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Food colorants: their past, present, and future

Tom Coultate*

*Leighton Buzzard, Bedfordshire, LU7 2RW; *e-mail: coultate.tom@gmail.com*

Richard S. Blackburn

School of Design, University of Leeds, Leeds, LS2 9JT

Abstract

Whether we are purchasing fresh vegetables from a market stall, ready meals from the supermarket, eating at home or in five-star restaurant, we use colour to tell us what to expect, in terms of taste, nutrition and safety. This review considers the techniques that have, over the years, been employed to modify the colour of our food, and the interactions of these techniques with questions of safety and nutrition. The demand for brightly coloured food resulted in the incorporation of some questionable inorganic and organic chemistry being used in our food. A limited number of synthetic dyes are still used in our food today, but health concerns and the general consumer-driven demand to move to "natural" colorants has brought about a shift-change in the way we colour food. On supermarket shelves the proliferation of products with labels that make a virtue of "No artificial colours" suggests that the future of the azo dyes and their various chemical cousins is strictly limited. Nature makes an abundance of colours and many of these are extracted and used as natural food colorants, however, they are subject to application limitations and stability problems. Significant research is ongoing by academia and industry into methods to stabilise and expand the application possibilities for the various approved natural food colorants, but most developments that the food colour manufacturers boast about nowadays are enhanced vehicles for delivering established natural pigments into food products.

Keywords: food dyes; food colorants; natural; artificial; Southampton six.

Introduction

Box 1
Coleslaw, active ingredients: ethanoic acid, α-D-glucopyranosyl-(1,2)-α-D-fructofuranose, *p*-hydroxybenzyl and indoylmethyl glucosinolates, *S*-propenyl and other *S*-alkyl cysteine sulfoxides, β-carotene, (and other carotenoids, E160), phosphatidylcholine.

For some years now the public have been pressing for more "natural" consumer products – pressure that is most frequently applied to the industry that provides our food and drink. Unfortunately no two consumers will ever agree on what they precisely mean by "natural", or "unnatural". For most consumers the key to the assignment of these adjectives is the word "chemical", associated with ingredients identified in chemical language or the dietary equivalent of the "mark of Cain", a three digit number preceded by the letter "E". One would be very surprised if a supermarket chain would ever succeed in selling coleslaw if they included the details shown in *Box 1* in the label.

Of all the different parameters by which the supposed healthiness of a food item is judged as we walk around the supermarket, "colour" is top of the list. Very few consumers are sufficiently well versed in nutrition to make valid appraisals of the nutritional data on the labels of their purchases, even if supermarket trollies were fitted with computers. We can rarely expect to sample the taste of food before we buy it, so that, other than the reputation or our past experience of the particular stall, shop or manufacturer, we are forced to rely on appearance as a guide to quality. If we are choosing raw fruit and vegetables then bright colours from anywhere in the spectrum are a recommendation in terms of freshness, vitamin content, and the supposed antioxidant benefits of many plant pigments. However, when their children joyfully reach the confectionery aisle, adults' attitudes change: *lurid* = *chemicals* = *"NO!"*

The Past

Box 2
"... for nothing is more common than to green pickles in a brass pan for the sake of having them a good green, when at the same time they will green as well by heating the liquor without the help of brass, or verdegrease [i.e. verdigris, mixed copper salts of acetate, carbonate, etc.] *of any kind, for it is a poison to a great degree…"* [1].

With due allowance for the changing components of our diet, these attitudes have changed little in the last 200 years or so. It was inevitable that the importance of colour of food would begin to rise in the 19th century as food preservation and processing started to move from the domestic kitchen, humble cottage, or stately mansion, to the world of tradesmen and manufacturers. The result was a commercial, as well as purely aesthetic, incentive to maintain the colours of processed and preserved food products as close as possible to the natural colours of their raw materials. The inevitable destruction of the natural, biological, pigments by the early techniques of food preservation had the obvious consequence and food suppliers began to enhance the appeal of their offerings with anything they could lay their hands on, notably the pigments used by the painters, of portraits and front doors. The potential hazards of "unnatural" colourings were not unnoticed. As early as 1782, in her book *'The Experienced English Housekeeper',* Elizabeth Raffald, an 18th century forerunner of Mrs Beeton, had drawn attention to the use of copper salts for enhancing the green colour of pickled vegetables, *Box 2* [1]. The chemistry of this effect is now the basis of the manufacture of sodium copper chlorophyllin (described below).

In 1820, Frederick Accum published his impressively titled, book "*Treatise on the Adulteration of Food and Culinary Poisons, Exhibiting the Fraudulent Sophistications of Bread, Beer, Wine, Spirituous Liquors, Tea, Coffee, Cream, Confectionery, Vinegar, Mustard, Pepper, Cheese, Olive Oil, Pickles, and Other Articles Employed in Domestic Economy, and Methods of Detecting Them*" [2]; this book enjoyed widespread success, with four editions published in two years. The growing public awareness of the issue that Accum initiated was reflected by in local newspaper reports such as the one in *Box 3* [3].

Box 3
"One pennyworth of red balls, called we believe, Nelson or Waterloo Balls, – there were no such fine names in our lick-finger days, – yielded on analysis 30 grains of an indissoluble matter resembling red-lead, which on being fused with the blow-pipe produced 24 grains of lead in a metallic state…" [3].

In 1850, the Chancellor of the Exchequer told Parliament that the adulteration of coffee with chicory could not be detected. Dr Arthur Hassall, a London physician, successfully contradicted this by simply identifying chicory fragments microscopically. With his assistant, Dr Henry Letheby, he went on to complete what was, for its time, a massive survey of food and drink adulteration [4]. Supported by the newly created Analytical Sanitary Commission, some 2500 samples were subjected to chemical analysis. Alum in bread, iron, lead and mercury compounds in cayenne pepper, copper salts in bottled fruits and pickles, Venetian red (iron oxide, Fe_2O_3) in sauces, potted meats and fish were all detected. Perhaps the most appalling of these results were those for confectionery, shown in *Box 4*. These results, published in the *Lancet*, created a considerable stir – even getting the mention in Reverend Charles Kingsley's classic "*The Water Babies*" [5] (*Box 5*).

Box 4
Of 100 samples of sweets:

59	contained lead chromate (yellow)
15	contained artificial ultramarine (blue), approx.: $Na_{6-10}Al_6Si_6O_{24}S_{2-4}$
12	contained red lead, *i.e.* lead oxide
11	contained gamboge (yellow; *see below*)
11	contained Prussian or Antwerp Blue *i.e.* crude ferric ferrocyanide
10	contained Brunswick Green, *i.e.* copper oxychloride
9	contained copper arsenite (green)
6	contained vermillion (red), *i.e.* mercuric sulphide

Box 5
"… as fast as they hide away the old trash, foolish and wicked people make fresh trash full of lime and poisonous paints, and actually go and steal receipts out of old Madame Science's big book to invent poisons for little children, and sell them at wakes and fairs and tuck-shops. Very well. Let them go on. Dr. Letheby and Dr. Hassall cannot catch them, though they are setting traps for them all day long..." [5]

Our legislators were not totally inactive in this field. Around the middle of the 19th century the Excise Department, the UK Government's tax raising arm, employed some sixty or so analytical chemists; their main activity was actually the examination of dutiable imported goods, particularly wine, spirits, tea and coffee, so that their primary objective was really the health of Exchequer rather than the health of the people. To a limited degree some commercial food manufacturers did respond to the publicity but not necessarily with the expected result. Recalling Elizabeth Raffald's 1782 warning against the practice of using copper salts when pickling green vegetables, in 1855 Crosse & Blackwell, major manufactures of pickles and sauces, bowed to public pressure and introduced enamelled vessels for boiling their vegetables. Unfortunately, for a time sales declined as it took the public a while to get used to the "healthy" brown colour [6]. Nowadays, with advances in food technology, pickle manufacturers have to include caramel to maintain the desirable brown colour! A similar situation arose many years later in the USA when, in the 1990s abortive attempts were made to market colourless, *i.e.* without added caramel, versions of traditional cola and beer [7].

In the UK a series of Acts of Parliament were enacted in the period up to the end of World War II that attempted to prevent food adulteration, with the first legislation specifically directed at food colours being passed in 1925. These regulations prohibited compounds of antimony, arsenic, cadmium, chromium, copper, lead, mercury & zinc, plus gamboge, but ignored completely the potential harm from the newer synthetic, so-called coal-tar, dyes. Gamboge is the powdered gum of certain Far-Eastern trees that is the traditional dye used for the saffron robes of Buddhist monks and also a powerful laxative! The readers of this journal will be familiar with the development of these dyes and their application in the textile industry, but by the beginning of the 20th century many of the 80 or so different textile dyestuffs had been borrowed by the food manufactures, regardless of the fact that little or nothing was known of their toxicity. The 1925 legislation was fundamentally flawed in that it was based on a "forbidden" list that could be easily sidestepped. It was not until the 1955 legislation finally introduced the much more secure concept of a "permitted" list, *i.e.* only substances *on* the list could be legally used.

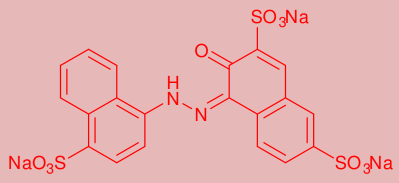
Readers interested in the parallel version of these, and subsequent, events, that took place in the USA, are recommended to read Adam Burrows' review [7].

