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Recent Developments in the Stabilization of Anthocyanins in Food Products

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ABSTRACT

The most recent findings on the influence of chemical structure, pH and copigments on colour stabilization of anthocyanin-containing media are reviewed. The pH is shown to be the most important factor affecting the colour of anthocyanins, but various means of stabilization are given. The discussion on copigmentation is based mainly on results of studies of this phenomenon in flowers. It is evident from the list of known copigments, however, that some of these compounds are commonly found in foods and there is sufficient evidence to show that they do play a role in the colour stabilization of food products. Most interesting is the recent discovery of acylated anthocyanins, which retain a stable colour in slightly acidic or neutral solutions. This discovery may prove to be of particular importance to the technology of foods, since with new and better sources of these compounds and with better understanding of their physicochemical properties they may find application in the colouring of food products.

INTRODUCTION

Colour is one of the most important attributes of food, appreciated for its intrinsic aesthetic value and as a basis for identification and quality judgement. Food selection or judgement of quality would be extremely difficult if colour discrimination was removed. It has been reported (Meggos, 1984) that liking or disliking a food is conditioned by its colour; attractive foods are sought out as pleasure-giving while unattractive foods are avoided as painful. Food colour and colour of the environment in which the food is seen can significantly increase or decrease our desire or appetite for it. By the process of experience, we learn what colours to expect and consider natural, and we predict rather precisely what properties a food or beverage will have from our knowledge of similar materials.

Before the development of processed foods, the colour effects in foods on the table were achieved by the seasonal use of fresh fruits and vegetables. With the introduction of processed foods, the need for colouring substances arose and the widely available, easily synthesized and inexpensive azo-dyes came to be used in manufacturing food products such as carbonated and still-beverages, dry mixes, baked goods, confections and dairy products. In the past two decades, however, because of their toxicological effects, several red dyes have been banned (Lancrenon, 1978; Meggos, 1984). Because of this, interest in the use of natural colorants in food is growing. A survey of the food colorants patents for the years 1971–75 compared with 1976–81 revealed an increase of more than 100% in the number of patents dealing with carotenoid, anthocyanin, betacyanin and haemoglobin type pigments, whereas the number of patents on synthetic compounds remained constant (Francis, 1984).

Anthocyanins are probably the best known of the natural food colours, since they form the reds and the blues of many fruits and vegetables. They provide the attractive red colour for many fruit juices, wines, jams and preserves. Yet, in spite of their obvious familiarity, they have not been used to any great extent as food colours. The reason for this may be threefold: they are not very stable chemically, they are difficult to purify and they have not been available commercially in large quantities. Also, anthocyanin preparations give about 100 times weaker colour magnitudes than do synthetic compounds (Riboh, 1977) and their colour is easily affected by a number of reactions occurring in food products (Hrazdina, 1974). On the other hand, it has long been known that anthocyanins form deep coloured complexes with some secondary plant metabolites such as flavonoids (Robinson & Robinson, 1931) and certain metals such as aluminium (Asen *et al.*, 1969). Upon formation of anthocyanin–flavonoid complexes, under relatively mild acidic conditions, the colour intensity of the solution

increases many fold (Asen *et al.*, 1972). Furthermore, during the last decade several new anthocyanins which are stable in water have been discovered (Asen *et al.*, 1977; Goto *et al.*, 1982) and the understanding of structural transformations of anthocyanins in aqueous solutions has improved (Brouillard, 1982). In this paper the influence of structure of the pigment, pH of the medium and presence of copigments on colour stabilization of anthocyanin will be discussed.

INFLUENCE OF STRUCTURE

The anthocyanins are glycosides of anthocyanidins and are based on the flavylium structure (Fig. 1). The chromophoric aglycones (anthocyanidins) are red polyhydroxylated salts which, due to their instability, are seldom found in their free form in plant tissues. Differences between the individual

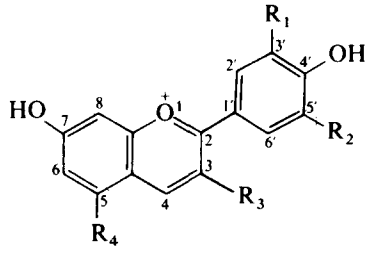

Fig. 1. The flavylium cation. R_1 and R_2 are H, OH or OCH_3, R_3 is a glycosyl or H and R_4 is OH or a glycosyl.

anthocyanins are the number of hydroxyl groups in the molecule, the degree of methylation of these hydroxyl groups, the nature and number of sugars attached to the molecule and the position of the attachment, and the nature and number of aliphatic or aromatic acids attached to the sugars in the molecule. The known naturally occurring anthocyanidins are listed in Table 1. Of these, six occur most frequently in plants. They are: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Harborne, 1967; Timberlake & Bridle, 1975).

