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Effect of light and tannic acid on the stability of anthocyanin in DMSO and in water

F. O. Bobbio, M. T. do Nascimento Varella & P. A. Bobbio

Departmento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Caixa Postal 6121, 13081-970 Campinas, SP, Brazil

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The stability to light of cyanidin-3-glucosyl arabinoside in DMSO and in water, in acid solutions with or without complexation with tannic acid was determined by measuring the decrease of the absorbance of the solutions. The values for the apparent first-order reaction rate constant and $t_{1/2}$ were calculated. The hypochromic effect observed in DMSO solutions when tannic acid was added as a co-pigment prevented the calculation of the complexation constant (K) for the anthocyanin–tannic acid, indicating that the predominating form in this system is the AH^+. Although loss of absorbance was considerably faster in DMSO than in water under the same conditions, tannic acid effectively retarded discoloration in both DMSO and water.

INTRODUCTION

Substituting synthetic colours by anthocyanins introduces several problems, among which the most serious is the lower stability of natural colours compared with synthetic types. A considerable stabilisation can be achieved by co-pigmentation as demonstrated by Maccarone *et al.* (1987) and a number of co-pigments were described by Asen *et al.* (1972).

A mechanism for co-pigmentation has been proposed recently by Mazza & Brouillard (1990), Brouillard *et al.* (1991) and Dangles & Brouillard (1992) and is based on kinetic and thermodynamic data from the system malvin–chlorogenic acid in buffered water solutions at pH values near 3·5. One point of interest is that a strong hyperchromic effect associated with a bathochromic effect takes place when co-pigmentation occurs and the co-pigmentation is effective at a pH where a low concentration of the AH^+ form exists.

This paper reports on a study of the stability of an anthocyanin in DMSO and in water and the effect of co-pigmentation of the anthocyanin by tannic acid and propyl gallate under the effect of light and low pH value.

MATERIALS AND METHODS

All reagents used were analytical grade. DMSO (anhydrous) (Merck) was of spectral grade. Tannic acid (Fisher) had a molecular weight of 1701·18. Concentrated 97·5% H_2SO_4 (Merck) was used to lower the pH when needed. The anthocyanin was isolated from *Euphorbia caracasana* and identified following standard procedures (Francis, 1982) as cyanidin-3-glucosyl-arabinoside.

Preparation of the solutions

Solution (a): 0·0280 g of Na_2HPO_4 and 0·9410 g of citric acid were dissolved in DMSO in a glass stoppered volumetric flask of 50·0 ml. In this solution were dissolved from 0·085 to 0·094 g of pure dry anthocyanin followed by 150 μl of H_2SO_4 to ensure a sufficiently acid medium (nominal pH 2·0).

Solvent (b); 2·0 ml of a 0·2 M solution of Na_2HPO_4 and 170 μl of H_2SO_4, were mixed in a 100 ml volumetric flask and the volume completed with a 0·1 M solution of citric acid. The pH of the final solutions was approximately 2·0; to 50·0 ml of this solution from 0·085 g to 0·094 g of pure dry anthocyanin were added.

The same amounts of anthocyanin were used to prepare a 50·0 ml water or DMSO solution, in which only H_2SO_4 was used to lower the nominal pH to approximately 2·0. The volume of H_2SO_4 used for the DMSO solution was 200 μl and for water solution 100 μl.

The solutions were distributed into screw-caps Pyrex tubes of 10 ml capacity. Half of the tubes were stored at 20 ± 1°C in the dark and half were placed between two parallel 'day-light' 40W tubes with nominal capacity of 2500 lux. The distance of each tube to the nearest lamp was 10 cm.

The same procedure was used for the preparation of the solutions to which tannic acid (water or DMSO) or propyl gallate (water) were added in a 1 : 1, 1 : 2 (mol : mol) ratio at pH 2·0 and 3·0.

Solutions of the anthocyanin were also, prepared at pH 3·5 and 4·3 in buffered water solutions and in DMSO where H_2SO_4 was used to obtain comparable acidity. Higher pH (5·0) produced immediate loss of colour.

Visible and UV spectra and absorbance at λ_{max} were recorded with a Beekman DU-70 spectrophotometer.

RESULTS AND DISCUSSION

It is accepted that solutions of anthocyanins in water lose colour through the initial formation of an hemiacetal (or pseudo base carbinol) at C_2 from the flavylium ion. The hemiacetal is the result of the nucleophilic addition of water molecules at C_2. In the resulting product, the extended double bond conjugation is disrupted causing a decrease in the absorbance (Adams, 1973).