The present – synthetic colorants

In the context of this article "the present" actually describes the 1960s to the present day – a period marked by much greater public interest in the entire food additives issue. Depending on one's point of view, Britain's membership of the EEC (now the EU) simplified, or complicated, food legislation questions. In 1957, thirty-two synthetic dyestuffs had been permitted for food use by UK legislation, but by 1973 nineteen of these had been removed from the list, with the addition of three. In 1979, just eleven of these were granted E numbers, permitting their regulated use in the EC. A few others were also permitted for UK use only, the rest of the rest Europe having no use for them [8,9]. Notable amongst these was Brown FK (E154; C. I. Food Brown 1), a mixture of six synthetic azo dyes, used in the past to enhance the brown colour of smoked fish such as haddock and kippers The "FK" looks as if it is one of the suffixes common in dyestuff terminology (*e.g.* in the name Ponceau 4R), but in fact is an abbreviation of "For Kippers". The safety of food colorants in the EU is currently evaluated by the European Food Safety Authority (EFSA) under Commission Regulation No 1129/2011 [10]. This is binding to all member countries of the EU.

In the United States, the 1960 Color Additives Amendment was the major legislation from this period that determining what colorants could be added to food [11]. Today the United States Food and Drug Administration (FDA) permits two major classifications of food colorants: certified (generally synthetic colorants) [12] and exempt from certification [13], which generally includes natural pigments, although there is no specific definition of what "natural" means [14].

The amounts of dyestuffs used are generally restricted, sometimes by the term *"quantum satis", i.e. "*no more than necessary", and none may be used in most fresh foods including, fresh fruit and vegetables, infant foods and raw meat and fish. The possession of an E number or FD&C number in no way implies that the use of a particular dyestuff in food is unregulated. For example amaranth (E123, C. I. Food Red 9; **1**) may only be used to colour aperitifs and fish roe, and erythrosine (E127, FD&C Red No. 3; C. I. Food Red 14; **2**) is restricted to maraschino cherries. Erythrosine is also the only approved synthetic colorant that can be directly printed onto eggs to give sufficiently water fast codes (used for communication of data such as best before date). In 1990, the FDA instituted a partial ban on erythrosine, with approval only given for use in food applications where the sodium iodide content of the products is below 0.1%.



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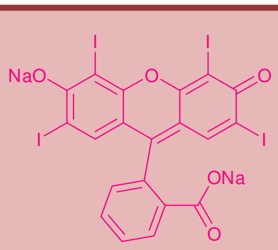
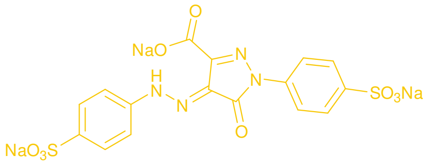
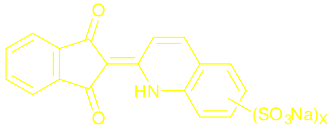
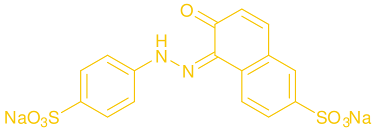
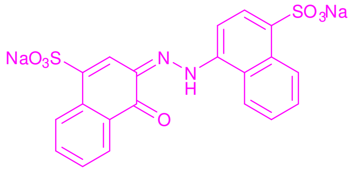
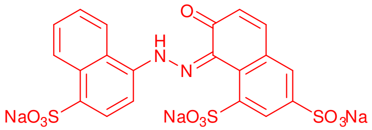
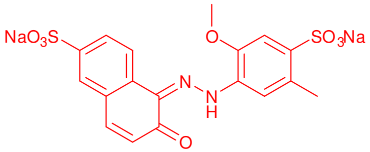


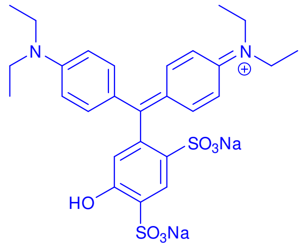
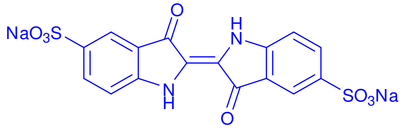
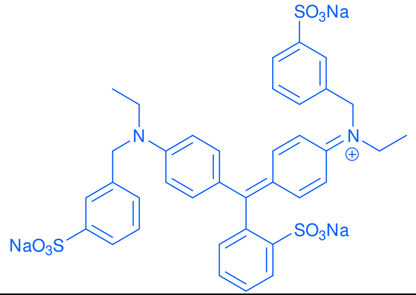
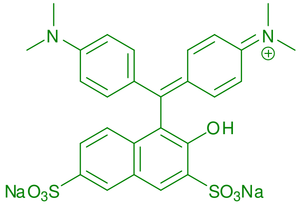
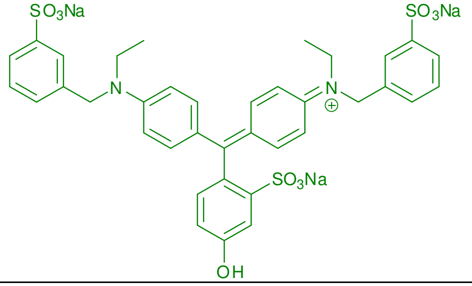
2

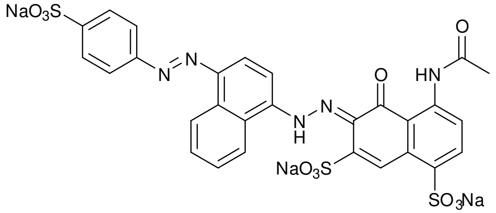
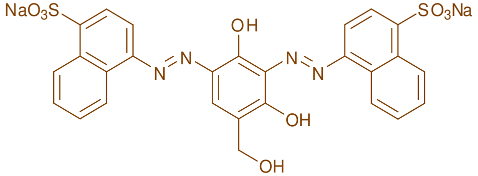
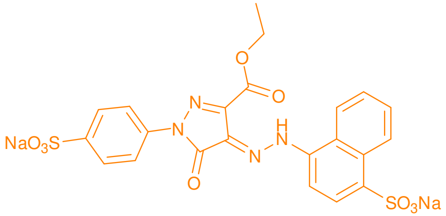
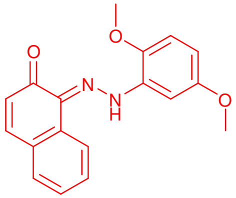
Until the 1990s most publicly expressed concern about the use of synthetic food colours had focussed on their potential carcinogenicity and almost invariably the withdrawal of permitted status had followed the results of test with laboratory animals. In recent decades attention has moved on to the possibility that some food colours might invoke allergic responses or other manifestations of intolerance, particularly in children. The yellow colour Tartrazine (**3**) has been the principal suspect and there is some evidence that it may invoke symptoms such as eczema or asthma in 0.01% to 0.1% of children. Surveys conducted in Britain in the 1990s revealed that as many as one in five people believed themselves to be allergic to certain foods, by their personal understanding of the word "allergic". However in one keynote investigation [15] proper diagnostic tests were applied to these individuals but only one in ten showed a measurable reaction to the food in question, *i.e.* 2% or less of the total adult population. Higher proportions of infants, up to 8%, may show a genuine food allergy.

Subsequently the possibility arose that consumption of Tartrazine and five other food colours, Quinoline Yellow WS (**4**), Sunset Yellow FCF (**5**), Carmoisine (**6**), Ponceau 4R (**7**), Allura Red AC (**8**) and was associated with higher incidence of hyperactivity (correctly termed Attention Deficit Hyperactivity Disorder; ADHD) in susceptible children. In 2007, studies conducted at the University of Southampton were published that provided some support for this suggestion [16]. The studies were unavoidably hampered by the difficulties, ethical as well as technical, of conducting double blind trials with relatively small numbers of young children and where the judgements of the effects were largely in hands of their parents. In spite of the support of the UK Food Standards Agency (FSA), the Southampton results failed to convince EFSA and the regulatory position of the suspect colours remained unchanged. However, the officials of FSA rose to the challenge and in 2010 introduced a seemingly innocuous regulation that required any food products sold in the UK that contained any of the six "Southampton colours" to include on the label "*May have an effect on activity and attention in children*". Manufacturers who removed these colours from their products were also encouraged to add their names to a freely accessible published list.

Table 1. Synthetic food colorants currently permitted for us by EFSA [10] and FDA [12].

Commercial name	E-number	FD&C	Permitted in EU or US	C.I. Name	Structure
Tartrazine	E102	Yellow No. 5	Both	C. I. Food Yellow 4	 3
Quinoline Yellow WS	E104	None	EU only	C. I. Food Yellow 13	 4
Sunset Yellow FCF	E110	Yellow No. 6	Both	C. I. Food Yellow 3	 5
Carmoisine	E122	None	EU only	C. I. Food Red 3	 6
Ponceau 4R	E124	None	EU only	C. I. Food Red 7	 7
Allura Red AC	E129	Red No. 40	Both	C. I. Food Red 17	 8

Patent Blue V	E131	None	EU only	C. I. Food Blue 5	 **9**
Indigo Carmine	E132	Blue No. 2	Both	C. I. Food Blue 1	 **10**
Brilliant Blue FCF	E133	Blue No. 1	Both	C. I. Food Blue 2	 **11**
Green S	E142	None	EU only	C. I. Acid Green 50	 **12**
Fast Green FCF	E143	Green No. 3	US only	C. I. Food Green 3	 **13**

Brilliant Black BN	E151	None	EU only	C.I. Food Black 1	 **14**
Brown HT	E155	None	EU only	C. I. Food Brown 3	 **15**
Orange B	None	Orange B	US only[a]	C.I. Acid Orange 137	 **16**
Citrus Red No. 2	None	Citrus Red No. 2	US only[b]	C.I. Solvent Red 80	 **17**

[a] Surfaces and casings of frankfurters or sausages, ≤ 150 ppm by wt. of finished product; [b] Skins of mature oranges, ≤ 2 ppm by wt. of whole fruit.