Since each anthocyanidin may be glycosylated and acylated by different sugars and acids, at different positions, the number of anthocyanins is 15–20 times greater than the number of anthocyanidins. The sugars most commonly bonded to anthocyanidins are glucose, galactose, rhamnose and arabinose. Di- and trisaccharides, formed by combinations of these four monosaccharides, may also glycosylate some anthocyanidins. The following

TABLE 1
Naturally Occurring Anthocyanidins

Flavylium salt	*Substitution pattern (R)*					
	3	*5*	*6*	*7*	*3'*	*5'*
Apigeninidin (Ap)	H	OH	H	OH	H	H
Luteolinidin (Lt)	H	OH	H	OH	OH	H
Tricitinidin (Tr)	H	OH	H	OH	OH	OH
Pelargonidin (Pg)	OH	OH	H	OH	H	H
Aurantinidin (Au)	OH	OH	OH	OH	H	H
Cyanidin (Cy)	OH	OH	H	OH	OH	H
Peonidin (Pn)	OH	OH	H	OH	OMe	H
Rosinidin (Rs)	OH	OH	H	OMe	OMe	H
Delphinidin (Dp)	OH	OH	H	OH	OH	OH
Petunidin (Pt)	OH	OH	H	OH	OMe	OH
Pulchellidin (Pl)	OH	OMe	H	OH	OH	H
Europinidin (Eu)	OH	OMe	H	OH	OMe	OH
Malvidin (Mv)	OH	OH	H	OH	OMe	OMe
Hirsutidin (Hs)	OH	OH	H	OMe	OMe	OMe
Capensinidin (Cp)	OH	OMe	H	OH	OMe	OMe

four classes of anthocyanidin glycosides are most common: 3-monosides, 3-biosides, 3,5-diglycosides and 3,7-diglycosides (Harborne, 1967; Timberlake & Bridle, 1975). Glycosylation of the 3'-, 4'- and 5'-hydroxyl group, however, has also been demonstrated (Yoshitama & Abe, 1977). In many cases, the sugar residues are acylated by *p*-coumaric, caffeic, ferulic, or sinapic acids, and in a few cases by *p*-hydroxybenzoic, malonic or acetic acids. Methoxyl substituents are found at the 3' and 5' positions and, less frequently, at positions 7 and 5 (Harborne, 1967; Momose *et al.*, 1977). It is noteworthy that, to date, no natural anthocyanin where all the three hydroxyl groups at the 5, 7 and 4' positions are substituted at the same time has been reported (Brouillard, 1983).

Sugars, acylated sugars, methoxyl and hydroxyl groups have a marked effect upon the colour and reactivity of anthocyanins. The colour of anthocyanins is also determined by the physicochemical milieu in which they are viewed. The same anthocyanin may have different colours, depending on the pH and concentration of the solution, the presence of copigments and other factors. On the other hand, different tissues may contain the same anthocyanin and yet display different coloration. Generally, as the number of phenolic hydroxyls increases, the colour changes from pink to blue. Methoxyl groups replacing hydroxyl groups reverse the trend. The hydroxyl group at C3 is particularly significant, as it

shifts the colour of the pigment from yellow–orange to red. This is exemplified by the difference in colour between the majority of the anthocyanins, which are red, and the 3-deoxyanthocyanidins—apigeninidin, luteolinidin and tricitinidin—which are yellow. The same hydroxyl, however, destabilizes the molecule, as illustrated by the fact that the 3-deoxyanthocyanidins are much more stable than the other anthocyanidins (Sweeny & Iacobucci, 1983). Similarly, the presence of a hydroxyl group at C5 and substitution at C4 both stabilize the coloured forms through the arresting of hydration reactions which lead to the formation of colourless species (Brouillard, 1982; Sweeny & Iacobucci, 1983). At a given pH, anthocyanin 3-glycosides are more coloured than 3,5- and 5-glucosides. Optical density comparisons, carried out by many investigators (Harborne, 1967; Timberlake & Bridle, 1975) show that 3,5- and 5-glycosides have only 50% of the absorption at 440 nm (when compared with the colour maximum) as do the 3-glycosides and the free anthocyanidins. Similarly, the intensity of the short-wave maximum is lower in the case of the 3,5-diglucosides than with 3-glucosides. Glycosylation also affects the stability of the pigment. For instance, the half-life (50% reduction in absorbance at λ_{max}) of a typical anthocyanin, cyanidin-3-rutinoside, is about 65 days at room temperature in 0·01M citric acid, pH 2·8. The corresponding free anthocyanidin, however, has a half-life of only 12 h (Iacobucci & Sweeny, 1983). Similarly, at pH 2·5 and 4·5, the stability of peonidin and malvidin is significantly lower than their corresponding 3-glucosides (Ohta *et al.*, 1980). The slow hydrolysis of the 3-*O*-sugar unit of anthocyanins under acidic conditions is supposedly responsible for the higher stability of these pigments. Attempts to improve the stability by methylation of free phenolic hydroxyl groups have been found to reduce stability instead. The presence of either a 4′-OH or a 7-OH in the molecule significantly stabilizes the pigment while methylation of these hydroxyls decreases it (Table 2).