The nucleophilic addition has its rationale based on the reactivity of the resonating structures of carbonium ions at C_2 and C_4. Although a nucleophile like HSO_3^- adds at C_4 and not at C_2 (Timberlake & Bridle, 1968), position four has been considered unfavourable for the nucleophilic addition (Brouillard, 1982) and, at equilibrium, only a very small amount of the C_4–OH intermediate was present.

The colour losses of anthocyanins are normally studied in water solutions for solubility reasons and also because the stability of anthocyanins in food systems is the main point of practical interest. It must also be mentioned that anthocyanins are practically insoluble in any aprotic solvent except DMSO which is a good, highly polar, solvent for many anthocyanins.

Having in mind the hemiacetal formation, DMSO was chosen as a solvent which could prove difficult to add at C_2, and therefore the colour of the anthocyanin would be stable in this solvent in the dark or when irradiated with 'day-light' lamps.

Since the experimental work was mainly carried out at low pH in water and acid solutions of DMSO (lower than the pK_a of the anthocyanin), where the concentration of AH^+ is larger than any other form, addition at C_2 and C_4 could take place through the protonated molecule of DMSO.

Results are presented in Table 1 as the periods of half life calculated from the values of k (reaction rate constant) including the effect of the buffer and H_2SO_4 in the dark and under light.

A fast reaction takes place in DMSO and there was no significant difference between solutions with buffer or sulphuric acid in water or between those with sulphuric acid or phosphate and citric acid added to DMSO solutions.

An attempt to run the experiment at pH 5·0 in water where the concentration of the AH^+ is drastically reduced, resulted in complete loss of colour after a few minutes under light.

To test the possibility of complexation of the anthocyanins in DMSO with copigments like tannic acid which effectively retarded discoloration of anthocyanins in water solutions (Bobbio *et al.*, 1992) under light and in the dark, tannic acid was added to the DMSO at nominal pH 4·3 and to the water solutions of anthocyanin at pH 4·3.

Some degree of protection was achieved since absorbance decreased by 37·8% from its initial value in the DMSO without tannic acid and by 16·7% with tannic acid after 52 h of reaction when then molar ratio copigment : pigment was 2 : 1. Much lower values were found in water where a 8 nm bathochromic shift was registered.

The values of the complexation constant (K) for water solutions as well as the n values (stoichiometric constants) at pH 3·5 using buffered solutions have been calculated following the method described by Brouillard *et al.* (1989, 1991). The low value of the constant (K) (31 ± 6 $\text{M}^{0\cdot5}$ at 20°C) indicates that copigmentation should be considered as quite incomplete but nevertheless a reasonable degree of protection is afforded by tannic acid (Table 2). The n value was 0·52 ± 0·01 suggesting that 2 molecules of the anthocyanin are associated with 1 molecule of tannic acid. In DMSO the hypochromic shift prevented the calculation of the K value.

It is to be noted that, although copigmentation was presumed to protect the anthocyanin from the nucleophillic attack of water at C_2 producing high hyperchromic and bathochromic changes such effects were not observed, although a considerable protection of the anthocyanin colour resulted from the addition of tannic acid in DMSO solution and the protective effect in water is apparently not in proportion to the low

Table 1. Half lives ($t_{1/2}$) in DMSO and in water under light or in the dark, in acid solutions

Solvent	$t_{1/2}$ (h)			
	H_2SO_4[a]		Buffer[b]	
	Dark	Light	Dark	Light
DMSO	7 ± 1 × 10[c]	1·1 ± 0·2 × 10[c]	7 ± 1 × 10[d]	1·1 ± 0·2 × 10[d]
Water	4·6 ± 0·6 × 10[a]	3·2 ± 0·3 × 10[a]	4·4 ± 0·3 × 10[b]	2·6 ± 0·2 × 10[b]

[a] H_2SO_4, used to lower the pH to 2·0.
[b] Phosphate–citric acid buffer, pH 2·0.
[c] H_2SO_4, used to prepare an acid solution.
[d] Na_2HPO_4 and citric acid added to the DMSO.

Table 2. Percentage of absorbance lost after 52 h of reaction under light in water (pH 4·3) and in acid DMSO

Solvent	Tannic acid	Without tannic acid
DMSO	16·7	37·8
Water	3·6	14·3

value of *K* in water solution. The possibility of DMSO or water addition at C_4 when C_2 is protected by tannic acid is being investigated although position four was considered kinetic and thermodynamically unfavourable (Brouillard *et al.*, 1982)

Propyl gallate was used as a possible inhibitor of colour loss of the anthocyanin in water at pH 2·0 and 3·0, using different proportions of the gallate and anthocyanin from 1 : 10 to 2 : 1. Propyl gallate had no effect under light or in the dark at either pH value.

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