This regulation was promptly adopted throughout the EU. The combined effect of these two measures was the rapid disappearance of the six colours from food products, particularly confectionery and soft drinks, in Britain and the rest of Europe. (It remains to be seen whether there has since been a detectable improvement in the attentiveness *etc.* of the EU's children.) It should be noted that all six "Southampton colours" are still permitted to be used in the EU [10] (Table 1); in the US, an FDA hearing on certified food colorants [17] concluded that "*a causal relationship between exposure to color additives and hyperactivity in children in the general population has not been established*". Tartrazine, Sunset Yellow FCF, and Allura Red AC are all still permitted by the FDA and widely used in food and drink products [12].

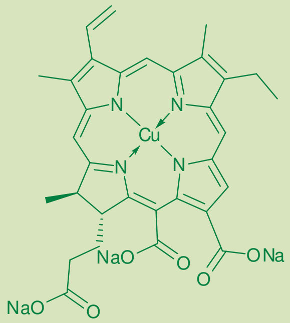
Changes in Europe were part of a general trend that had been developing for many years. The original 1979 list of E-numbered additives already included 11 colours that most consumers would describe as "natural" rather than "chemical" or "synthetic". The degree of processing or modification that is compatible with the word "natural" is of course an issue for philosophers and the advertising industry, not the rest of us. In addition to the "Southampton six" and erythrosine discussed above, EFSA and FDA still allow the use of several other synthetic colorants, including Patent Blue V (**9**), Indigo Carmine (**10**) and Brilliant Blue FCF (**11**); in addition, Green S (**12**), Brilliant Black BN (**14**), and Brown HT (**15**) are allowed in the EU (but banned in US), and Fast Green FCF (**13**), Orange B (**16**), and Citrus Red No. 2 (**17**) are allowed in the US (but banned in EU) (Table 1).

The present – natural food colorants

Green

The green colour that had been the subject of Elizabeth Raffald's concern illustrates some of the issues that have arisen as natural colorants have begun to replace their synthetic predecessors. This is of course chlorophyll (E140), the plant pigment responsible for capturing the energy of visible light in the fundamental process of photosynthesis [18]. Chlorophyll a (**18**) is the dominant form of the green pigment found in most higher plants, including the fruit and vegetables we eat – the green colour arises from the pyrrole-based ring structure around a central Mg^{2+} ion and the extended phytol side chain facilitates the close association with the other lipids of chloroplast membranes. For use as a food colour chlorophyll is extracted from a variety of plants, including grasses, alfalfa, spinach and stinging nettles. It is quite stable in the protective environment of the membranes in chloroplasts in the cells of plant leaves, however, when subjected to prolonged heating in even mildly acidic environments (*e.g.* school dinner cabbage) it is converted to a greyish brown derivative, pheophytin, wherein Mg^{2+} is replaced by two protons [19]; pheophytin also gives uncoloured canned peas their colour. Cooks have traditionally preserved the colour of leafy green vegetables by adding a pinch of sodium bicarbonate, to the saucepan to keep the pH above 7. Sadly this ruins the texture and destroys the ascorbic acid (vitamin C). Fortunately modern vegetable processing techniques such as HTST (high temperature short time) can often minimize the formation of pheophytin. However, other food products have to rely on synthetic colours, *e.g.* the mixture of Tartrazine and Green S, which will always be associated with canned peas. The naturally-derived alternative to these is sodium copper chlorophyllin (E141; C. I. Natural Green 3; **19**), a bluish green colorant synthesised from chlorophyll with Mg^{2+} replaced by Cu^{2+} and the ester link cleaved to remove the phytol side chain – this colorant is used in a broad range of foods in the EU [20]. Sodium copper chlorophyllin is much more stable to heat and low pH; it is best described as only "fairly" soluble in water so that it does not penetrate well through the outer layers of vegetables such as peas. It seems unlikely that its range of use will ever extend far beyond the jellied confectionery where it is most frequently encountered today and its status as "natural" does not appear to have been questioned. With due respect to Elizabeth Raffald's concerns, it should be pointed out the contribution of copper chlorophyllin to the copper intake of consumers is a tiny fraction of the current figure of 1.4 mg per day (males aged 19-64) recommended by qualified nutritionists [21]. The FDA permits the use of sodium copper chlorophyllin in citrus-based dry beverage mixes only at a limit of ≤ 0.2% by wt. of the dry mix [13].

18



19

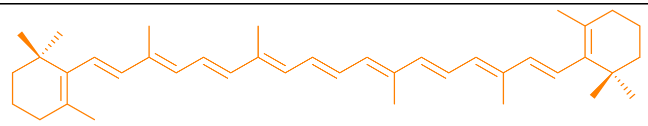
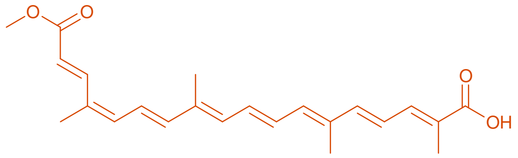
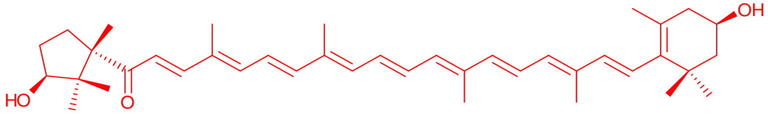
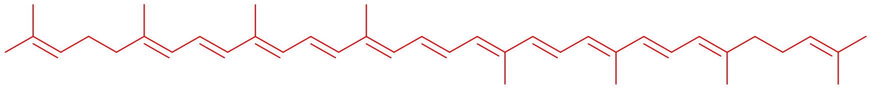
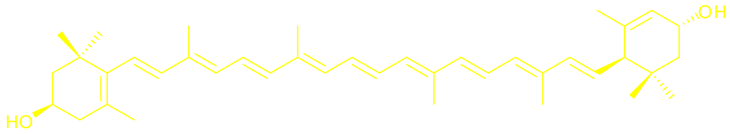
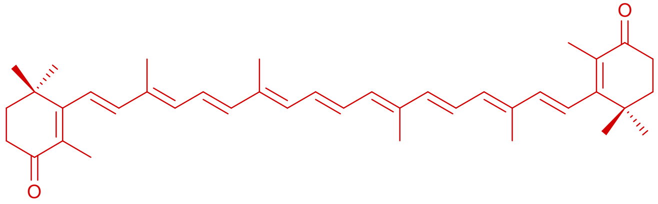
Yellow and orange

The carotenoids are responsible for the yellow, orange, and some red, colours of many fruits and vegetables, and more than 700 carotenoid pigments have been identified in nature [19]. These tetraterpenoid pigments are characterized by a long chain of conjugated double bonds, and the π-electrons delocalised along the polyene chain are responsible for their absorbance in the visible spectrum [22], however, this electron-rich, highly saturated system does render the chromophore vulnerable to oxidation during processing and storage [23]. The structures of several important naturally occurring carotenoids are shown in Table 2, most of which are permitted food additives in EU [10] or as in the US as colorants exempt from certification in the form of extracts [13]. Several carotenoids are commercially synthesised for use as food colorants on an industrial scale, by both purely chemical routes and also by fermentation of appropriate yeasts or algae, these are β-carotene (**20**), lycopene (**23**), and canthaxanthin (**25**) [24]. Capsanthin (**22**) is extracted from the fruits of *Capsicum annuum* (paprika, bell, jalapeño, cayenne peppers) or *Capsicum frutescens* (piri piri, tabasco peppers) by percolation with a variety of solvents, primarily hexane, which are removed prior to use [25]. In the EU, capsanthin (E160c) is the listed food colorant, whereas in the US paprika oleoresin is listed as the colour additive; oleoresins are semi-solid extracts composed of a resin in solution in an essential and/or fatty oil, obtained by evaporation of the solvent(s) used for their production [26].

Food carotenoids are actually quite stable to food processing temperatures. In the environment of plant tissues they are well protected from the bleaching action of atmospheric oxygen by the natural antioxidants present, notably ascorbic acid, vitamin C, and tocopherols, vitamin E. Carotenoids are widely used as food colorants, especially in dairy products (ice cream and desserts) and baked goods (cakes, *etc.*) [27,28]. In all of these products carotenoids can be delivered dissolved in the fatty components of the recipe. They are not required in spreads such as margarine as the addition of a small proportion of unhydrogenated palm oil, naturally very rich in β-carotene, late in the manufacture of these products, will give the desired result. These products have to be fortified with vitamin A to bring them up to the same vitamin levels as butter. In aqueous environments, such as yoghurt, confectionery and cloudy soft drinks, very small crystals of carotenoids can be added in a carrier such as glycerol [28].

One particularly interesting carotenoid colorant is the orange-red *cis*-bixin (**21**). At first sight this appears unqualified to be a carotenoid/tetraterpenoid, being deficient in the carbon atom department, however being naturally synthesised in the plant from tetraterpenoid precursors, it is officially an *apocarotenoid*. It is a major constituent of the traditional colouring matter annatto, the powdered resinous coating of the seeds of a widely grown tropical shrub achiote (*Bixa orellana*), traditionally used as the colouring in red cheeses such as Red Leicester due to its lipid solubility. Its modest solubility in water is attributed to its carboxylic acid group, but saponification of the methyl ester at the other end of the molecule produces the water soluble pigment norbixin [11]. Bixin is specifically prohibited from spices such as curry powder where its bright colour could give a false impression of quality. Heat treatment of *cis*-bixin can result in the formation of the *trans* isomer, which is more lipid soluble and has a colour shift to more yellow tones [29].

Table 2. Carotenoids widely used in Europe [10] for food coloration and related details of food colorants exempt from certification by FDA [13].

