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## PLANT PIGMENTS

# Polarographic Measurement and Thermal Decomposition of Anthocyanin Compounds

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Pelargonidin-3-monoglucoside, petunidin-3-monoglucoside, and malvidin-3-monoglucoside usually yielded two polarographic waves in HCl-sodium citrate or tartrate supporting electrolytes. The electron change was one for each of the two waves. The half-wave potentials varied according to pH. The length of time required to destroy 90% of the P-3-G upon being heated at 38°, 49°, and 71° C. followed a first-order reaction. The presence of ascorbic acid, H<sub>2</sub>O<sub>2</sub>, FeCl<sub>3</sub>, and NaNO<sub>3</sub> significantly altered the destruction rates as compared with control solutions. At 100° C., Pt-3-G and M-3-G also decomposed according to a first-order reaction. Polarographic wave height was related to the anthocyanin content of strawberry juice. Interactions among constituents of the food, heat, and anthocyanin content may markedly affect the stability of anthocyanins in food.

CHELATION by copper of anthocyanins (20) and the stability of anthocyanins to heat (6, 15) have been studied in our laboratories as part of a program concerned with the microbiological activity of anthocyanin pigments (19). This report records the half-wave potentials of P-3-G, M-3-G, and Pt-3-G and the stability of P-3-G in the presence of redox compounds.

Zuman (23) determined the half-wave potentials of six anthocyanin compounds. He listed one half-wave potential for each compound except delphinin, which possessed two polarographic waves. Somaatmadja, Powers, and Hamdy (20) observed that M-3-G also yielded two waves. The effects of oxidizing and reducing agents, pH level, heat, and light on the stability of anthocyanin compounds have been extensively investigated (2, 4-6, 9, 11-13, 16, 22), but only some investigators (3, 7, 8, 10, 14, 21) have reported that anthocyanins de-

compose according to a first-order reaction. Pratt, Powers, and Somaatmadja (17) observed that strawberry and grape anthocyanins were more stable in the juice than as extracted, purified pigments.

### Experimental

The P-3-G was obtained from strawberries (10); the Pt-3-G and M-3-G were isolated from Cabernet Sauvignon grapes by a paper chromatographic procedure (19). A 0.1M HCl solution buffered to different levels with sodium citrate and a tartrate supporting electrolyte were used for the polarographic determinations. Different pH levels were used to relate half-wave potentials to pH. After obtaining satisfactory polarograms of the isolated pigments, polarographic analyses were made using strawberry juice as the substrate to learn whether anthocyanin compounds could be measured in mixed systems.

The stability of P-3-G in the presence

of FeCl<sub>3</sub>, NaNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, ascorbic acid, and cystine was determined at 38°, 49°, and 71° C. The concentrations used were 25 mg. per 100 ml. Quantitative measurements of the P-3-G degradation were made according to the method of Meschter (11), and the destruction rates were established by calculating the decimal reduction time (7).

For Pt-3-G and M-3-G the destruction rate curves were plotted to determine the decomposition rate.

### Results

**Half-Wave Potentials.** Two polarographic waves resulted when P-3-G, M-3-G, and Pt-3-G, isolated from strawberries and grapes, were analyzed. Two waves also resulted when strawberry juice was used. Table I shows the half-wave potentials of P-3-G at pH 1.0 to 6.0. M-3-G yielded three waves (-0.196, -0.247, and -0.372) at pH 3.5, but only two waves above pH 3.9. Pt-3-G yielded two or three waves ac-

**Table I. Half-Wave Potentials of Pelargonidin-3-monoglucoside at pH Levels from 1.0 to 6.0**

pH	Half-Wave Potential	
	1st wave	2nd wave
1	-0.08	-0.41
2	-0.11	-0.42
3	-0.13	-0.43
4	-0.15	-0.44
5	-0.18	-0.45
6	-0.20	-0.46

ording to pH, and the half waves were 0.08 less negative than that of the M-3-G. Plotting of  $\log(i_d - i)/i$  against the applied potential established that the electron change was 1 for each wave. The half-wave potential of the second wave of P-3-G was in the same range as those reported by Zuman (23), although a different supporting electrolyte was used. Zuman's polarograms show two waves at some pH levels, but he listed only one half-wave potential. Somatmadja, Powers, and Hamdy (20) observed two waves for malvidin-3-monoglucoside. The authors' half-wave potential values agree with his. Table II shows the effect of concentration of strawberry juice on wave height, while Figure 1 shows the effect of pH on wave height. Comparisons could not be made between the pigment and strawberry juice in supporting electrolytes of identical concentrations because the pH was not the same, but the half-wave potentials appeared to be the same at any given pH.

