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Anthocyanins as natural food colours—selected aspects

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The anthocyanins have a long history as part of the human diet, these and other flavonoids, are receiving renewed attention for their positive health attributes. The principal commercially available anthocyanin food colorants, grape, elderberry, red cabbage and roselle are considered and others mentioned. Factors affecting the usage of extracts, in terms of colour hue and stability are discussed. Chemical aspects of anthocyanin colour are summarised briefly and the advantages of acylation for colour durability noted as a promising area for future development. Special emphasis is placed on quantitative information where applicable. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The appreciation of good quality food and drink is one of life's great pleasures. Colour plays an important role in our enjoyment of foodstuffs, it is appreciated both for its aesthetic role and as a basis for the assessment of quality. In this latter respect, colour gives visual cues to flavour identification and taste thresholds, influencing food preference, food acceptability and ultimately food choice.

Most food nowadays is processed in some way before reaching the consumer, and manufacturers have a need to replace colour lost during processing or to colour products which would otherwise be colourless and unappealing. With increasing public concern about the safety of synthetic colorants, natural pigment extracts are assuming greater prominence. Europe presently has 13 permitted pigments derived from natural sources, which may be used for food coloration. These are curcumin (curcuminoid), lutein (xanthophyll), beta-carotene, bixin/norbixin, capsanthin/capsorubin (carotenoids), betanin (betalaine), carminic acid and carmine (anthraquinones), chlorophyll and copper chlorophyll/chlorophyllin (porphyrins), caramelised sugar and malt extract (melanoidins) and anthocyanins (flavonoid). This overview is confined to some aspects of the latter group of food colorants, with emphasis on quantitative data, where appropriate.

ANTHOCYANINS

The anthocyanins comprise the largest group of water soluble pigments in the plant kingdom and are especially characteristic of the angiosperms or flowering plants, which themselves provide our major source of food crops. In food plants, anthocyanins are widespread occurring in at least 27 families, 73 genera and a multitude of species.

Diet and health

Anthocyanins have presumably been on earth since the appearance of the flowering plants and early man must have consumed large amounts of these pigments, in a diet being based largely on wild fruits and berries. Anthocyanins are particularly associated with fruits, but also occur in vegetables, roots, tubers, bulbs, legumes and cereals; also, certain anthocyanin-containing leaves and flower parts are traditionally consumed in some parts of the world. It is reasonable to assume, therefore, that human beings are well conditioned to ingesting anthocyanins; further, it may be argued that the present day diet with its increasing dependence on processed foods, has become deficient in anthocyanins. The addition of natural anthocyanin extracts, to give colour to processed foodstuffs, could be regarded in this context as maintaining current levels, if not actually redressing the balance, which may be desirable in view of their beneficial effects.

The average daily anthocyanin intake with food in the U.S.A. in 1971, was estimated as 215 mg/day in the

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summer and 180 mg/day during winter (Kuhnau, 1976). In a more recent study of 569 Italian subjects, anthocyanin consumption varied from 25–215 mg/person/day, depending on gender and age; greatest intake was, perhaps not surprisingly, from red wine (Alberti-Fidanza *et al.*, 1996). These data give an indication of the quantities of anthocyanins consumed, but of course country to country variations will occur according to individual diets and preferences. Certainly, anthocyanin intake would be high in wine-drinking countries and also in the so-called under developed countries, where wild fruits and berries abound and are a part of the staple diet.

Existing evidence indicates that the anthocyanins are not only non-toxic and non-mutagenic, but have positive therapeutic properties (Saija, 1994), for instance, in ophthalmology (Pourrat, 1977), for treatment of various circulatory disorders (Bettini *et al.*, 1985) and inflammatory diseases (Vincieri *et al.*, 1992). The more recent appreciation of the antioxidant properties of anthocyanins and related flavonoids has resulted in a burgeoning literature on their role in the reduction of risk of coronary heart disease (e.g. Waterhouse, 1995). Special reference in this context to the role of flavonoids in red wines, (rather than the alcoholic content of wine—Frankel *et al.*, 1993; Kondo *et al.*, 1994), in preventing or ameliorating some chronic diseases, has led to active investigation of the so-called 'French Paradox' (e.g. Kinsella *et al.*, 1993; Leake, 1995). These latter properties in particular, have raised the profile of the anthocyanins and flavonoids in general—a fascinating group of plant secondary metabolites.