Common name	E-number	Colour Index name	FDA	Structure
β-carotene	E160a	C. I. Food Orange 5	Natural and synthetic permitted	 20
bixin	E160b	C. I. Natural Orange 4	Annatto extract permitted	 21
capsanthin	E160c	C. I. Natural Red 34	Paprika and paprika oleoresin permitted	 22
lycopene	E160d	C. I. Natural Yellow 27	Lycopene, tomato extract or concentrate permitted	 23
lutein	E161b	--	*Tagetes* (Aztec marigold) meal and extract permitted for chicken feed only	 24
canthaxanthin	E161g	C. I. Food Orange 8	Canthaxanthin permitted for general use ≤ 66 mg/kg of food and for salmonid fish feed ≤ 80 mg/kg	 25
crocetin	[a]	--	Saffron permitted	 26

[a] Crocetin is the main colorant in saffron; saffron is often referred to as E164 but this designation has no official basis and is not recognised by the FSA or EFSA; legislators currently treat saffron as a food flavouring ingredient that just happens to provide colour. In the US, the FDA consider saffron as a food colorant.

The spice saffron is essentially the dried stigmas of the autumn crocus (150,000 per gram of spice) and contains another apocarotenoid, crocetin (**26**), which also owes its modest water solubility to carboxylic acid groups. Crocin is the diglycoside of crocetin, where the carboxylic acid moieties are each esterified with a gentiobiose disaccharide group, and is also found in saffron, being highly water soluble as a result of the sugar substituents [11].

Suffice to say that most carotenoids, or apocarotenoids, have never had significant issues of toxicity raised against them. Although widely used in the USA & Asia, canthaxanthin (**25**) is not permitted in EU except for "Saucisses de Strasbourg" – some concerns have been raised in Europe over its safety following its misuse as a (sun)tanning aid. In fact certain carotenoids have well established status as essential nutrients – dietary β-carotene, and its α- and γ- isomers, is converted in the liver into retinol, vitamin A (Figure 1), notable for its role in vision. Night blindness is the best known, although not the most important, symptom of deficiency. Interestingly, The British government's World War II campaign that suggested that the success of RAF's night fighters against the Luftwaffe was down to the pilots' carrot consumption was not, as is often assumed, a ploy to divert enemy attention from British radar developments. Actually it was a cunning, and successful, plan to encourage children to eat more carrots to compensate for the shortages of vitamin A rich dairy products in their wartime diet [30]. Unfortunately the efficiency of conversion of dietary β-carotene to retinol is not high as it is poorly absorbed from plant foods, especially when they are eaten raw.

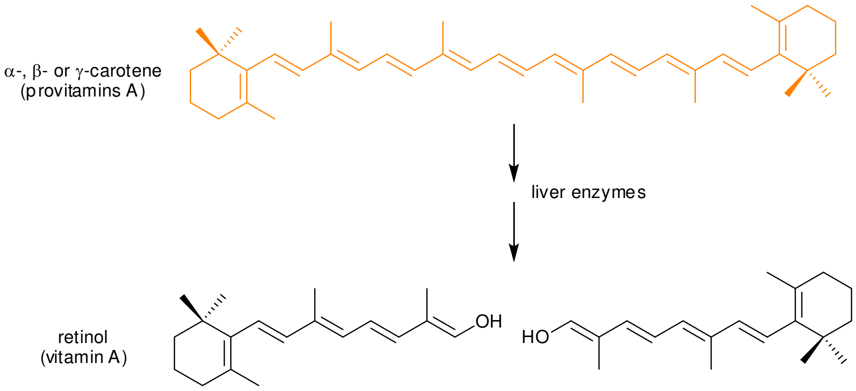
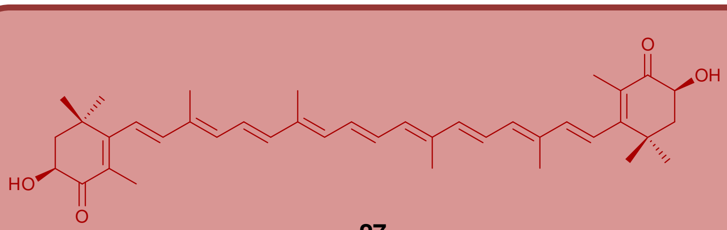


Figure 1. The relationship between vitamin A and α-, β- and γ-carotenes. The carotenoid structure shown here is that of β-carotene.

Lutein (**24**) is a xanthophyll carotenoid that plays an important role in maintaining eye health and protection against sunlight [31]. Humans cannot synthesize xanthophylls and uptake is therefore dependent on the consumption of certain fruits, vegetables or animal products such as eggs [32]. Irreversible blindness linked to age-related macular degeneration (AMD) has been associated with dietary lutein deficiency [33]. Lutein, extracted from the petals of marigolds (*Tagetes erecta*), is widely available as an expensive dietary supplement, but is not widely used as food colorant [34].

Astaxanthin (**27**) is a lipid-soluble xanthophyll pigment and is present in most red-coloured aquatic organisms. Like saffron, astaxanthin is often awarded an unofficial E number (E161j) but it is not used as an additive in human food. Astaxanthin occurs in shellfish & some fish, notably salmon and sea trout. The industrial production of astaxanthin for use in the feed of farmed salmon and trout comes from both synthetic and natural sources (krill and the cultivated freshwater microalga *Haematococcus pluvialis*. Natural astaxanthin is generally recognized as safe (GRAS) by the FDA, permitted in salmonid fish feed ≤ 80 mg per kg of finished feed, but there is little clinical evidence to support its use as an “antioxidant” dietary supplement [35].



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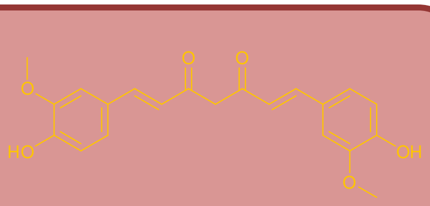

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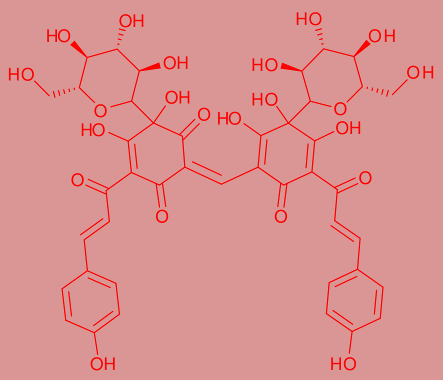

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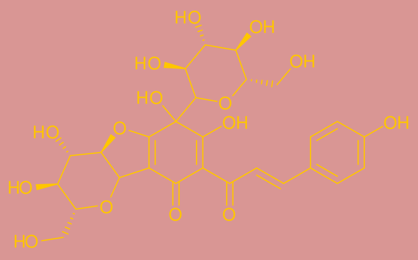

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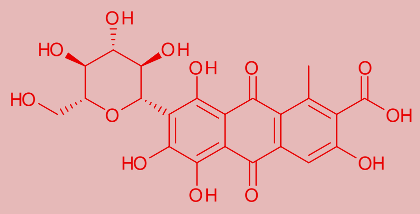
Besides the carotenoids there are two other natural yellow colours with a long history of food use, turmeric and safflower. Turmeric is the dried powdered root of *Curcuma longa*, used for centuries as a spice (especially in Asian cookery where it contributes an earthy, slightly bitter taste as well as its yellow colour) and in Asia as a textile dye. It is the characteristic ingredient of the English pickle known as piccalilli and is an essential component of curry powders. The greenish yellow oil-soluble pigment in turmeric is curcumin (E100i; C. I. Natural Yellow 3; **28**), which is becoming popular in a variety of manufactured foods, not just pickles, but also ice cream and other dessert products. Turmeric and turmeric oleoresin are food colours exempt from certification by the FDA [31]. One of its possible advantages is its reputation for medicinal benefits, which include an as yet unproven role in slowing down the progress of Alzheimer's disease [36]. Amongst its practical disadvantages in food colorant roles are its poor water solubility and its light sensitivity.

Like turmeric, safflower is another colorant with traditional roles in both textiles and food. The thistle-like safflower (*Carthamus tinctorius*) grows readily in arid regions and is cultivated as an oilseed source in India, Mexico and the USA. Its red flower petals contain three water soluble pigments: red-coloured carthamin (**29**) is destroyed by light, oxygen and low pH, but its yellow precursors, safflor yellow A (**30**) and safflor yellow B are more stable, but still tend to fade in bright light; all three contain unusual C-glycoside linkages between the glucose residue and the aglycon component. In spite of their instability, these pigments, in the form of dried flower petals, are traditional colorants in a number of characteristically British soft drinks and are being considered as a natural alternative to quinoline yellow. Like saffron, safflower petals are treated as a food ingredient, rather than additive, which just happens to coloured.

Red and purple

The red and purple natural colours are particularly interesting and offer plenty of scope for applications as colorants. Most red, purple and black fruit owe their natural colour to a group of pigments known as anthocyanins. However, before considering those in detail a brief diversion to a red pigment of animal origin with a long history of use as food colorant is in order. Cochineal (E120) has been an object of commerce throughout recorded history as a dye for textiles – Cortés, writing in 1520, marvelled at hanks of cotton "in all colours" in a Mexican market [37]. As a textile dye, cochineal was applied with an alum mordant and optionally post-mordanted with other metal mordants providing a range of bright crimson colours [38].



