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Stability of anthocyanic pigments from *Panicum melinis*

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The two major pigments from the inflorescences of *Panicum melinis* were identified as cyanidin-3-caffeoylarabinosylglucoside and a non-acylated cyanidin-3-glycoside. The stabilities in both light and dark of the partially pure pigments as well as the pure ones were studied at pH 2·0. The stability of the crude extract of *P. melinis* was studied under the same conditions. Light strongly affected color stability of all fractions which showed good stability in the dark. The decreasing order of instability to light was: partially purified anthocyanins, crude extract, pure anthocyanins.

INTRODUCTION

The substitution of synthetic coloring matter by natural pigments like anthocyanins in the food industry faces a twofold problem: scarcity of commercially useful sources and instability of many of the natural pigments. Anthocyanins, particularly red ones, have few possible commercial sources (Francis, 1989) and with few exceptions are discolored by light and pH (Mazza & Brouillard, 1987).

Since *Panicum melinis* (a grass with deep red inflorescences) varieties could be a valuable source of red anthocyanins the authors wish to report, in this paper, the identification of the major anthocyanin in a wild variety of the plant, and report on the stability of the crude extract of the inflorescence as well as on purified fractions under the effect of light at pH 2·0 under nitrogen.

MATERIALS AND METHODS

Extraction of the anthocyanins

A variety of *P. melinis* that grows wild was harvested in May and June of 1988 and the inflorescences were extracted overnight with 0·05% HCl in methanol at 5°C ($[H^+] = 6 \times 10^{-4}$) in the dark under nitrogen. The extract was decanted and the process repeated twice. The methanolic extracts were combined and concentrated at 30°C under reduced pressure until a 5·8% concentration of anthocyanins was reached. After removal of the chlorophyll with Et_2O the extract was stored under N_2 at −18°C. The pigments content in the extract was measured by the method of Fuleki and Francis (1968).

Purification, separation and identification of the anthocyanins

The crude extract was chromatographed on a column of PVP according to Wrolstad and Struther (1971) with acidified MeOH : H_2O (70 : 30) as solvent, and Whatman 3 mm paper, using the following development systems: 1% HCl (conc. HCl–water, 3 : 97); BAW (*n*-butanol–acetic acid–water, 6 : 1 : 2) and BFW (*n*-butanol–formic acid–water, 15 : 2·5 : 2·4) and HPLC carried out at 35°C on a Nucleosil C^{-18} column, with isocratic elution using methanol, water, acetic acid (20 : 70·4 : 9·6). The pure major anthocyanin was identified by spectroscopic and chemical methods fully described in the literature (Francis, 1982). The acid in the acylated anthocyanin was identified by HPLC performed at 35°C on a Bondapak C^{-18} column with isocratic elution using citric acid–acetonitrile–water (2 : 16 : 82). Cinnamic acids were used as standards.

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Stability of the anthocyanins

Solutions of the pure anthocyanins and of the extracts were made in citrate–phosphate buffer pH 2·0, and distributed into tightly sealed screw cap tubes, flushing the head space with N_2. Half of the tubes were placed at 10 cm from a 40 W, 2·500 lx daylight lamp at 20 ± 1°C and half were placed in the dark at the same temperature.

The loss of absorbance measured spectrophotometrically at time intervals for each system up to 240 h was used as a measure of color stability, by establishing the values of first order reaction rate constants and the corresponding values of $t_{1/2}$ for each system.

RESULTS AND DISCUSSION

Identification of the anthocyanins

The crude methanolic extract of *P. melinis* pigments proved to be a complex mixture of several anthocyanins and yellow flavonoids.

Column chromatography on PVP yielded two major red bands well spaced apart, and four minor ones not recovered.

The two partially purified fractions, namely F_1 and F_2, contained yellow flavonoids as impurities and were further purified by paper chromatography. Using BAW as solvent, F_1 and F_2 were split into two zones each: A_1, B_1 and A_2, B_2. In each case both A_1 and A_2 were the nearest to the starting line. Spectral data, paper chromatography in different solvents and HPLC data demonstrated the identity of zones A_1 to A_2 and B_1 to B_2. Therefore in this work these fractions will be designated simply as A and B. Judging by color intensity, zone A was present in higher amounts and was identified as cyanidin-3-caffeoylarabinosylglucoside. Zone B present in smaller amounts was partially identified as a non-acylated cyanidin.

The color properties of the yellow flavonoids eluted from the paper, are those of flavonols as established by Harborne (1967).

Comparative stability of the different fractions

The results in Table 1 show that the half life of each system is considerably decreased by the effects of light and furthermore, the increased purification of the isolated fractions resulted in a decreased resistance to the effects of light on the anthocyanins.

The presence of flavonols loosely associated, through complexation, with F_1 and F_2, with F_2 being associated with apparently larger amounts and possibly different types of flavonols than F_1, can explain the increased stability of F_2 over F_1 and of both over the pure anthocyanins of fractions A and B. By the same token the different migration of F_1 and F_2 on PVP might be attributed to a less loose association between flavonols and F_2 and to a larger proportion of the flavonols in zone F_2.

The existence of complexes between anthocyanins and flavonols has been reported in several papers (*inter alia* Timberlake & Bridle, 1977; Scheffeldt & Hrazdina, 1978; Chen & Hrazdina, 1981; Osawa, 1982), as well as the effect of the ratio of the anthocyanins to the flavonols on the formation of such inter-molecular complexes.

The half life of the cyanidin-3-caffeoylarabinosylglucoside, pigment A, has the same values as for the non-acylated cyanidin, pigment B, indicating that monoacylation of anthocyanins does not necessarily improve color stability. This result is in agreement with results reported by Mazza and Brouillard (1987).

Although Table 1 indicates that the half life of the anthocyanins is increased by its complexation with the flavonols and that the increase is related to the amount of flavonols present, the data for the crude extracts show a lower than expected value for its half life. This could be due to the presence in the crude extract of compounds that would hinder the association of the anthocyanins with flavonols or to a preferred interaction between flavonol molecules forming a more stable association.

A different reaction pathway must occur in the absence of light and the formation of complexes between flavonols and anthocyanins is no longer capable of protecting the anthocyanins. It seems relevant to point out the similarity of the values for the $t_{1/2}$ in the purified fractions (F_1, F_2, A and B) and the lower value for the crude extract richer in flavonols but also in several non-identified compounds that could interact very slowly with the anthocyanins.

Table 1. Effect of light on the pigments from *P. melinis* in different degrees of purification, at 20 ± 1°C and pH 2·0

	Extract		F_1		F_2		A		B	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
k^a	0·8941	0·0187	0·5040	0·00440	0·0969	0·00464	1·6202	0·00461	1·669	0·00462
$t_{1/2}$ (h)	78	3706	137	15750	715	14933	43	15002	42	14998
k_{light}/k_{dark}	47·5		115·0		21·9		348·9		357·1	

[a] Reaction rate constant: $k \times 10^{-2}$ (h^{-1}).
Reaction time 240 h.

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