Food colorant sources, extraction and processing

One of the most comprehensive reviews, in English, of anthocyanins as food colorants, is that produced by Francis (1989). Therein is described, in detail, the extraction and processing of pigments from grapes and other plant sources and methods for their evaluation and colour stability testing. More recent data featuring most food plant sources is provided by Mazza & Miniati (1993). These and earlier publications (Markakis, 1982; Francis, 1993) provide an abundance of data on most of the plant species seriously considered as having potential for a supply of anthocyanin food colours. This section contains additional information and some individual observations on processing aspects, concerning some of the principal commercially available anthocyanin colorants.

Grape (*Vitis* sp.)

The anthocyanin content of grapes is of the order 30–750 mg/100g, and as with other plant material, varies greatly according, to cultivar, season and environmental factors. Grape pomace (*V. vinifera*) is a relatively inexpensive source, since it is a by-product of the wine industry. The simplest and most cost effective method of pigment production is by aqueous extraction of the pomace, which yields high volume, low strength solutions (1–2%); further spray drying gives an upper concentration limit of about 5% pigment. Historically, the oldest and most abundant anthocyanin extract is enocyanin or 'enocianina' (Dieci, 1967), produced from red grape pomace and marketed in Italy since 1879. Traditionally, pomace from deeply pigmented grape cultivars such as Lancellotta, Lambrusco, Alicante and Salamina, is extracted batch-wise with decreasing concentrations of sulphur dioxide (SO_2) for some months. After concentration, the liquor may be refrigerated to remove potassium tartrate, centrifuged and formulated as a liquid or a spray-dried solid. Other workers have experimented with different extraction solvents (Philip, 1974; Metivier *et al.*, 1980; Hang, 1988).

In the French 'Sefcal' process (Combe & Ladet, 1979), grape pomace is continuously extracted with 0.2% SO_2 solution at 80°C in a Frilli counter-flow diffuser. This system minimises the volume of solvent required; with recycling of the distillate, one litre of solvent is used for each kilogram of pomace. In 1980, three extraction plants were operating, in France, southern Italy and Tunisia, producing 12–18 tons of colour concentrate per day, with a total capacity of treating 100 tons of pomace per day (Dupuy *et al.*, 1982).

Anthocyanins may also be purified and concentrated by ultrafiltration on cellulose acetate membranes, a procedure which, ideally, separates proteins and higher molecular weight carbohydrates from smaller molecules (sugars, phenolics, anthocyanins etc.). However, the tendency for coloured oligomeric anthocyanins to aggregate may cause them to be retained by the membrane. Equipment design and membrane replacement are factors that need to be evaluated if continuous flow is used for long periods. Adsorbent resins (Applexion Technology) are also used, particularly in France, for extracting grape wastes (e.g. use of the non-ionic polymeric resin Amberlite XAD-4).

The main anthocyanins in the European grape, *Vitis vinifera*, are 3-glucosides, 3-acetylglucosides and 3-p-coumaroylglucosides of malvidin (Mv), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and cyanidin (Cy) (Wulf & Nagel, 1978). During extraction and processing, a proportion of these monomeric anthocyanins become degraded and condensed with other flavonoid material, to form polymeric pigments. Thus, analysis by high performance liquid chromatography (HPLC) of, for example, enocyanin, yields a simplified anthocyanin chromatogram consisting largely of the five main 3-glucosides, with unresolved polymeric pigment(s) causing an elevated baseline underlying the later-eluting acylated anthocyanins (Bakker & Timberlake, 1985).

Hybrid grapes exhibit an even more complex pigment pattern than *V. vinifera*, since they contain additionally the five corresponding 3,5-diglucosides and their acylated derivatives (Hrazdina, 1975; Baublis *et al.*, 1995).

Although a dry powder product extracted from Concord grape lees (*V. labrusca*) or filter cake press has now been discontinued, a highly pigmented concentrated Concord juice is still available for colouring purposes, as is a concentrate from Rubired grapes, reported as having eight times the colouring potential of existing red grape concentrates (Francis, 1995). Grape pigments are a potent source of food colourings, but they are used to a lesser extent in Japan, since the imposition of import tax makes them more expensive; hence there is much interest in other potential sources of food colours in this part of the world.