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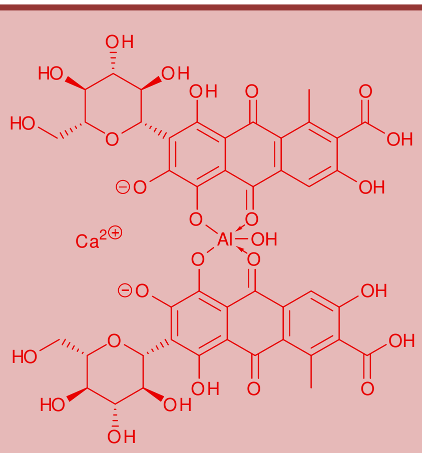

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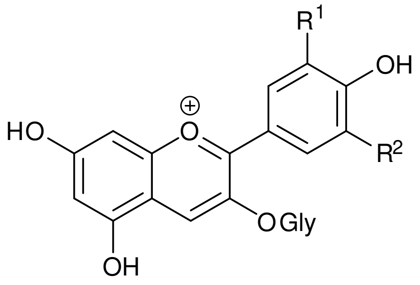
Cochineal is obtained from aphid-like insects (notably *Dactylopius coccus*) that are harvested from cactus plants, notably the prickly pear (*Opuntia ficus-indica*), in various parts of world, particularly Peru, the Canaries and southern Africa. Cochineal's principal colorant is the anthraquinone carminic acid (C. I. Natural Red 4; **31**), which contains an interesting C–C glycosidic bond that is stable to acid hydrolysis; it exists in the insect as the potassium salt [39]. The free form is used and in aqueous solution at pH 4 is yellow-orange, but the primary use in food is as either aluminium or calcium lakes, with colours ranging from magenta red to purple [40], with the most famous being the bright red aluminium lake carmine (C. I. Natural Red 4:1; **32**). Carmine is heat stable and maintains its colour in the pH range (6-8) of food products such as cakes (notably Battenberg) and some confectionery where it has a long history of use, with no safety questions against it. Unfortunately it suffers from two serious drawbacks: firstly it is very expensive; secondly, and even more problematic, is that its insect origins rule it out for Jewish, Muslim and vegan consumers. There would obviously be a case for the chemical synthesis of a "nature identical" version, if only it could be achieved economically. Carmine and cochineal extract are food colours exempt from certification by the FDA [13].

The red, purple and blue colours of fruit, flower petals and some (but not all) vegetables colours are owed to a class of glycosides known as anthocyanins (**33**), which are the largest group of polyphenolic pigments in the plant kingdom, responsible for the red, purple, blue and orange coloration, accumulating solely in the vacuoles of plant cells [41] More than 20 different anthocyanidins (the aglycons) have been identified in nature, all based on the flavan nucleus, but the six different aglycons shown in Table 3 are the most common components found in foods, leading to many anthocyanins, due to the diversity of glycosylation [42]; in total more than 600 anthocyanins have been identified in nature [43], their colors are determined by the number of hydroxyl groups (and degree of methylation) and the nature, number and position of sugar moieties [44]. The sugar component(s) usually attached at the 3-*O*-position may also carry phenolic compounds, notably *p*-coumaric, sinapic, ferulic or caffeic acids [45]. The great variety of actual colours one observes in flower petals or raw fruit is to a large extent the result of complex formation with metal ions and/or other phenolic substances present in the plant tissues. The sugar residues are essential in nature since without their interference the phenolic hydroxyl groups would tend to bind to the surface of enzyme proteins. Their resulting inactivation would have devastating results for the metabolism of plant. On the plus side the phenolic groups are the source of the anthocyanins' astringency, essential to the flavour of red wine. Figure 5 also shows the structures of two examples that illustrate their potential for variety in different plant species. Anthocyanins are widely permitted as natural sources as food/beverage colorants within Europe (E163), Japan, the US, and many other countries [46]. Grape skin extract has been used as a colorant for more than 100 years, first being applied to enhance wine colour [47]. In the US, “Grape-color extract” is obtained as a by-product in processing Concord grapes (*Vitus labruscana*), but its application is limited by the FDA to non-beverage food use [13].

The usefulness of anthocyanins as colorants is principally limited by pH. In acid environments, as typically found in intact fruit or fruit juices, and at ordinary cooking temperatures, even if followed by prolonged room temperature storage, anthocyanins keep their colour; traditionally bottled or canned fruit bear witness to this stability. Modern commercial jam making processes also do little damage although the prolonged high temperatures used by commercial and domestic jam makers in the past were fairly destructive. It is the availability of enzymatically hydrolysed starch syrups that has facilitated the dramatic improvement in colour and flavour of commercial jam in recent decades [48]. Anyone who has poured cream (~pH 6.8) over cooked blackcurrants (juice ~pH 3-4) will be familiar with the colour change that results (Figure 2). American style blueberry muffins also demonstrate a colour change combined with the yellow colour of egg yolk (Figure 3); the brownness of the crust, which splits to reveal the blue green interior, is the result of the combined effects of the neutral pH and the high temperature of baking.

Table 3. Structures and absorption maxima for common anthocyanins.

 **33**	**Anthocyanin**	**R^1**	**R^2**	**$^*\lambda_{max}$**
	pelargonidin	H	H	503
	cyanidin	OH	H	517
	peonidin	OCH_3	H	517
	delphinidin	OH	OH	526
	petunidin	OCH_3	OH	526
	malvidin	OCH_3	OCH_3	529

$^*\lambda_{max}$ values shown are for corresponding 3-*O*-glucoside at pH 3.

Figure 2. Cooked blackcurrants, with juice, mingling on the plate with some fresh cream.

Figure 3. American style blueberry muffins. Initially the muffin batter (~pH 6-7 dictated by the egg proteins, raising agents, *etc.*) has a green shade associated with the blue and purple of the anhydrobase forms (A and A^{-}) of the anthocyanins blended with yellow of the egg yolk. In the oven, the surface temperature is sufficiently high to decompose the anthocyanins and leave the crust the brown shade dominated by the Maillard reaction of the flour and egg proteins with the sugar.

The source of this problem is the complex pattern of ionisation of the anthocyanin chromophore, illustrated for red cabbage anthocyanins in Figure 4, where it is observed that as the pH rises above 3 anthocyanin solutions start to lose their strong red colour as the proportion of the colourless carbinol pseudobase increases. As neutrality is approached the purple and then blue forms of the anhydrobases come to dominate. In aqueous solution of pH <3, the anthocyanin is red and the flavan nucleus exists mainly as the very stable flavylium cation (AH^{+}). Increasing pH leads to kinetic and thermodynamic competition between two reactions. When pH increases, the flavylium form is in equilibrium with the purple/blue quinonoidal base (A) by a deprotonation reaction (pK_{a1} ~ 3.7) and also with the respective anionic quinonoidal base (A^{-}) at high pH pK_{a2} ~ 7) that has a blue colour [42,49-52]. Furthermore, above pH = 2, the flavylium cation is prone to water addition (hydration) at C-2 (pK_{h} 2-3) yielding the colourless carbinol pseudobase (B), which may undergo ring opening to chalcone pseudobases (C) that exist in either *cis*- (C_{cis}) or *trans*- (C_{trans}) form, and both isomers are yellowish [53].

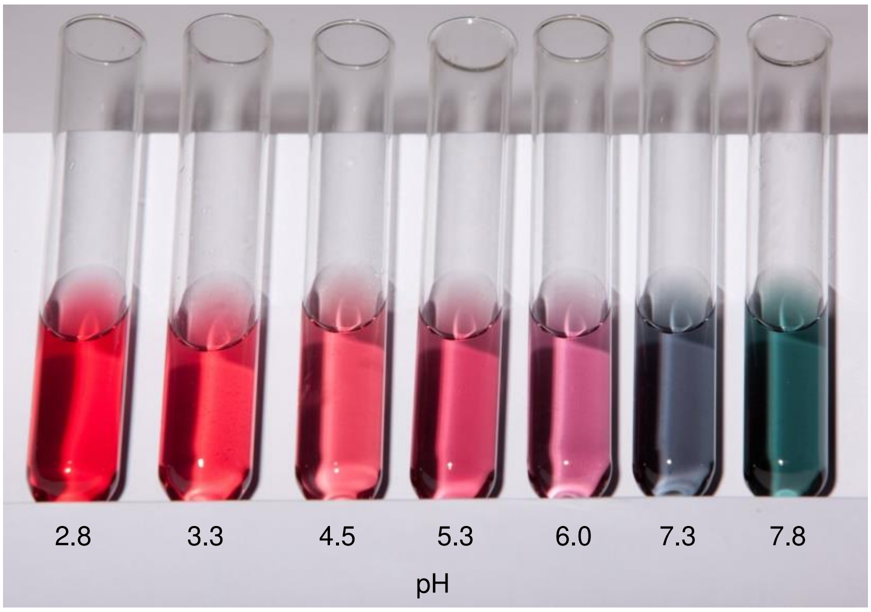


Figure 4. Aqueous solutions of red cabbage anthocyanins in buffers at a range of pH values.

