color shade of carminic acid. Carmine is soluble in alkaline, neutral, and slightly acidic solutions, but will precipitate at low pH. It can, however, be dissolved in hot hydrochloric acid.
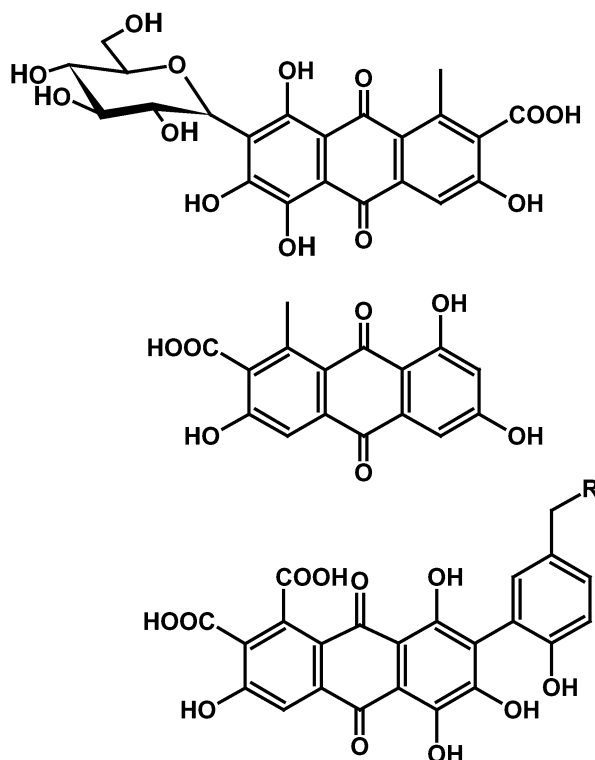

Fig. 6 Structures of carminic acid (top), laccaic acid D (middle) and laccaic acids A (R = CH₂NHCOCH₃), B (R = CH₂OH), C (R = CH(NH₂)COOH), and E (R = CH₂NH₂).

Spirulina
Cyanobacteria contain the green chlorophyll *a* and a blue pigment called phycocyanin. Just like chlorophyll, phycocyanins are photosynthetic pigments absorbing the red light that chlorophyll does not strongly absorb. Phycocyanins are water-soluble proteins containing the covalently bound chromophore phycocyanobilin (Fig. 7)—a linear tetrapyrrole (compare to chlorophyll, Fig. 2). The blue colorant is known by the name spirulina, which is also the name of a dietary supplement rich in proteins consisting of dried cyanobacteria (also called microalgae). Spirulina has also been consumed for centuries as a nutritious food in Africa and South America. Some confusion exists surrounding the name spirulina. Spirulina (the edible cyanobacterium) is often described as the species *Spirulina maxima* and *S. platensis*. However, the cyanobacteria used for human consumption is not *Spirulina* spp. but the related genus

Arthrospira (both of the family Oscillatoriaceae). The name spirulina (for the edible cyanobacterium and the colorant) is so well established that it will probably persist for some time to come. Spirulina (the dried cyanobacteria) may be consumed in the Western world as a dietary supplement, but the (extracted) pigment is allowed neither in the EU nor in the USA as a food colorant. However, it is used as such in other parts of the world, e.g., Australia and the Far East.
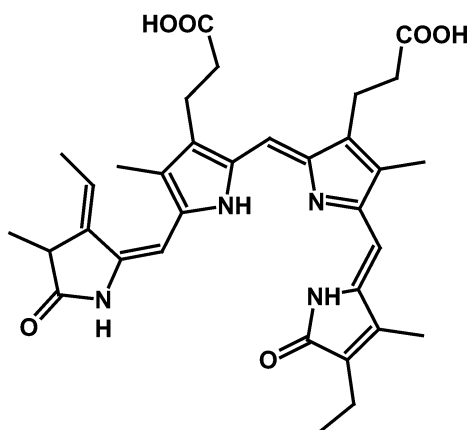

Fig. 7 Structure of phycocyanobilin.