Elderberries (*Sambucus nigra*)

Manufacture of an elderberry colorant in Russia has been described (Mazza & Miniati, 1993). Anthocyanins found in elderberries are all cyanidin glycosides, comprising the 3-sambubioside, 3-glucoside, 3-sambubioside-5-glucoside and 3,5-diglucoside. Amounts vary between 200 and 1000 mg/100 g fresh weight (FW) (Bronnum-Hansen *et al.*, 1985). Of these pigments, cyanidin 3-sambubioside has been reported as the most stable during processing (Drdak & Daucik, 1990). The use of stabilised danewort (*S. ebulis*) pigments has also been investigated, after treatment to remove offending taste and smell (Kovakova *et al.*, 1993). Additionally, Cy 3-p-coumaroylglucoside-5-glucoside was found in fruits of *S. racemosa* (Lamaison *et al.*, 1979). Fruits of *S. canadensis* may also have potential for food colouring following the finding that this acylated anthocyanin was the major pigment present (69.8%) (Johansen *et al.*, 1991). Recent NMR work on *S. canadensis* pigments has revealed two further acylated pigments (Nakatani *et al.*, 1995). Acylation is known to confer additional stability to an anthocyanin, making it more stable than the corresponding non-acylated pigment (Saito *et al.*, 1995).

Red cabbage (*Brassica oleracea*)

Red cabbage extract is available from European sources, but much of the published data emanates from Japan, where many patents describing the preparation and application of red cabbage colorants were granted to investigators in the 1980s. Currently the colorant San Red RC is available commercially. A more recent patent (Mano *et al.*, 1990) describes a two stage process of chromatography of an extract on a hydrophilic polyvinyl gel to give a purple colorant, followed by chromatography on phenol-formaldehyde polymer or polyamide (Duolite XAD 761) to produce a heat and light resistant red product. Red cabbage extracts have been treated with polymeric resin (Mano *et al.*, 1988) and anion exchange (Tamura *et al.*, 1992) to remove unpleasant odour. Stabilisation has been achieved by adding flavonols, water-soluble antioxidants and phosphates (Washino & Moriwaki, 1990), anionic pigments (Kobayashi *et al.*, 1988) and sugars (Onishi & Kotake, 1988) and by copigmentation with water soluble flavonoid glycosides (Nishimura *et al.*, 1990). Using HPLC, Idaka (1987, 1988) separated more than 15 anthocyanins from *B. oleracea* and fully characterised all but four of these complex anthocyanins. They are based on Cy 3,5-diglucoside and Cy 3-diglucoside-5-glucoside acylated at the 3- position with ferulic, p-coumaric and sinapic acids. This work confirmed and extended the findings of Nakatani *et al.* (1987) and Ikeda *et al.* (1987). The extensive acylation accounts for the superior stability and colour characteristics of these red cabbage pigments. The colour quality of two cultivars was generally maintained during storage of the cabbages at 4°C for 84 days (Lachman *et al.*, 1991); they contained 69 and 94mg/100g (FW) expressed as Cy 3-glucoside.

Roselle (*Hibiscus sabdariffa*)

The anthocyanins from the calyces of this tropical plant are ideal for producing brilliant red colourings in gelatin, jams, jellies and fruit beverages (Mazza & Miniati, 1993). Extraction and processing have been described previously (Francis, 1989). Dp 3-sambubioside is the major pigment responsible for the reddish-violet colour. Cyanidin 3-sambubioside is also present with lesser amounts of Dp and Cy 3-glucosides. The relative proportion of Dp to Cy 3-sambubioside is 70.9 and 29.1% (Pouget *et al.*, 1990). In dry calyces there was about 1.5g total anthocyanin/100g dry weight, expressed as Dp 3-glucoside (Du & Francis, 1973).

Other sources

The following commercial product sources were profiled by Mazza & Miniati (1993) and are summarised here, with additional detail where available.

1. Blood Orange (*Citrus sinensis*). This European source is now well established in Sicily and southern Italy. The juice is pigmented mainly by cyanidin 3-glucoside (50%) and its acetylated derivative (18%) (Maccarone *et al.*, 1985) and a spread of 8 minor components. The 'Moro' orange contains approximately 200mg anthocyanins per 100ml juice.
2. Black Chokeberry (*Aronia melanocarpa*) is a small dark coloured fruit found in north America and eastern Europe. Following experimental trials and interest in Poland and Scandinavia a product from this fruit is now offered commercially. The highly pigmented berry is also cultivated in Austria; it is coloured by cyanidin glycosides (1% fresh weight) and also has a high sugar content (26%) (Strigl *et al.*, 1995*a,b*).
3. Sweet potato (*Ipomoea batatas*). A patented product from sweet potato tubers is marketed in

Japan where breeding programmes for optimum colour have been carried out. The pigments are based on acylated cyanidin and peonidin 3,5-diglycosides.