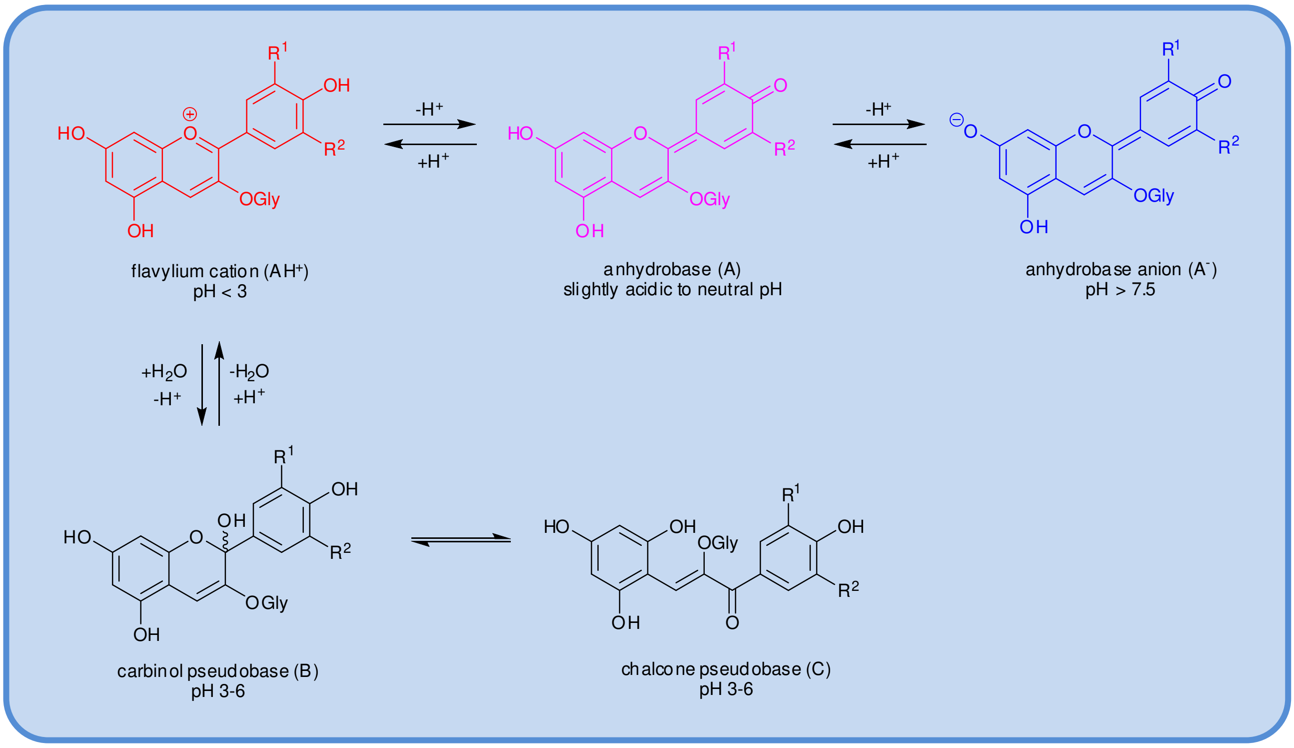


Figure 5. Effect of pH on anthocyanin structure and resultant colour. Not shown here are the less well defined series of irreversible reactions, occurring at high temperatures, leading to the breakdown of the chalcones – the effects of these are visible in the muffin crust in Figure 3.

Co-pigmentation, *i.e.* interactions between polyphenolics, is the phenomenon resulting in anthocyanins appearing bluer, brighter, or more intense in intact plant tissues. It can also help to stabilize anthocyanins against light induced degradation pathways [54]. The characteristic colour of strawberries (*Fragaria spp.*), whose anthocyanins are almost exclusively based on pelargonidin, is attributed to co-pigmentation with other polyphenols. In particular, copigmentation depends upon intermolecular hydrogen bonding between flavonoid catechol moieties and carbonyl groups of anthocyanin anhydrobases [41]. These so-called stacking interactions may also involve self-association (anthocyanin + anthocyanin), self-association of acylated anthocyanins and the formation metal-anthocyanin complexes [51]. Anthocyanin copigmentation is a noncovalent complexation that has been shown to be the main mechanism by which certain colours, particularly blue, violet, and red, are stabilized and modulated in flowers, vegetables, fruit and food products derived from them (*e.g.* wine and jams) [51].

These pH driven changes are not a problem in low pH products such confectionary and preserves, but present a real challenge to manufacturers of dessert products including milk proteins, such as ice-cream and yoghurts. The anthocyanins of black carrots (*Daucus carota* subsp. *sativus* var. *atrorubens*), a vegetable becoming popular in gourmet circles, can provide a partial solution; the pK_a values of its various transitions are shifted a little towards neutrality, increasing the range of food products in which it can be used, making them applicable as neutral pH colorants. It has been demonstrated that red cabbage extracts containing highly acylated anthocyanins can produce blue hues similar to indigo carmine [55]. This increased stability is a result of intramolecular copigmentation effects resulting from acylation of the glycosides with hydroxycinnamic and hydroxybenzoic acids (Figure 6) [56]. Acylation also affords greater heat stability at higher pH values in comparison with non-acylated anthocyanins [57]. In addition to pH, water in the food system affects degradation – the more water is able to interact with the anthocyanin, the lower the stability [58,59].

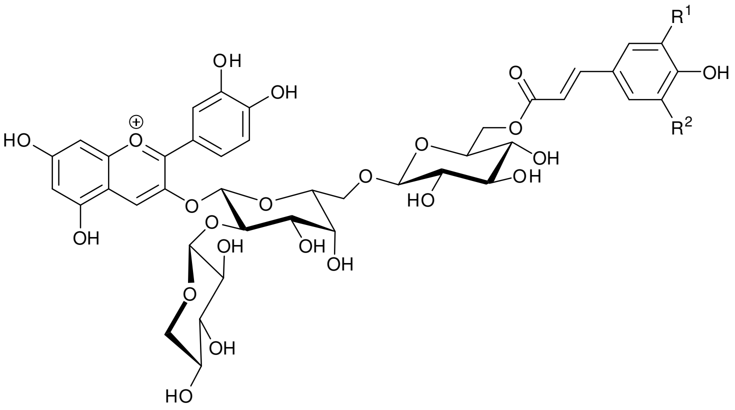
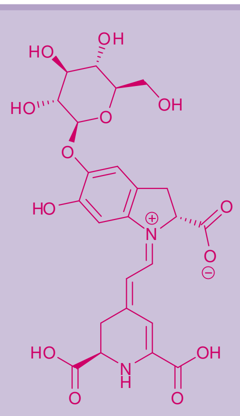


Figure 6. Chemical structures of acylated anthocyanins in black carrots: cyanidin-3-*O*-(2''-*O*-xylosyl-6''-*O*-sinapoylglucosylgalactoside) ($R^1 = R^2 = OCH_3$), cyanidin-3-*O*-(2''-*O*-xylosyl-6''-*O*-feruloylglucosylgalactoside) ($R^1 = H$, $R^2 = OCH_3$), and cyanidin-3-*O*-(2''-*O*-xylosyl-6''-*O*-*p*-coumaroylglucosylgalactoside) ($R^1 = R^2 = H$).

Toxicological data on anthocyanins support the view that these pigments pose no threat to human health [60]. In fact, dietary consumption of anthocyanins has been associated with many beneficial effects, including anti-inflammatory and anti-carcinogenic activities, reduced incidence of cardiovascular disease, control of obesity and diabetes alleviation [61].

Other useful red pigments that are often overlooked are those from beetroot, betalains. Until the chemical structure of these compounds was properly established they were often described as “nitrogenous anthocyanins”, but in fact they are quite unlike anthocyanins being indole rather flavan based. Of the two groups of betalains, it is the dominant purplish-red betacyanins (*e.g.* betanin, E162, **34**) that provide the characteristic colour of beetroot (*Beta vulgaris*) and are of interest as food colorants [48]. In the US, beet juice (as vegetable juice) and beet powder (dehydrated beets) are food colours exempt from FDA certification. The indole ring of the betacyanins normally carries a glucose residue, but in the yellow vulgaxanthins (**35**) the ring is “unravelled”. These indole-derived pigments are unique to the botanical order *Caryophyllales* (also known as *Centrospermae*) none of whose members ever contain anthocyanins, even in their flower petals. Although they bear no obvious relationship to beetroot two other plant genera have also been recognised as sources betacyanin pigments. One is the prickly pear cactus (*Opuntia ficus-indica*) native to Central and South America, which is also host to the cochineal insects mentioned previously that are a source of cochineal. The flower petals of members of the genus *Amaranthus*, also a member of the *Caryophyllales* have been used as source natural textile pigments centuries. The synthetic dye C. I. Food Red 9 (E123; **1**) was named “amaranth” because the similarity of its colour to the natural amaranth pigments. Compared with the anthocyanins, red colour of the betalains is much less affected by rising pH. The response of its ionisable groups to pH changes has no influence on the conjugated double bond system that is the source of its colour. It is very stable to the highest temperatures of food processing and culinary operations as long as it is in an acidic environment. Malt vinegar provides just such an environment when beetroot is preserved by pickling. However in neutral or alkaline environments it breaks down at cooking temperatures to unattractive brown compounds. This property means that it shares with anthocyanins their unsuitability for colouring baked goods such as cakes or biscuits.



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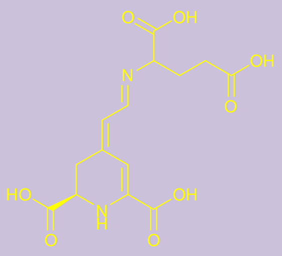

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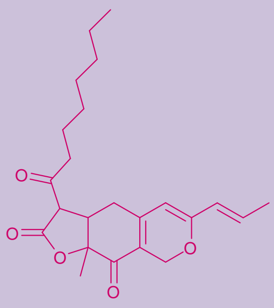

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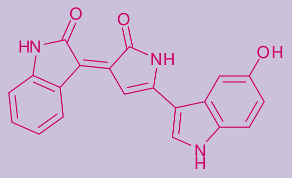

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The problems posed by introducing novel sources of natural pigments are typified by *anka*, a red food colour popular in many Far Eastern countries. Various moulds belonging to the genus *Monascus* are cultivated on steamed rice or other cooked starchy material [62]. The mould cells contain several structurally related pigments, *e.g.* rubropunctamine (**36**), which collectively impart a purplish red to the rice. No attempt is made to extract the pigments from the mould cultures; the red rice is simply dried and powdered for direct use in food preparation. The colour is undoubtedly "natural", as are the miscellany of other fungal metabolites that may be present. There is a strong possibility that at least some of these could present a hazard to consumers, as do the fungal toxins associated with contaminated peanuts and other crops. While *anka* remains a popular, and legal, food ingredient in many Far Eastern countries, the FDA and EFSA are disinclined to legalise it. This would require extensive testing on a variety of animal species and much more defined, and regulated, manufacturing processes.

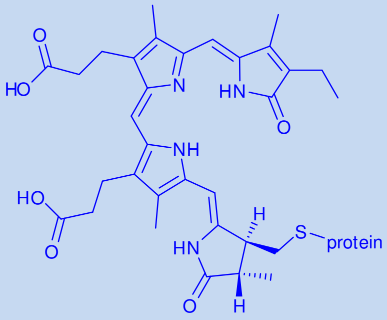
Another purple pigment that has attracted recent interest for food applications is violacein (**37**), a naturally-occurring bis-indole pigment with antimicrobial properties [63] that occurs in several species of bacteria, including *Chromobacterium violaceum* [14]. The water-soluble pigment is stable between pH 1-11 and was showed no significant degradation in a yoghurt system after 1 month storage at 4 °C [64]; it has not yet been approved for use in food [14].