In recent years, a few acylated anthocyanins have been found which display remarkable stability in neutral or weakly acidic aqueous solutions. The first pigment identified in this series was platyconin. It was isolated by Saito *et al.* (1971) from the petals of Chinese bell-flowers *Platycodon grandiflorum* and, on the basis of chemical evidence, assigned the structure of delphinidin 3-dicaffeoylrutinoside-5-glucoside (Saito *et al.*, 1972). Goto *et al.* (1983), however, established by ^{1}H-NMR spectroscopy that the stereostructure of platyconin is 3-*O*-(6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl)-7-*O*(6-*O*-(*trans*-4-*O*-(6-*O*-(*trans*-4-*O*-(β-D-glucopyranosyl)-caffeoyl)-β-D-glucopyranosyl) caffeoyl)-β-D-glucopyranosyl) delphinidin (Fig. 2). Other anthocyanins which display extraordinary colour stability throughout the whole pH range are: dicaffeoyldelphinidin 3,7,3′-triglucoside from *Senecio*

cruentus (Yoshitama, 1981); dicaffeoylpeonidin-3-sophoroside 5-glucoside from *Ipomoea tricolor* (Asen *et al.*, 1977, 1979); caffeoylferuloyl-*p*-coumaroyldelphinidin-3-rutinoside 5,3′,5′-triglucoside from *Lobelia erinus* (Yoshitama and Abe, 1977); tricaffeoyldelphinidin 3,7,3′-triglucoside and caffeoylferuloylcyanidin 3,7,3′-triglucoside from *Tradescantia reflexa* (Yoshitama, 1978); caffeoylferuloylcyanidin 3,7,3′-triglucoside and tricaffeoylcyanidin 3,7,3′-triglucoside from *Zebrina pendula* (Stirton & Harborne, 1980).

TABLE 2
Effect of Structure on the Stability of Selected Pigments at 25°C in 0·01 M Citric Acid, pH 2·8 (Adapted from Iacobucci & Sweeny, 1983)

Compound	λ_{max} (*nm*)	*Half-life* (*days*)
3-hydroxy-4′,5,5′,7-tetramethoxyflavylium	512	0·04
3,4′,5,5′,7-pentahydroxyflavylium	512	0·5
3,4′,5,5′,7-pentamethoxyflavylium	512	6
3-rutinose-4′,5,5′,7-tetramethoxyflavylium	512	13
3-rutinose-4′-5,5′,7-tetrahydroxyflavylium	512	65
4′,5,5′,7-tetramethoxyflavylium	488	170
4′,5,7-trihydroxyflavylium	547	400
4′,7-dihydroxyflavylium	458	400
4′-hydroxyflavylium	436	400
4′-methoxyflavylium	437	35
7-hydroxyflavylium	428	300
7-methoxyflavylium	427	8

The stability of these anthocyanins in neutral solutions is currently attributed to the presence of two acyl groups, one of which must be situated above the pyrylium ring and the other beneath it. Hydrolysis of the acyl groups gives the corresponding deacylated anthocyanins which, upon their dissolution in slightly acidic or neutral media, are rapidly and almost completely converted to colourless forms (Yoshitama, 1978; Brouillard, 1983). Therefore, since monoacylated anthocyanins do not show colour stability, the presence of two or more acyl residues linked to the sugars is a fundamental structural requirement for good colour stability in neutral solutions. It remains to be seen if there will be application of these acylated anthocyanins to the colouring of foodstuffs, although Asen *et al.* (1979) were granted a patent for the possible use of peonidin 3-(dicaffeoylsophoroside)-5-glucoside as a food colorant.