The following sources are also of interest to Japanese producers, some have been patented: *Perilla spp.* (leaves)—pigments based on acylated cyanidin 3,5-di-glucoside. Purple corn (*Zea mays*) leaves and seeds—pigments based on pelargonidin and cyanidin 3-glucosides with malonic acid acylation. Boysenberry (*Rubus sp.*)—a loganberry derivative containing cyanidin glycosides (Torre & Barritt, 1977) is also under consideration as a potential food colour source (161mg anthocyanins/100g fresh fruit).

Plant tissue culture (PTC)

Investigation into anthocyanin production by tissue culture is the most rapidly growing field in the search for food colorants. Seitz (1988) tabulated the occurrence of anthocyanins in tissue and cell cultures. Since 1988, over one hundred references to patents and papers have appeared in the chemical press, including many potential food colour sources. However, at the present time, none has been exploited for production commercially.

Applications—quantitative and qualitative aspects

There are few recommended methods of measuring anthocyanin content. One example is that used under Danish legislation (Knuthsen, 1987), whereby anthocyanin pigment is calculated as a percentage of total sample weight from the specific absorption (A) 1%/1 cm = 500 at maximum absorption (c. 520 nm) in buffer pH 1.0; this corresponds to molar absorptivity value (ϵ) of 26 500.

Significant properties of anthocyanin extracts are colour density and colour hue. These can be affected by pH, SO_2, heat, light, metals, copigmentation and 'thin film' effects, and others (Markakis, 1982). Quality control is necessary to standardise extracts of varying strengths and qualities from many sources. Anthocyanins are red-coloured in acidic solutions, thus it is not surprising that the main use of grape pigments on a volume basis, is in beverages and soft drinks (which are themselves high volume, low-price products). Dosage of about 3 kg of a 1% anthocyanin extract to 1000 litres of beverage (ca. 30 mg/l anthocyanin) can impart a deep red colour. Used in sugar confectionery, grape extract (0.4%w/w) produces a clear ruby red colour in boiled sweets; the exact tone may be adjusted with colorants from other fruit sources. It is possible that sweetened products may benefit from some colour enhancement, particularly in mildly acidic conditions (pH 4), since certain sugars have been shown to increase anthocyanin colour intensity (Lewis *et al.*, 1995). In dairy products and ice creams, addition of 0.3–0.5%w/w colorant imparts a mauve-black colour (Rayner, 1991). With increasing pH values anthocyanin colour becomes paler, however, if the product being coloured contains components capable of acting as copigments, colour may be retained and also light-stabilised to a certain extent (Bobbio *et al.*, 1992). Colour fluctuation is caused also by SO_2, widely used as a preservative in beverages; anthocyanin colour may be bleached initially, but then partially restored as SO_2 becomes oxidised. Resistance to bleaching by SO_2 is offered by many grape extracts, which contain mainly oligomeric or polymeric pigments. These innately stable products are effective reddish-blue colorants and almost all blackcurrant drinks in the UK are coloured with grape pigment. The main anthocyanins in grapes are malvidin glycosides, which are among the most colour stable anthocyanins, due to the dimethoxylation in the molecule, a feature which additionally maximises the colour potential if copigmentation occurs (Davies & Mazza, 1993).

Anthocyanin extracts have limited use in beverages which are slightly hazy or cloudy, since absorption onto particulate matter can cause a blueing effect. Other applications of anthocyanin extracts include coloration of acid fruit preparations, jams and preserves. Formulations for these need to be adaptable, since they depend on the nature and quality of the fruit, whether it be fresh, frozen or sulphited. Grape extracts have only limited use for masking brownness in fruit products, as they themselves absorb to some extent in the spectral brown region (420–440 nm).