Blue

There is a general absence of naturally blue foods (blueberries are certainly not blue). The result of this absence has resulted in blue being traditionally regarded as a taboo colour in food packaging, notably associated with the blue glass bottles of traditional remedies emphatically labelled, "Not to be taken internally!" The synthetic blue food colorants, Indigo Carmine, Brilliant Blue FCF, and Patent Blue V, were not included in the "Southampton Six", but have nevertheless become unacceptable to the public in the EU, especially in children's confectionery; FD&C Blue No. 1 and

No. 2 are, however, still widely used in the US. In response to the public disquiet, Nestlé replaced blue *Smarties* with white ones. To the surprise of many in the confectionery industry this change generated uproar in the social media from people who wanted their blue *Smarties* back! Fortunately there was one potential source of natural blue pigments – blue-green algae.

In spite of their name, blue-green algae are actually photosynthetic bacteria, the *Cyanobacteria* (or *Cyanophyta*). Besides chlorophyll, their photosynthetic machinery includes a protein, *C*-phycocyanin (**38**), which contains a bright blue chromophore, which structurally resembles bilirubin, a breakdown product of haemoglobin in mammals. By good fortune, there was one species, *Arthrospira platensis* (once classified in the genus *Spirulina*), that was already being widely cultivated. This occurs naturally in tropical and subtropical lakes rich in carbonate and bicarbonate (pH 8-11) and is readily cultivated large pools with either sunlight or artificial light. It forms thick mats of filaments that are easy to harvest. Dried cells have been sold in tablet form for many years as a high protein food supplement and numerous studies have claimed additional health benefits for its consumption. For a long time it was marketed as a "natural" source of vitamin B_{12} suitable for vegans, but it has now been established that the variant of the vitamin that it contains cannot be utilised by humans [65]. It has recently been marketed as a "superfood" (whatever one of those might be). The blue protein pigment is known commercially as "spirulina", but there are no details of the process for its extraction and purification from the filaments of alga cells, however, its long history of human consumption has meant that its food colour use has not provoked any safety issues or public outcry [66,67]. Technically it has proved an excellent colour for confectionery, *etc*., although its application is limited as it has poor light stability and high heat sensitivity, degrading readily above 45 °C [68]. Spirulina's regulatory status is proving problematical for legislators. If it is to be approved as a food additive (complete with E number), alongside the other natural colours, anthocyanins, carotenoids, *etc*., spirulina would have to undergo time-consuming and expensive toxicological scrutiny. The alternative is the rather dubious classification of "colouring food stuff", the best known example being saffron. The regulatory fate of spirulina has now been in the EFSA's in-tray for quite a while but in the meantime the UK authorities are simply turning a blind eye to its growing popularity in confectionery! Concentrated solutions of spirulina can now be found on supermarket shelves for domestic use as a blue food colour for cake icing, *etc.* It is being left to individual UK local authorities to challenge spirulina's unregulated use but any prosecution would have to demonstrate that its use was injurious to health. Unsurprisingly no prosecutions have yet taken place. In the US, spirulina extract is exempt from certification by the FDA for coloration of confections (including candy and chewing gum), frostings (icing), ice cream and frozen desserts, dessert coatings and toppings, beverage mixes and powders, yogurts, custards, puddings, cottage cheese, gelatine, breadcrumbs, and ready-to-eat cereals (excluding extruded cereals) [13].



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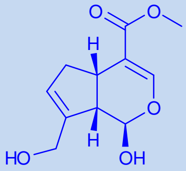

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Some recent research as explored the possible application of nature's techniques for colouring flower petals blue [69-71] by associating anthocyanins with other phenolics or aluminium ions; at the right ratio of Al^{3+} or Fe^{3+} cation to anthocyanin, such chelates develop blue colours above pH 2.5 [72]. However the inherent problems by posed by the nature of the food matrix, and the need for long term stability, makes this a very difficult objective, especially when consumers' enthusiasm for blue food and drink is so limited.

An iridoid glycoside pigment called geniposide is present in the fruit of *Gardenia jasminoides*; the aglycon , genipin (**39**), is formed by enzymatic hydrolysis and treated with amino acids to produce the blue pigment gardenia blue [62,73], which is approved for use in Japan, but not EU and US [11,74].

Brown

Brown colours arise in food by two distinct processes associated by consumers with quite different groups of foods. Furthermore one of the processes, associated with fresh fruit and vegetables, is mostly undesirable, the other associated with carbohydrate rich baked foods is mostly desirable. The first of these is described as "enzymic browning". This occurs when physical impact, fungal or insect attack, damages plant cell membranes and allows the cytoplasm, with its array of enzymes, to mix with the contents of the cell vacuoles, which contain a variety of phenolic compounds [75]. The cytoplasmic enzyme phenolase catalyses the oxidation of the phenolics to *o*-diphenols and then *o*-quinones; these are highly reactive and spontaneously polymerise to the brown pigments that characterise the cut surfaces of potatoes, bananas, *etc.*, exposed to the air [76]. Though aesthetically unappealing and providing a negative indicator to consumers, this process provides an essential defence against mould growth. Conversely, there is one group of phenolase substrates, the catechins, which are particularly abundant in the leaves and shoots of a species of the tropical and subtropical shrub, *Camellia sinensis*, *i.e.* tea; in this case, the enzymic browning reactions are a necessary, and desirable, part of the production process [77].

Much more relevant to this article are the products of non-enzymic browning, the chemical reactions associated with reactions of carbohydrates when they are heated. When strongly heated in the absence proteins or amino acids, sugars give rise to brown pigments in a complex and poorly defined sequence of reactions known as caramelisation. When subjected to high temperatures in the presence of proteins, in cooking or baking, a similar sequence of reactions occurs – the Maillard reaction, which is now recognised as the source of desirable colours and flavours in cooked (especially baked) foods where carbohydrates and proteins occur together.

For centuries cooks have been partially burning sugar in metal pans over strong heat to generate a sticky brown material, caramel, that can be used to both colour and flavour food. The residue from sugar refining, molasses, was used for the same purpose. Caramel (C. I. Natural Brown 10) for commercial use in food colouring is manufactured by controlled heating of carbohydrates either alone (Class I, E150a), in the presence of alkali and sulfite (Class II, E150b), in the presence of ammonia (Class III, E150c), or in the presence of ammonia plus sulfite (Class IV, E150d). However, they do not qualify for the epithet “natural” unless their manufacture and use resembles traditional, domestically prepared “burnt sugar” [78]. Large quantities of caramels are used in a wide range of food and drink products including beer, spirits, cola, confectionery, soup, dairy products, *etc.* – in bulk terms they are by far the most heavily used class of food colorants. Of the various classes of caramel, only Class III has raised concern on grounds of toxicological safety and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established 200 mg per kg body weight as an acceptable daily intake (ADI). Beer is by far the most significant source of Class III caramel in the diet, but exceeding the ADI would require daily consumption of over 20 litres, its alcohol content would raise more significant health issues.

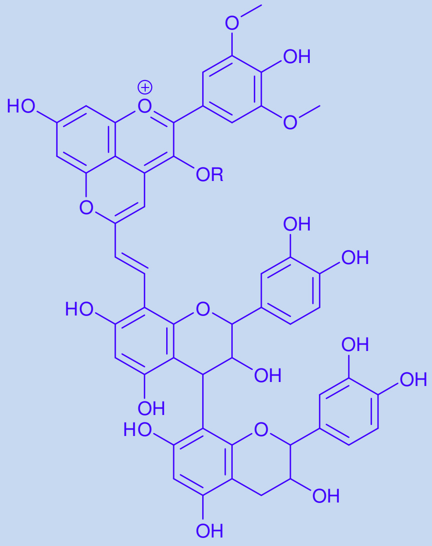
A novel approach to the issue of whether caramels can be regarded as natural has been adopted by one manufacturer of tradition caramels in the USA. A variety of fruit or vegetable juice concentrates are intensively cooked and further concentrated to become brown colouring materials with residual flavour elements characteristic the original raw material. Not containing any ingredients that might be construed as “synthetic”, “artificial” or “unnatural” they can marketed without reference to food additive legislation.

The future

Anthocyanins are seen as an area of significant potential for food colorants and there is an ongoing global search for edible plants that contain these pigments, particularly those with desirable colours and good stability [11]. Research groups have developed biotechnological methods to produce anthocyanins through plant tissue cultures as an alternative to crop production [79], although none have been commercialised.

Researchers have also examined the possibility of using biological engineering techniques as possible methods to produce anthocyanins in large quantities. The tomato (*Solanum lycopersicum*) is seen as an excellent vehicle for such engineering, despite anthocyanins normally being absent in tomato fruit [80]. Ectopic fruit-specific expression of transcription factor proteins from snapdragon (*Antirrhinum majus*) resulted in accumulation of anthocyanins throughout tomato fruit in high concentrations; the most abundant anthocyanins expressed were shown to be an acylated delphinidin glycoside and an acylated petunidin glycoside [81]. These 'purple' tomatoes have significant potential as foods with high nutritional benefit and also for producing reliable, high quantities of anthocyanins for other applications, including as food colorants. However, although ectopic expression can be caused by natural processes, the issue of genetic modification (GM) may significantly limit their application as both food and source of food colorant in the Europe due to EU restrictions on GM; the last approval for a GM food crop in the EU came in 1998. Despite being developed at the John Innes Centre in the UK, large-scale production is now under way in Canada as Canadian regulations are seen as more supportive of GM [82]. A key question is whether a GM product that may have health benefits will influence public opinion; a major survey across the European Union in 2010 found opponents outnumbered supporters by roughly three to one.