Monascus

Red rice has been consumed for centuries in the Far East, where it has been used as a colorant, a flavor, and a preservative, in particular for meat. Red rice is rice that has been fermented with fungi of the genus *Monascus*. It is most often powdered and sold as such. However, the pigments may also be extracted. At least six pigments are known to be produced by *Monascus*: the yellow monascin and ankaflavin, the orange monascorubrin and rubropunctatin, and the red monascorubramine and rubropunctamine (Fig. 8). The levels of yellow, orange, and red pigments are controlled by a number of factors, e.g., pH, specific strain, and nitrogen source [10]. The pigments are soluble in ethanol but poorly soluble in water. However, they react with amines, including amino acids, to produce water-soluble derivatives. The pigments' high affinity for amino acids make them ideal for coloring meat (instead of nitrite-curing) and surimi. Both red and yellow monascus preparations are commercially available. Monascus is neither allowed in the EU nor the USA as a food colorant.
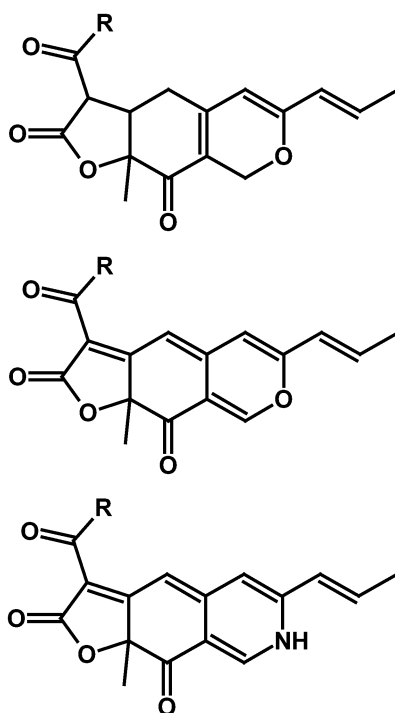
Fig. 8 Structure of monascus pigments. Top: Monascin (R = pentyl) and ankaflavin (R = heptyl). Middle: Rubropunctatin (R = pentyl) and monascorubin (R = heptyl). Bottom: Rubropunctamine (R = pentyl) and monascorubramine (R = heptyl).

Gardenia

Cape jasmine or gardenia (*Gardenia angusta*—often referred to as *G. jasminoides* though this is not the accepted name) is native to Southeast Asia. Its fruits contain the yellow water-soluble carotenoid crocin (Fig. 1) known from saffron (vide supra). The fruits also contain a series of iridoids, of which geniposide (Fig. 9) is the most abundant. The colorless iridoids can be converted into pigments of a variety of colors of which blue and red pigments are the most important. The first step in making the pigments is preparing the aglycone genipin (Fig. 9) by employing a β-glucosidase. The next step involves reacting the aglycone with amines, amino acids, or protein hydrolysates. This leads to formation of the pigments, which are water-soluble polymeric substances. Blue, green, yellow, and red gardenia are commercially available. Yellow gardenia is crocin, and blue and red gardenia are the polymeric iridoids; green gardenia is made by mixing yellow and blue gardenia. None of the gardenia colorants are allowed in the EU or the USA.
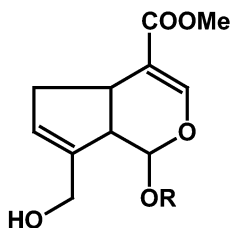

Fig. 9 Structures of geniposide (R = glucose) and genipin (R = H).

Stability

Pigments are no different from other compounds in that heat, light, and oxygen are detrimental to their stability. Actually, pigments are rather unstable because they strongly absorb light and are highly unsaturated molecules. However, there are large differences in stability. Vegetable carbon and caramel are very stable pigments, withstanding heat, light, and oxygen very well. Carminic acid and carmine are also very stable pigments, though not as stable as vegetable carbon and caramel. At the other end of the stability scale, turmeric is rapidly bleached by light and beetroot pigments turn brownish even under mild heating conditions. Even within a class of pigments, large differences in stability are observed. Thus, lycopene is much more unstable than β-carotene.

Knowing the limitations of each pigment means that a specific pigment can be avoided for certain applications, in which the conditions are unfavorable for the pigment, and that alternatives can be sought, or that attempts can be made to increase the stability of the pigments by formulation.