Anthocyanins become paler on heating, because the equilibrium between the four anthocyanin species shifts towards the colourless carbinol base and chalcone forms. Under perfect conditions, the original colour should be regained on cooling if sufficient time (a few hours) is allowed for reconversion of the chalcone. In practice, in the presence of oxygen, some pigment browning or degradation usually occurs and this is most noticeable in the cooling cycle after pasteurisation of fruit products. Jams may be allowed to cool naturally from 80 to 20°C during the course of a few days; samples taken from the hot pan early may then differ in colour from those taken later in the process. At the other end of the temperature range, an interesting situation arises if anthocyanins are used to colour, for example, water ices. At such low temperatures the formation of blue quinonoidal forms is favoured, resulting in a product which may be red before freezing but blue afterwards. Physical factors, such as suspended particulates and internal reflections and refractions of light within the short path-length outer layers of ice crystals compound the blueing effect. This 'thin layer' or 'meniscus' effect is especially noticeable at the meniscus or periphery of a glass of young red wine, which appears bluer than the bulk of the wine. This observation can be modelled and measured in the laboratory, using tristimulus colorimetry with a selection of varying path-length cells (Timberlake & Bridle, 1983).

Some precipitation problems may occur by interaction of anthocyanin extracts with proteins. Thus, extracts which contain other phenolics or oliogomeric pigments above a certain level cannot be used to colour jellies as they form precipitates with gelatin. However, overall the stability of anthocyanin extracts is good and many thousands of tons of the 1–2% grape extracts are used for colouring all classes of foodstuffs, including high-boiling sugar confectionery and bakery toppings (biscuits and cakes). A summary of quality requirements for natural food colours would include consistency, safety and stability. In practice, these three requirements are interactive, and each must be satisfied to produce a colour that is 'fit for the purpose'.

Chemistry and colour

The primary, pH-dependent, anthocyanin equilibrium structure is the flavylium cation, from which secondary structures are derived either by proton transfer to produce bluish quinonoidal bases and corresponding ionised quinonoidal bases, or by hydration, to form colourless carbinol pseudobases which can tautomerise to colourless retro-chalcones (Brouillard & Dangles, 1994). Tertiary structures can result from molecular associations of primary or secondary forms, either with themselves (self-association) or with another molecule in solution (*inter*-molecular copigmentation). Quaternary structures which result from medium (solvent) effects on the colour of any of the preceding structures, are likely to be of significance only in strongly alcoholic beverages.

Unlike synthetic colorants which generally obey the Beer–Lambert law and yield 100% of their colour irrespective of pH, the colour produced by anthocyanins is dependent on many factors. Total anthocyanin colour is developed only in strongly acidic solutions. In isolation, anthocyanins have little colour above pH 3.5, but in natural media they become much more coloured by copigmentation with other (plant) components, which may themselves be colourless. This physico-chemical phenomenon was first described over sixty years ago (Robinson & Robinson, 1931). In acid solution (pH less than 2) anthocyanins display a negative deviation from Beer's law (colour increases less than linearly with increase in concentration). However, at higher pH values (e.g. in foodstuffs) a positive deviation from Beer's law is observed (Timberlake & Bridle, 1983). This greater than linear augmentation of colour results from self-association and copigmentation. On dilution of the product the colour is proportionately less than the dilution factor. Thus, the implication is that anthocyanin colour is arbitrary, being the net result of competing effects, and pertains only under the actual conditions and concentrations in the product itself.

Acylated pigments

The presence of one or more acyl groups in an anthocyanin molecule, hinders hydrolysis of the red flavylium cationic form to the colourless carbinol base, allowing preferential formation of the blue quinonoidal bases, thereby resulting in pigments that are less sensitive to pH changes (i.e. they remain coloured in mildly acidic or neutral media). Dangles *et al.* (1993) quantified this effect using pelargonidin 3-sophoroside-5-glucoside acylated with up to three moles of caffeic acid. The pK_h values (where K_h is the hydration constant) increased with increasing acylation, confirming that the pigments became intrinsically more coloured (at a given pH). Acylated pigments are more stable than their non-acylated analogues (Saito *et al.*, 1995); Yoshida *et al.* (1991) attributed the remarkable stability of the mono-acylated pigment alatanin C from purple yam to an *intra*-molecular sandwich-type stacking. The relevant factors involved are threefold: the position of the acyl group on the anthocyanin, the position of the sugar moiety and also the length of the sugar spacer. The exploitation of acylated anthocyanins with improved colour and stability to heat, light, pH and SO_2, coupled with the development of successful PTC, are possibly the two most promising areas for advancing anthocyanins as natural food colours.

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