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ANTHOCYANIN STABILITY: A CHEMOMETRICAL APPROACH

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The stability of cyanidin 3-glucoside towards five different factors (pH, anthocyanin concentration, sodium chloride concentration, ascorbic acid concentration, Oxygen) was studied during 60 days storage at room temperature using a 2^{4-1} reduced factorial design. The influence of each individual parameter on anthocyanin stability was found to vary as a function of time. Sodium chloride and ascorbic acid were, respectively, the variables most contributing to and against anthocyanin stability. Results suggest there may be some degree of interaction between anthocyanin/ascorbic acid, and between pH/Oxygen, affecting anthocyanin degradation.

KEYWORDS: anthocyanins, stability, chemometrics, multivariate experiment, reduced factorial design.

INTRODUCTION

The study of anthocyanin stability towards different physical and chemical parameters has been mostly performed through the systematical variation of one-factor-at-the-time. This approach requires the performance of many experiments and fails to account for interactions between factors. The use of a chemometrical approach allows the simultaneous investigation of a larger amount of variables with a smaller number of trials, and may detect the existence of interaction between factors.

The purpose of this study was to quantify the impact of five factors on the stability of cyanidin 3-glucoside (cy3glc) upon a 60-day storage period at 23ºC, and to identify possible interactions between parameters (Cabrita, 1999).

EXPERIMENTAL

Experimental design: The following stability factors were selected to participate in the study: pH, sodium chloride concentration, ascorbic acid concentration, cy3glc concentration and Oxygen. A 2-level variation was used for each factor:

level	pH	[NaCl]	[cy3glc]	[AA]	O_2
(-)	3.1	1.0 M	0.025 mg/ml	0.050 mg/ml	0 min
(+)	4.0	5.0 M	0.100 mg/ml	1.250 mg/ml	1 min

The number of variables was reduced by combining anthocyanin and ascorbic acid concentration into one factor, the ratio [AA]/[cy3glc]. The experimental design was based on a 2^{4-1} reduced factorial design, corresponding to a half-fraction with 8 samples. The resulting design matrix defines which level will be actually assumed by the individual factors in each sample, i.e., either a high (+) or low (-) level:

experiment	pH	[NaCl]	[AA] / [cy3glc]	O_2	interactions		
	1	**2**	**3**	**1.2**	**1.3**	**2.3**	**1.2.3**
1	-	-	-	+	+	+	-
2	+	-	-	-	-	+	+
3	-	+	-	-	+	-	+
4	+	+	-	+	-	-	-
5	-	-	+	+	-	-	+
6	+	-	+	-	+	-	-
7	-	+	+	-	-	+	-
8	+	+	+	+	+	+	+

Materials and Methods: A set of 8 samples containing adequate amounts of NaCl, ascorbic acid and cy3glc (Table 2), was prepared. Then, each sample was dissolved with 10 ml of the corresponding buffer solution, either pH 3.1 or pH 4.0. The UV-Visible spectra of each sample was recorded 1 hour after dissolution, and then after 1, 2, 5, 8, 15 and 60 days. Absorbance values at $\lambda_{Vis\text{-}max}$ (510-520 nm) were registered for each sample. Sample tubes were sealed and stored in the dark at room temperature (23ºC) between measurements. After every UV-Visible measurement session, samples assigned a high (+) level for the oxygen variable

were sparged with a gentle stream of oxygen during 1 min, and then sealed and stored. PLS (Partial Least Square) coefficients were calculated using SIRIUS 6.5.