Formulation

Formulation is the process in which the extracted colorant (e.g., an oleoresin) is mixed with other components. Formulating colorants serves a number of purposes. The simplest formulation is to mix two or more colorants to get a different shade. This may be with the purpose of getting a completely different color as in mixing yellow and blue gardenia (vide supra), or to obtain just the right shade by mixing the orange paprika with the more yellow carotene in different proportions. Another reason for mixing colorants is to obtain the same color shade as an existing color (e.g., to replace a synthetic or nature-identical colorant with natural colors or to obtain a more stable or cheaper solution than existing colors), an example of which is blends of annatto and turmeric to replace β-carotene in margarine.

Colorants may also be formulated to increase the range of applications. Apart from crocin, the available yellow pigments are lipid-soluble carotenoids, which cannot be used directly in water-based systems like soft drinks. By emulsifying the carotenoid-containing oleoresin, a dispersion of carotenoid in water can be obtained. This dispersion will be opaque unless a microemulsion is prepared.

Formulation of colorants may also be done to enhance handling of them. Thus, many oleoresins are highly viscous liquids or semi-solids, but by diluting them with vegetable oil a more easily handled colorant of lower strength is obtained (this may also ease dosing). In some applications, a powder is preferred over a liquid. It is possible to spray-dry many colorants using maltodextrin as a carrier to get a powder that can easily be dissolved or dispersed in water. Vegetable carbon is a very dusty powder, for which reason it is often sold as a sugar syrup suspension. The suspension is very viscous, making it hard to pump. By coating the carbon particles with hydrocolloids and suspending them in water, a less viscous product is obtained.

Increased stability is also an important reason for formulating colorants. Often, pigment formulations are added antioxidants to inhibit color fading. The most often used antioxidants are α-tocopherol and ascorbic acid. The pigments may also be coated (microencapsulated) with hydrocolloids such as gelatin, gum arabic, pectin, and others which, besides making oil-soluble colors dispersible in water, create a physical barrier around the pigment, protecting it from degradation. Light stability of microencapsulated turmeric is manyfold increased compared to the nonencapsulated pigment.

Another way of formulating colorants is through lake formation. Lake formation is the process of precipitating soluble pigments with aluminum cations in the presence or absence of calcium cations. Being essentially alumina, lakes are soluble at low and high pH. Lake formation is widely used for synthetic dyes, but lakes of natural colorants are not very common. Carmine is a lake of carminic acid (vide supra), and recently an anthocyanin lake, which is blue, was prepared [11]. Anthocyanin in its blue form (around neutral pH) is usually quite unstable (vide supra), but lake formation confers a high degree of stabilization.

Future prospects

Development of "new" food colorants can be divided into two categories: those covered by existing legislation and those that are not. Colorants covered by existing legislation are not really new, but encompass subjects such as new sources of known pigments (e.g., a newly discovered fruit rich in carotenoids), new ways of formulating existing pigments (e.g., blue anthocyanin), and improvement of existing sources (e.g., by breeding). Breeding and selection of high-yield strains will most likely only lead to minor improvements. Major improvements could be achieved by genetic manipulation, but this is not allowed in the EU and the naturalness of the colorant would be (partly) lost.

Production of colors by fermentation has a number of advantages: cheaper production, possibly easier extraction, higher yields, no lack of raw materials, and no seasonal variations. Fermented colors are already used today: *D. salina*, *B. trispora*, spirulina, and monascus. It is not unlikely that new, fermented colors such as lycopene from *B. trispora* will become allowed in the near future. A giant leap forward in color production could be achieved by combining genetic manipulation and fermentation. Microorganisms could be made to produce colorants in high yield by inserting genes coding for the colorant—even colorants not naturally produced by microorganisms (e.g., turmeric) could be made in this way. Colorants made in this way will probably face some hurdles, at least in Europe where genetically modified food is generally viewed with a large degree of skepticism by the consumers. Finally, new colorants would have to be approved by the authorities, which is very costly because of the various toxicological studies needed to confirm the safety of a new food additive.

The above gives a fairly compressed overview of the most important natural food colorants. The reader is referred to a number of excellent textbooks for more detailed information on plant pigments and food colorants [12–16].

ACKNOWLEDGMENT

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