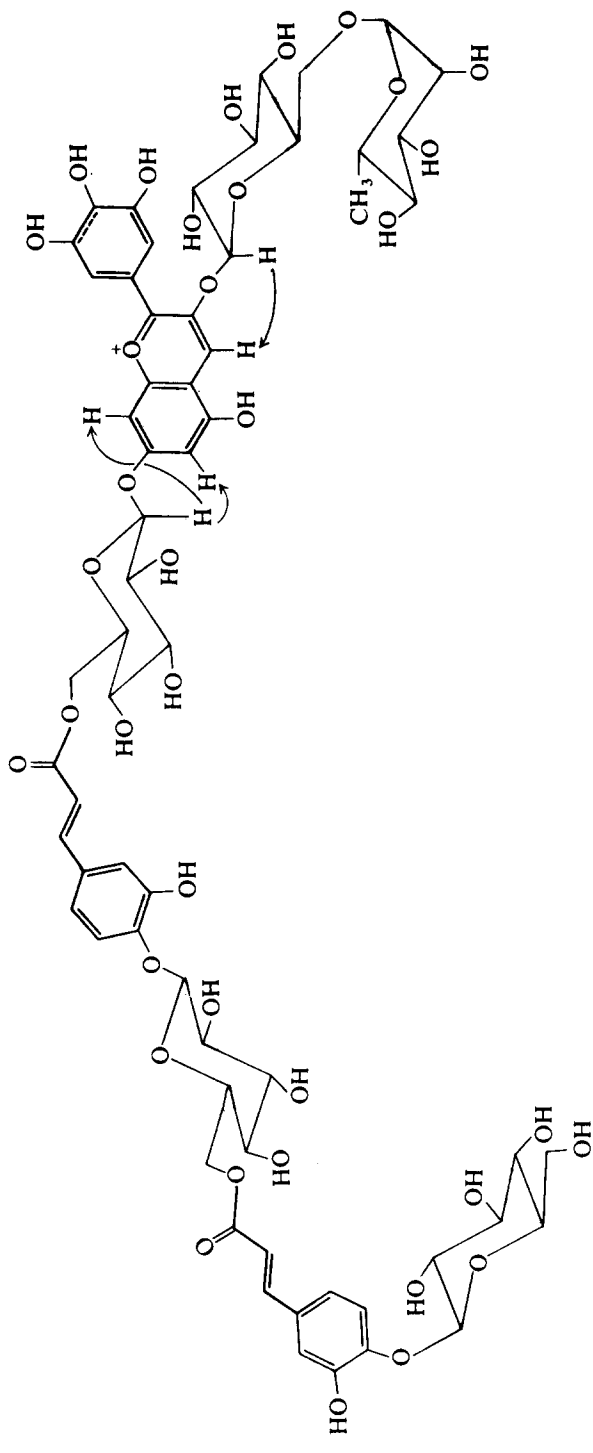

Fig. 2. Stereostructure of platyconin as established by ^{1}H-NMR spectroscopy. (From Goto *et al.* (1983) by permission of Pergamon Press.)

INFLUENCE OF pH

Non-acylated and monoacylated anthocyanins (i.e. most anthocyanins) behave like pH indicators, being red at low pH, bluish at intermediate pH and colourless at high pH. The nature of the chemical structures which these anthocyanins can adopt upon changing the pH has been clarified recently (Brouillard & Dubois, 1977; Brouillard & Delaporte, 1977, 1978; Brouillard *et al.*, 1978; Brouillard *et al.*, 1979; Brouillard, 1982). In acidic aqueous solution, four anthocyanin species exist in equilibrium. They are: the quinoidal base A, the flavylium cation AH^+, the pseudobase or carbinol B, and the chalcone C (Fig. 3). Interconversion between these four structures takes place according to Scheme I (Brouillard and Delaporte, 1977; Brouillard, 1982).

$$AH^+ \overset{K_a}{\rightleftharpoons} A + H^+ \quad \text{Acid–base equilibrium} \quad (1)$$

$$AH^+ + H_2O \overset{K_h}{\rightleftharpoons} B + H^+ \quad \text{Hydration equilibrium} \quad (2)$$

$$B \overset{K_T}{\rightleftharpoons} C \quad \text{Ring-chain tautomeric equilibrium} \quad (3)$$

Scheme 1

where: K_a, K_h and K_T are the equilibrium constants for acid-base, hydration, and ring chain tautomeric equilibria, respectively. $K_a = ([A]/[AH^+])a_{H^+}$; $K_h = ([B]/[AH^+])a_{H^+}$, $K_T = [C]/[B]$, and a_{H^+} is the activity of the hydronium ion ($pH = -\log a_{H^+}$).

At pHs below 2, the anthocyanin exists primarily in the form of the red (R_3 = O-sugar) or yellow (R_3 = H) flavylium cation (AH^+). As the pH is increased a rapid proton loss occurs to yield the red or blue quinoidal forms (A). The quinoidal form usually exists as a mixture, as the pK_a of the 4′,7- and (if present) 5-OH groups are very similar (Brouillard, 1982). On standing, a further reaction occurs; that is, hydration of the flavylium cation (AH^+) to give the colourless carbinol or pseudobase (B). This in turn, can, at an even slower rate, equilibrate to the open chalcone form (C), which is also colourless. The relative amounts of cation (AH^+), quinoidal forms (A), pseudobase (B) and chalcone (C) at equilibrium vary with both pH and the structure of the anthocyanin (Figs 4 and 5). For malvidin 3-glucoside (Fig. 4), for instance, the red cation (AH^+) is the sole structure only when the pH of the solution is less than 0·5. With increasing pH its concentration decreases as hydration to the colourless pseudobase (B) occurs, the equilibrium being characterized by a pK_h value of 2·6, when equal amounts of both forms exist. At this pH, however, small amounts of the colourless chalcone (C) and the blue quinoidal base (A) are also present, and the proportions of these and the