**Thermal Destruction Rates.** The 90% destruction values—*D* values in hours—of P-3-G for the different addi-

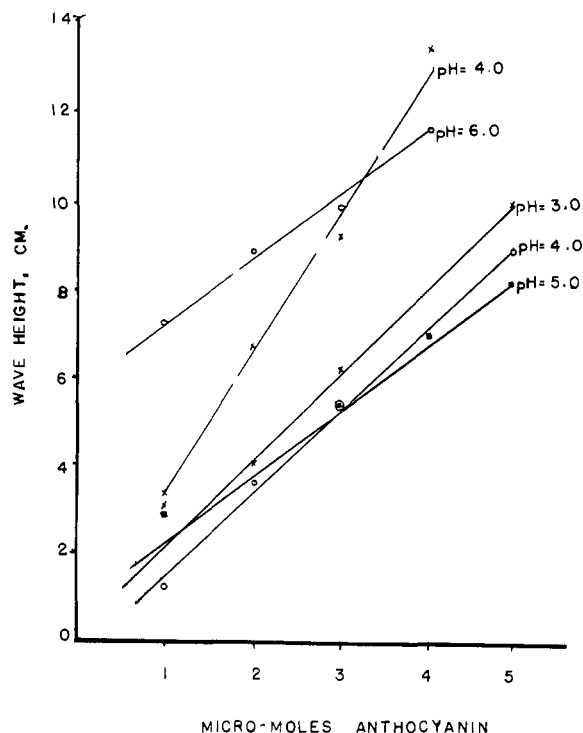
**Table II. Effect of Concentration of Strawberry Juice on Wave Height**

Strawberry Juice	Wave Height, Cm.
100%	4.0
Juice diluted to 80%	3.3
Juice diluted to 50%	1.3

**Table III. *D* Values (90% Destruction Values) at 38°, 49°, and 71° C. for Pelargonidin-3-monoglucoside Isolated from Strawberries**

Additives, <sup>a</sup> Mg./100 ml.	<i>D</i> Values, Hours		
	38° C.	49° C.	71° C.
No additives (control)	5833	1435	90
Ascorbic acid	1536	764	75
NaNO <sub>3</sub>	5799	1647	131
H <sub>2</sub> O <sub>2</sub>	All samples discolored immediately		
FeCl <sub>3</sub>	...	67	...
Ascorbic acid + H <sub>2</sub> O <sub>2</sub>	2200	1085	156
Ascorbic acid + NaNO <sub>3</sub>	1518	784	70
Ascorbic acid	...	574	...
Ascorbic acid + H <sub>2</sub> O <sub>2</sub> + FeCl <sub>3</sub>	2092	462	40
Ascorbic acid + NaNO <sub>3</sub> + FeCl <sub>3</sub>	937	423	29
NaNO <sub>3</sub> + FeCl <sub>3</sub>	...	90	...
Cystine	4634	1704	80
Ascorbic acid + 1(-) cystine	965	670	55

<sup>a</sup> When more than one substance was added, each substance was added at 25 mg./100 ml. Concentration of P-3-G used in each treatment was 25 mg.-%.



**Figure 1. Effect of pH on the polarographic wave height of M-3-G and Pt-3-G**

The M-3-G is represented by solid lines and Pt-3-G by the broken lines

tive treatments are presented in Table III. Analysis of variance of these data demonstrated a highly significant difference among the additive treatments; this is illustrated in Table IV, which shows the effects of treatment at 49° C. The addition of 25.0 mg.-% of H<sub>2</sub>O<sub>2</sub> brought about discoloration of the pigment at a rate too rapid for measurement. In all statistical calculations involving this treatment, the *D* values were

**Table IV. Analysis of Variance for 90% Destruction Values (*D* Values) at 49° C. for P-3-G Isolated from Strawberries**