Significant research at The University of Porto on the isolation and hemi-synthesis of anthocyanin-derived pigments found in aged Port red wines has led identification of a family of rare pyranoanthocyanin pigments (**40**) that present a blue colour (λ_{max} = 575 nm) at acidic pH [83]. These pigments were comprised of a pyranoanthocyanin moiety linked to a flavanol by a vinyl bridge formed through the reaction of anthocyanin-pyruvic acid adducts with vinyl-flavanol adducts. The extended conjugation of the π electrons throughout all the pigment molecule is likely to confer a higher stability on it and is probably the origin of the intense blue colour. The researchers also identified a new family of pyranoanthocyanin dimers (**41**), displaying an extraordinary turquoise-blue colour (λ_{max} = ~730 nm), in aged Port wine [84]. These compounds comprise two pyranoanthocyanin moieties linked together through a methyne bridge formed from reaction between carboxypyranoanthocyanins and methylpyranoanthocyanins. However, the compounds have poor water solubility, limiting their industrial application as food colorants. Most recently the researchers produced a new blue portisin-derived (vinylpyranoanthocyanin) pigment (**42**), resultant from the reaction of A-type vitisin (carboxypyranomalvidin-3-glucoside) with 4-(dimethylamino)-cinnamic acid [85]. The presence of an amino group in the structure is responsible for extensive π-delocalization and the associated intense blue colour (λ_{max} = 638 nm).



R = glucose or coumaroylglucose

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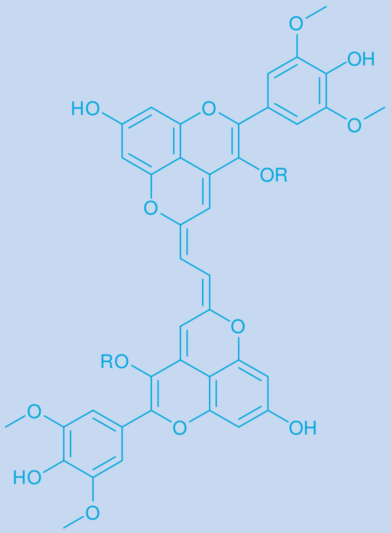

R = glucose or coumaroylglucose

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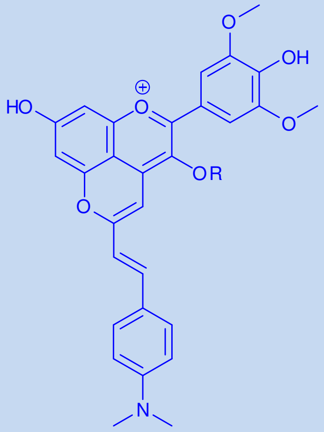

R = glucose

42

In an attempt to increase the stability of anthocyanins, Porto researchers attempted acylation of anthocyanins. Compared to other flavonoids, chemical modifications in anthocyanins is highly challenging, because of the positive charge in the flavylium form, the various equilibrium forms in solution, low solubility in organic solvents, and susceptibility to degradation in neutral and basic conditions. Cruz *et al*. [86] successfully acylated of malvidin-3-glucoside with stearoyl chloride to provide the stearic acid derivative, but the reaction was not regioselective and mono-, di, and tri-ester derivatives were isolated that were very difficult to separate. In contrast, the research group showed that regioselective synthesis (where only the primary –OH on the glucose moiety was derivatised) of a malvidin-3-glucoside-oleic acid ester derivative was achieved by enzymatic catalysis with *Candida antarctica* Lipase B (*Cal*B), giving only one ester product [87]; the derivative was more lipophilic and more stable to oxidation.

Research on lipophilic food colorants has focused on green chemistry approaches to extract lycopene from tomatoes [88-94], lutein from marigolds [95,96], curcumin from turmeric [97-100], bixin from annatto [101], capsanthin from peppers [102,103], using more environmentally friendly and less toxic solvents. This is desirable from a sustainability and toxicological perspective, but can also give higher yields with better colour and flavour [104]. ‘Greener’ solvents and methods include supercritical carbon dioxide [90,92,95,96,99,101-103,105,106], microwave-assisted extraction [89,97,99,107], ultrasound-assisted extraction [89,91,93,94,98,99,108,109], enzyme-assisted extraction [92,110], and continuous counter-current extraction [111]. Food processing waste is an attractive source of food colorants and these novel extraction methods have been employed usefully in extracting β-carotene from tomato waste [94], lycopene from tomato waste [94,105,106], and lycopene from papaya waste [109]; other interesting source materials have been explored such as gac fruit (*Momordica cochinchinensis*) as a source of lycopene and β-carotene [107].

Other sources of food waste have also examined as a potential source of food colorants. Guyot *et al.* [112] developed a new bright yellow and highly water-soluble dye from apple pomace, the major by-product of apple juice and cider production, as a potential natural alternative to tartrazine. The yellow pigment (POPj) is formed in high yields by oxidation of phloridzin (found in high concentrations in apple pomace) by polyphenol oxidase (PPO) enzymes (Figure 7). POPj has a bright yellow colour at pH 3 to 5, turning to orange for higher pHs and was only weakly degraded by prolonged storage at ambient temperature.

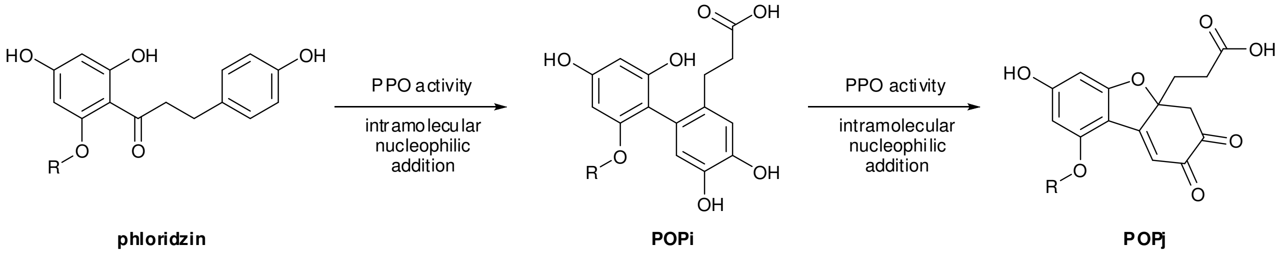

Figure 7. Synthesis of the yellow pigment POPj by oxidation of phloridzin by polyphenol oxidase (PPO) enzymes.

Microalgae are being developed as a new source of food colorants as they are the major source on earth of certain natural pigments [113]. Different chlorophylls are readily available in most microalgae. In addition, β-carotene is found in green microalgae (*Chlorophyta*), brown microalgae (*Diatomophyceae*), cryptomonads (*Cryptophyta*), euglenoids (*Euglenophyta*), and dinoflagellates (*Dinophyta*); xanthophylls are seen in blue-green microalgae (cyanobacteria), green microalgae, brown microalgae, cyanobacteria, and euglenoids; astaxanthin is present in green microalgae; and phycobiliproteins are found in cyanobacteria and cryptomonads [113]. Several factors have to be considered in microalgal pigment production in order to achieve the highest levels of pigments in the cells. Light and temperature are the most important factors that affect overall biomass production [114]; *Arthrospira* spp. (cyanobacteria) prefer low light intensities in production of phycobiliproteins [115], whereas β-carotene production in *Dunaliella salina* (a green microalgae) is greater in higher light intensities [116]. Optimum temperature for the production of most pigments from most microalgae was in the relatively narrow region of 25-36 °C [113]. However, it has been demonstrated that higher temperatures between 55-60 °C were optimal for the production of total carotenoids from green microalgae [117,118]. The potential concentrations of such pigments in microalgal sources significantly outweigh vegetable/fruit sources – spinach has a chlorophyll content of 0.06 mg kg^{-1}, whereas *A. platensis* biomass contains 1.15 mg kg^{-1}, nearly 20x the concentration [113]. New, clean extraction techniques described above offer significant potential in being translated to extraction of pigments from microalgae.

Conclusion

Whether we are purchasing fresh vegetables from a market stall, ready meals from the supermarket, eating at home or in five-star restaurant, we use colour to give us clues as to what to expect, in terms of taste, nutrition and safety. This is very different to our approach to the colour of the other things we buy. The colour of a coat doesn't tell us how warm, or waterproof, it will be. The colour of a car doesn't tell us how fast, or safe, it will be. This review has considered the techniques that have, over the years, been employed to modify the colour of our food, and the interactions of these techniques with questions of safety and nutrition. On supermarket shelves the proliferation of products with labels that make a virtue of "No artificial colours" suggests that the future of the azo dyes and their various chemical cousins is strictly limited. If the fate of the "Southampton Six" is any guide, it will be consumer choice rather than legislation that wields the axe. Neither should we be too optimistic about the outcome of searches for more "new" natural colours. The new developments that the food colour manufacturers boast about nowadays always seem turn out to be no more than enhanced vehicles for delivering established natural pigments into food products.

It appears likely that the food colouring issue will actually follow in the pioneering footsteps of food seasoning, *i.e.* added salt. Public awareness of the impact of excessive salt, *i.e.* sodium chloride, on human health is beginning to catch up with Chinese physicians who around 2500 BC were warning their patients that that too much salt in their food would "harden their pulse" [119]. Since the 1980s various medical authorities around the world have stressed the relationship of hypertension to salt intake and food legislators are now taking an interest. In Britain it was recognised that bread is a major contributor to our salt intake and with a little encouragement from the FSA our bakers have responded. Over the last few years salt levels in ordinary white bread have gradually been halved, from around 2% to 1%. Consumers do not appear to have noticed, let alone complained! If this line of reasoning does also apply to food colour then consumers might be expected eventually to accept less vivid supermarket food. If this trend ultimately leads to the demise of canned processed peas and some cup-cake toppings then so be it.

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