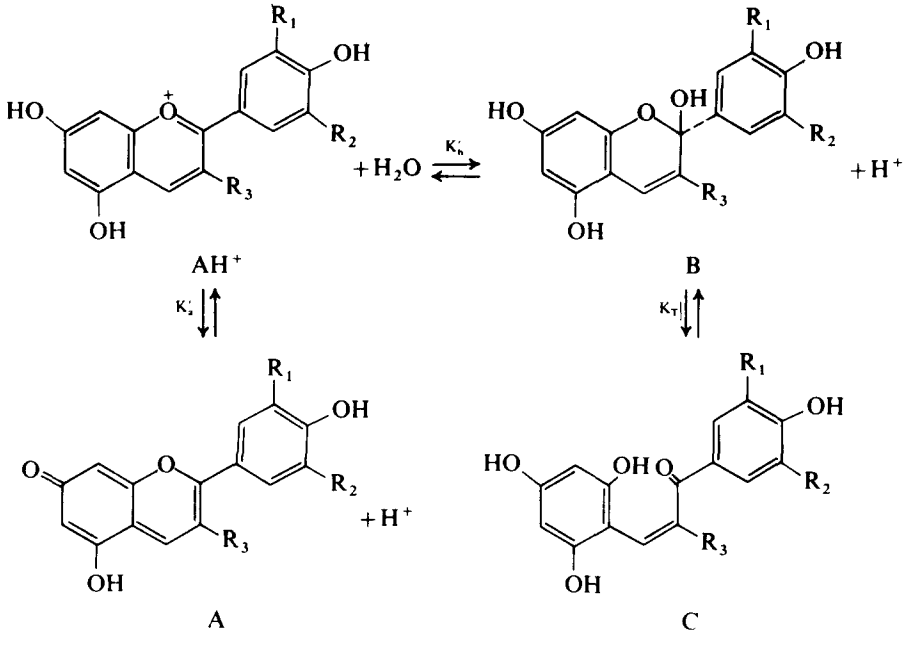

Fig. 3. Structural transformations of anthocyanins.

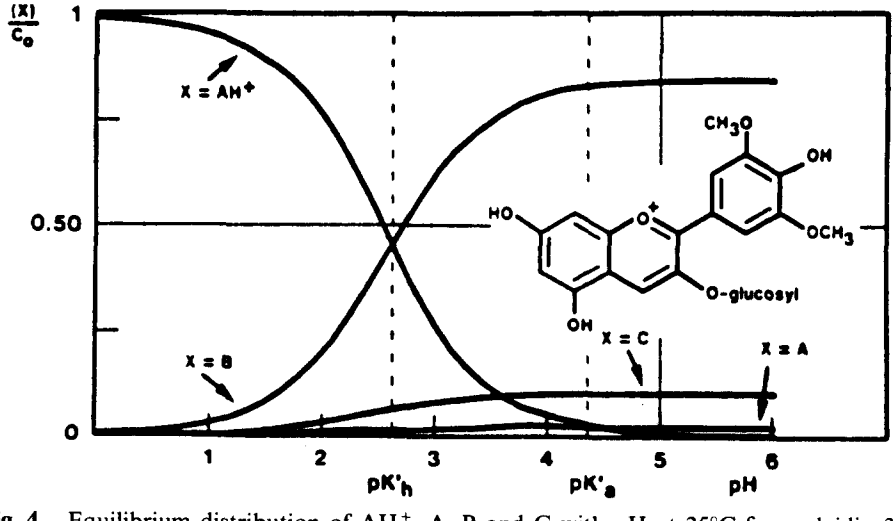

Fig. 4. Equilibrium distribution of AH^+, A, B and C with pH at 25°C for malvidin 3-glucoside. (From Brouillard, 1982.)