Source of Variation	Degrees of Freedom	Mean Square
Replications	1	215,060 <sup>a</sup>
Ascorbic acid—no ascorbic acid	1	105,787 <sup>b</sup>
FeCl <sub>2</sub> —no FeCl <sub>2</sub>	1	3,373,650 <sup>a</sup>
NaNO <sub>3</sub> —no NaNO <sub>3</sub>	1	125,847 <sup>b</sup>
Ascorbic acid—no ascorbic acid X FeCl <sub>2</sub> —no FeCl <sub>2</sub>	1	1,838,051 <sup>a</sup>
Ascorbic acid—no ascorbic acid X NaNO <sub>3</sub> —no NaNO <sub>3</sub>	1	3,814
FeCl <sub>2</sub> —no FeCl <sub>2</sub> X NaNO <sub>3</sub> —no NaNO <sub>3</sub>	1	84,827 <sup>b</sup>
Ascorbic acid—no ascorbic acid X FeCl <sub>2</sub> —no FeCl <sub>2</sub> X NaNO <sub>3</sub> —no NaNO <sub>3</sub>	1	1,793
Error	7	14,906

<sup>a</sup> Significance exceeds 0.01 probability level.

<sup>b</sup> Significance exceeds 0.05 probability level.

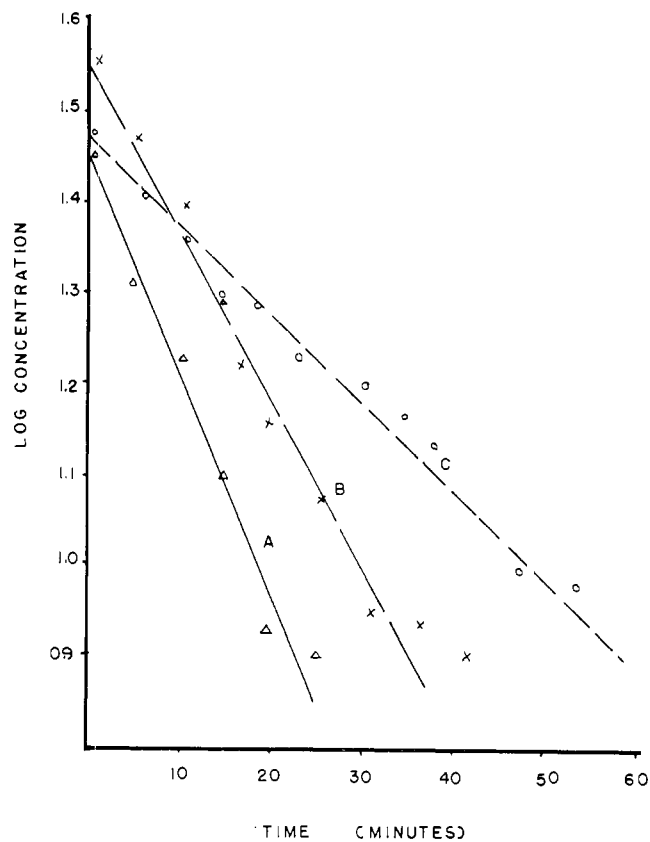


Figure 2. Kinetics of heat degradation of anthocyanins at 100° C.

(A = M-3-G, B = Pt-3-G, and C = P-3-G)

considered to be zero. When test solutions contained 25 mg.% of ascorbic acid, the destruction of the pigment at each temperature tested was more rapid than in the control batches. The slopes of thermal destruction curves were such that the control and ascorbic acid treatments intersected at approximately 79° C.

Cystine and  $\text{NO}_3$  did not significantly affect the stability of the P-3-G. The  $\text{Fe}^{+3}$  treatment reduced the  $D$  values very significantly. Somaatmadja (18) showed that the presence of  $\text{Fe}^{+2}$  significantly affected the decomposition rate of M-3-G. In the combination  $\text{FeCl}_3$ - $\text{NaNO}_3$  treatment,  $\text{NaNO}_3$  had a statistically significant protective effect at 49° C.

The slopes of the destruction rate

curves of M-3-G, Pt-3-G, and P-3-G in pure solution at 100° C. were  $-0.020$ ,  $-0.0153$ , and  $-0.011$ , respectively. These curves are presented in Figure 2.

The model studies may have practical applications. The fact that nitrate ions protect anthocyanin from the destruction by  $\text{Fe}^{+3}$  is interesting in view of the apparent relation of high nitrate content to detinning of cans. Culpepper and Caldwell observed (3) the effect of metals dissolved from the can on the instability of anthocyanin in canned peaches. The demonstration of interactions between heat, redox compounds other than anthocyanins, and anthocyanins may also have practical applications in which foods such as jams, jellies, fruit juices, and canned foods are subjected to different amounts of heat.

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