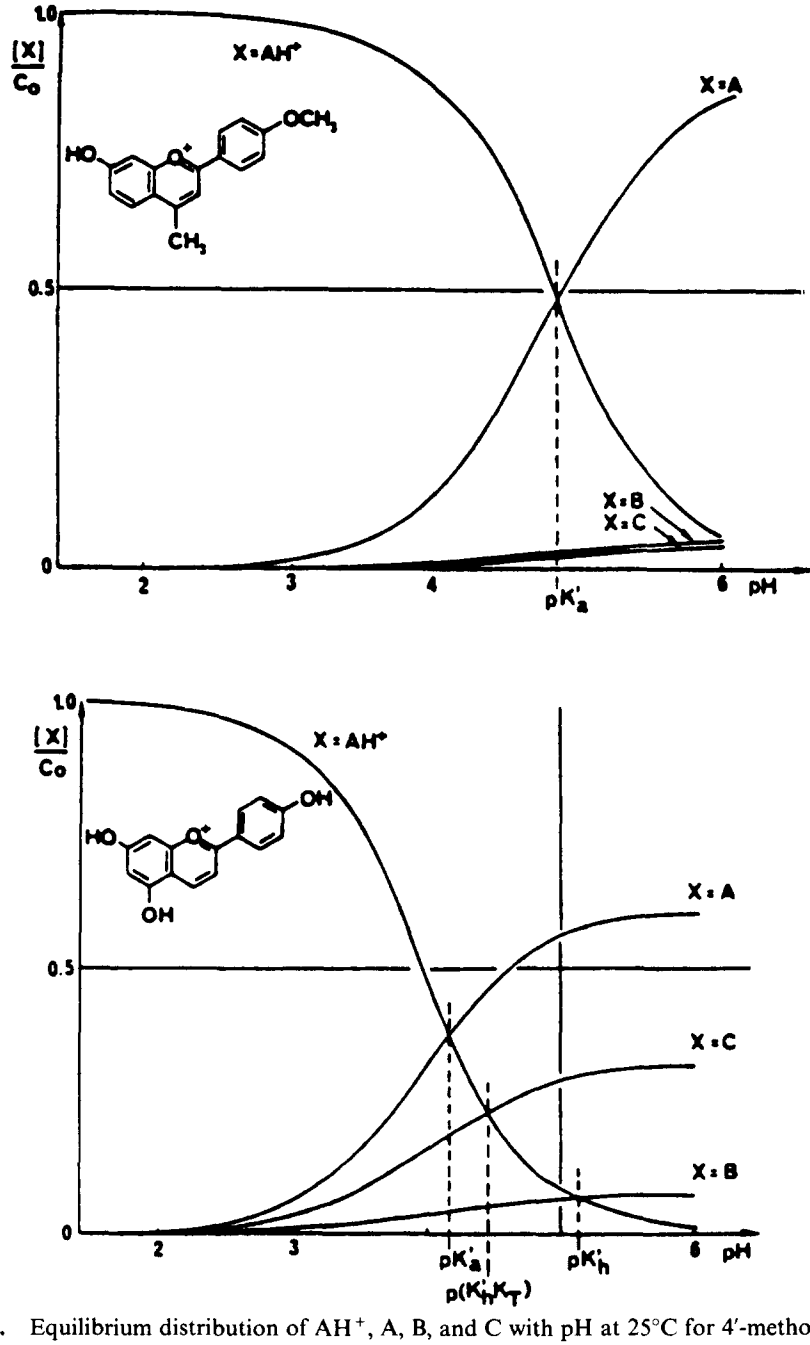

Fig. 5. Equilibrium distribution of AH^+, A, B, and C with pH at 25°C for 4′-methoxy-4-methyl-7-hydroxyflavylium chloride (upper) and apigeninidin chloride (lower). (From Brouillard, 1982.)

carbinol form (B) increase with increasing pH at the expense of the red cationic form (AH^+) up to about pH 4·5. Between pH 4 and 5·5 very little colour remains in the anthocyanin since the amounts of the two coloured forms (AH^+ and A) are very small. Above pH 5·5 the only coloured species present is the quinoidal form. It is thus clear that malvidin 3-glucoside itself does not confer much colour to a solution in which the pH ranges from 4 to 6.

Malvidin 3,5-diglucoside exhibits even less colour than malvidin 3-glucoside. This occurs because the pK_h value for the equilibrium between the cation form and the pseudobase of the diglucoside is approximately one pH unit lower than that of the monoglucoside (Brouillard & Delaporte, 1977). Also solutions of malvidin 3,5-diglucosides are essentially colourless above pH 4·0.

3-Deoxyanthocyanidins, which exist primarily in the cation, quinoidal and chalcone forms at pHs above 4, are exemplified by 4′-methoxy-4-methyl-7-hydroxyflavylium chloride and 4′,5,7-trihydroxyflavylium chloride or apigeninidin (Fig. 5). As can be noted, 4′-methoxy-4-methyl-7-hydroxyflavylium chloride exists only as the cation form up to a pH of 2·5, and primarily as the quinoidal isomer at pHs above 5. Similarly, apigeninidin exists only as the cation form at pHs 0–2·5, as a mixture of cation, quinoidal, chalcone and pseudobase at pHs between 2·5 and 6 and mostly as the quinoidal isomer at pHs above 6. The presence of a C4 substitution such as the methyl group, in the case of 4′-methoxy-4-methyl-7-hydroxyflavylium chloride, and the presence of a hydroxyl group at C5, in the case of apigeninidin, are presumed to be the cause of the high colour stability of these pigments in neutral solutions (Brouillard, 1982; Sweeny & Iacobucci, 1983). Pigments such as the last two discussed give rise to solutions that are coloured, regardless of the pH, and may find applications as food colorants.

In the course of the kinetic studies, Brouillard (1982) has also found that, on heating an anthocyanin solution, the equilibria are driven toward the chalcone form (C) and the resulting decrease in coloured forms (AH^+ and A) occurs. On cooling and acidification, the quinoidal base (A) and the carbinol base (B) are quickly transformed into the cationic form (AH^+), but the change of the chalcone form (C) is relatively slow. It takes approximately 12 h for the chalcone form of 3,5-diglucosides and 6–7 h for the chalcone form of 3-glucosides to reach equilibrium with the corresponding flavylium form at 25°C (longer at lower temperatures). Nonetheless, the fact that the reactions shown in Scheme 1 are reversible, and thus that the colourless forms of anthocyanins can be transformed into the coloured cationic and quinoidal forms, is highly significant and should be exploited in the manufacturing of food products such as fruit juices.

TABLE 3
Copigmentation of Cyanidin 3,5-Diglucoside (2×10^{-3}M) at pH 3·32[a]

Copigment (6×10^{-3}M)	λ_{max} (*nm*)	$\Delta\lambda_{max}$ (*nm*)	*A/mm at* λ_{max}	% *A increase at* λ_{max}
None	508	—	0·500	—
Aurone				
Aureusidin[b]	540	32	2·135	327
Alkaloids				
Caffeine	513	5	0·590	18
Brucine	512	4	1·110	122
Amino acids				
Alanine	508	0	0·525	5
Arginine	508	0	0·600	20
Aspartic acid	508	0	0·515	3
Glutamic acid	508	0	0·530	6
Glycine	508	0	0·545	9
Histidine	508	0	0·595	19
Proline	508	0	0·625	25
Benzoic acids				
Benzoic acid	509	1	0·590	18
o-Hydroxybenzoic acid	509	1	0·545	9
p-Hydroxybenzoic acid	510	2	0·595	19
Protocatechuic acid	510	2	0·615	23
Coumarin				
Esculin	514	6	0·830	66
Cinnamic acids				
m-Hydroxycinnamic acid	513	5	0·720	44
p-Hydroxycinnamic acid	513	5	0·660	32
Caffeic acid	515	7	0·780	56
Ferulic acid	517	9	0·800	60
Sinapic acid	519	11	1·085	117
Chlorogenic acid	513	5	0·875	75
Dihydrochalcone				
Phloridzin	517	9	1·005	101
Flavan-3-ols				
(+)-Catechin	514	6	0·890	78
Flavone				
Apigenin 7-glucoside[b]	517	9	0·840	68
C-glycosyl flavone				
8-C-Glucosylapigenin (vitexin)	517	9	1·690	238
6-C-Glucosylapigenin (isovitexin)	537	29	1·705	241
6-C-Glucosylgenkwanin (swertisin)	541	33	2·835	467
Flavonones				
Hesperidin	512	13	1·095	119
Naringin	518	10	0·985	97

TABLE 3—*contd.*

Copigment (6×10^{-3}M)	λ_{max} (*nm*)	$\Delta\lambda_{max}$ (*nm*)	*A/mm at* λ_{max}	% *A increase at* λ_{max}
Flavonols				
Kaempferol 3-glucoside	530	22	1·693	239
Kaempferol 3-robinobioside-7-rhamnoside (robinin)	524	16	1·423	185
Quercetin 3-glucoside (isoquercitrin)	527	19	1·440	188
Quercetin 3-rhamnoside (quercitrin)	527	19	1·588	217
Quercetin 3-galactoside (hyperin)	531	23	1·910	282
Quercetin 3-rutinoside (rutin)	528	20	1·643	228
Quercetin 7-glucoside (quercimeritrin)	518	10	1·363	173
7-*O*-Methylquercetin-3-rhamnoside (xanthorhamnin)	530	22	1·576	215

[a] From Asen *et al.* (1972).
[b] Formed a slight precipitate.

INFLUENCE OF COPIGMENTS

From consideration of pH, it appears that ordinary anthocyanins do not confer much colour since they exist largely in colourless forms over a wide pH range. In nature, however, anthocyanins are associated with highly coloured materials and are, therefore, not in the colourless carbinol state; this implies that the coloured forms must be stabilized by some unusual physico-chemical factors. One of these factors is related to the presence of compounds generally called copigments (Robinson & Robinson, 1931; Asen *et al.*, 1972). The names of 37 of these compounds are presented in Table 3. As can be noted they are flavonoids, alkaloids, amino acids and nucleotides. Other compounds can also act as copigments, and anthocyanins themselves can serve as copigments of other anthocyanins (Osawa, 1982).

Generally, copigments by themselves do not significantly contribute to the colour. Their main effect is to produce, with almost all natural anthocyanins under suitable conditions, a bathochromic wavelength shift and an increase in the absorbance of the visible band. The effect of copigments increases with increasing anthocyanin concentration and the ratio of copigment to anthocyanin (Asen *et al.*, 1972; Scheffeldt & Hrazdina, 1978). By suitable combinations of anthocyanins and copigments at appropriate concentrations and pH, Asen *et al.* (1972) were able to reproduce the spectra of flower petals. Such considerations help us explain the striking colours of many flowers and fruits when, at the pH of the cell-sap, they may be expected to have little or no colour. In particular, the occurrence of purple and blue flowers and fruits can be attributed mostly to the stabilization of the quinoidal base by copigmentation (Asen *et al.* 1970, 1972).

The most efficient copigments so far discovered are flavonols (quercetin and rutin), aureusidin (an aurone) and particularly C-glycosyl flavones such as swertisin (Somers & Evans, 1977). Flavonolsulphonic acids are also good copigments, presumably due to the added attraction of the negative charge of the sulphonic acid groups to the flavylium cation (Sweeny *et al.*, 1981). The structure of the anthocyanin–copigment complex has been a subject of some controversy, with some workers favouring a horizontal (end-to-end) hydrogen stabilization of the quinoidal base (Scheffeldt & Hrazdina, 1978; Chen & Hrazdina, 1981) while others (Sweeny *et al.*, 1981; Brouillard, 1983) support the vertical stacking complex first proposed by Goto *et al.* (1979). It is unlikely, however, that in water the anthocyanin–copigment interaction is due to hydrogen bonds since water is an excellent hydrogen bond donor and acceptor. Also, formation of an end-to-end complex does not prevent the pyrylium ring from suffering water attack. Association most likely occurs by a stacking process related to hydrophobic forces. This provides good protection against water nucleophilic addition and subsequent colour loss.

Copigmentation is affected by several factors, among which pH is an important one. It has been demonstrated that the copigment effect occurs from pH values close to 1 to neutrality (Asen *et al.*, 1970; Yazaki 1976; Williams and Hrazdina, 1979). The changes in absorbance as a function of pH, observed in model experiments for malvin in the absence and in the presence of the quercetin glucoside, spiraeoside (Yazaki, 1976), can be interpreted in the light of the mechanism proposed by Brouillard (1982) to describe the intermolecular copigmentation effect. At pH 1, malvin exists essentially in the flavylium form, and the 15 nm shift in the spectral maximum, observed in the presence of the copigment, is due to the interaction of the malvin flavylium cation with the copigment. The molecular extinction coefficient at the visible maximum of the complex is identical to that of the uncopigmented cation. At pH 2–3, there is an important colour loss for malvin alone and a significant colour retention for the solutions containing the anthocyanin and the copigment. Thus, the copigment effect is to reduce the production of the colourless carbinol pseudobase. At pH 4–6, the solutions containing only malvin are practically colourless, whereas the solutions with both malvin and copigment are still coloured. In this pH range quinoidal bases are formed, and again colour retention is due to a decrease in the amount of the carbinol pseudobase in the solutions.

Other factors which affect copigmentation are: type and concentrations of anthocyanin, concentration of copigment, temperature and metals. Thus, in studies of the effect of rutin on several malvidin glucosides at pH 3·2, the colour of the 3,5-diglucoside increased 10 times but that of the 3-glucoside only 1·5 times under comparable conditions (10^{-5} M anthocyanin,

$1{\cdot}2 + 10^{-2}$ M rutin; Scheffeldt & Hrazdina, 1978). To date, the effect of concentration of anthocyanin and copigment has been investigated only for a few compounds. Nonetheless, the results so far reported suggest that, for most anthocyanins and copigments, there is an optimum molar ratio of pigment to copigment at which intensity and stability are maximized. Jurd & Asen (1966), for instance, reported no change in the colour, spectra, or stability of cyanidin 3-glucoside at $3{\cdot}5 \times 10^{-5}$ M from the addition of 1 to 10 molar equivalents of quercitrin at pH 3–6. In this experiment, the molar ratio of the anthocyanin to copigment was probably below the minimum required for copigmentation. This supposition is supported by the result of Asen *et al.* (1972) who reported a bathochromic shift of 21 nm when the concentration of cyanidin 3,5-diglucoside was 10^{-2}M and the molar ratio of quercitrin to the anthocyanin was 3:1. However, in order to obtain a similar shift in the spectrum at the concentrations of 5×10^{-3} and 10^{-4}M for cyanidin 3,5-diglucoside, the molar ratios of the copigment to pigment had to be increased to 4:1 and 100:1, respectively. Similarly, the results reported by Scheffeldt & Hrazdina (1978) on the effect of anthocyanin concentration on copigmentation with rutin, suggest that, for a given concentration of copigment, there is a corresponding molar ratio of anthocyanin to copigment below which the colour intensity of the pigment–copigment solution decreases. The exact nature of the complex formed at pH 3 remains unknown, but the recent studies by Hoshino *et al.* (1980, 1981*a*, 1982) at pH 7·0 have provided very strong evidence that a vertical stacking of the anthocyanin quinoidal bases is occurring at this pH. The stacking would seem related to that shown by nucleosides, as the aggregates give strongly intensified circular dichroism curves with increasing concentration (Hoshino *et al.*, 1980, 1981*b*, 1982).

Other mechanisms of colour stabilization are: metal complexing and condensation of anthocyanins with catechin and acetaldehyde. Stable anthocyanin–metal complexes have been reported by Salt & Thomas (1957) for tin, Somaatmadja *et al.* (1964) for copper and Jurd & Asen (1966) for aluminium. Other authors have noted the presence of metal ions (Mg, Fe, K) in the structure of stable acylated anthocyanins (Takeda & Hayashi, 1977; Takeda, 1977). More recent reports, however, indicate that the presence of metal ions is not required to stabilize the colour of these metalloanthocyanins (Hoshino *et al.*, 1980) and Ca, Fe, Al and Sn afford some protection to common anthocyanins, but these complexes are generally unstable and decompose with time.

Condensation of anthocyanins with flavan-3-ols, such as catechin, can be induced by adding acetaldehyde. The reaction yields highly coloured new anthocyanin–catechin complexes linked by CH_3CH bridges (Timberlake & Bridle, 1976, 1977). Addition of catechin and acetaldehyde to crude extracts

of fruit anthocyanins also enhances and stabilizes their colour at pH 4–6 (Timberlake & Bridle, 1980; Green & Mazza, 1986). However, the identity and properties of these acetaldehyde-bridged polymers have not been determined.

CONCLUSIONS

The most recent findings on the influence of chemical structure, pH and copigments on colour stabilization of anthocyanin-containing media have been discussed. pH is shown to be the most important factor affecting the colour of anthocyanin, but various means of stabilization are given. The discussion on copigmentation is based mainly on results of studies of this phenomenon in flowers. It is evident from the list of known copigments, however, that some of these compounds are commonly found in foods and there is sufficient evidence to show that they do play a role in the colour stabilization of food products. Further work to identify new and more effective copigments, as well as to determine the optimum concentration and the identity and properties of the pigment–copigment complexes, is, however, needed. Most interesting is the recent discovery of acylated anthocyanins which retain a stable colour in slightly acidic or neutral solutions. This discovery may prove to be of particular importance to the technology of foods since, with new and better sources of these compounds and with better understanding of their physicochemical properties, they may find application to the colouring of food products.

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