RESULTS AND DISCUSSION

The results were presented as percentages of retained colour measured at determined intervals during a period of 60 days.

exp.	day 0	day 1	day 2	day 5	day 8	day 15	day 60
1	100	98	95	86	76	56	18
2	100	104	102	90	76	47	0
3	100	103	104	104	105	106	98
4	100	130	137	139	138	133	31
5	100	99	97	84	71	34	0
6	100	102	96	85	67	36	0
7	100	104	109	109	109	110	82
8	100	97	94	76	56	0	0

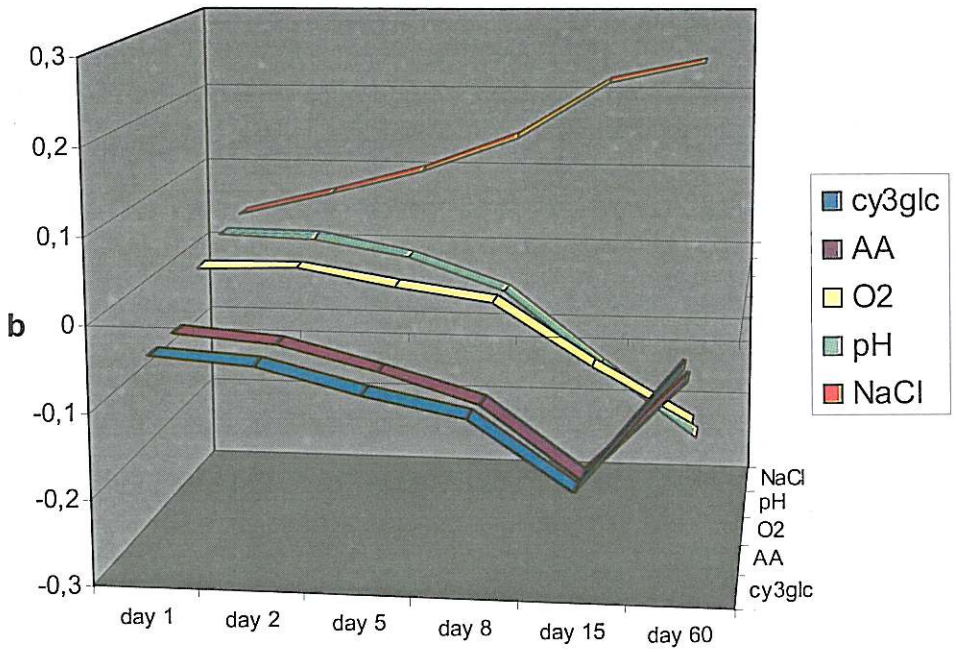
Results indicate that the simultaneous variation of several experimental factors may produce both beneficial and deleterious effects on colour and anthocyanin stability. In order to determine the influence of each parameter on stability, the absorbance values registered each day (A_t) were modelled by PLS to give a model of the type:

$$Y_{(t)} = b_0 + b_{pH}\cdot pH + b_{NaCl}\cdot[NaCl] + b_{cy3glc}\cdot[cy3glc] + b_{AA}\cdot[AA] + b_{oxygen}\cdot O_2$$

where Y stands for sample response after day t and b_i (i = pH...oxygen) are the regression coefficients estimated for each factor:

$Y_{(t)}$	b_0	b_{pH}	b_{NaCl}	b_{cy3glc}	b_{AA}	b_{oxygen}
$Y_{(1)}$	0.047	0.035	0.039	-0.040	-0.040	0.014
$Y_{(2)}$	0.042	0.031	0.069	-0.051	-0.050	0.016
$Y_{(5)}$	-0.035	0.010	0.102	-0.081	-0.081	-0.004
$Y_{(8)}$	-0.129	-0.030	0.146	-0.116	-0.104	-0.021
$Y_{(15)}$	-0.347	-0.112	0.217	-0.202	-0.185	-0.095
$Y_{(60)}$	-0.713	-0.209	0.241	-0.082	-0.042	-0.163

Generally, the regression coefficients estimated by PLS-modelling were sorted in order $b_{NaCl} > b_{pH} > b_{oxygen} > b_{cy3glc} > b_{AA}$ during most of the experiment period, except at day 60, when b_{pH} became the most negative parameter.



CONCLUSIONS

Results suggest that the contribution of each variable to the stability of cy3glc varied as a function of time. The concentration of NaCl was the only factor with a protective effect on colour of cy3glc throughout all the storage period. The concentration of ascorbic acid was the most deleterious parameter to stability, only surpassed at day 60 by the effect of pH. NaCl concentration, followed by ascorbic acid concentration and pH, were the most influencing parameters on the colour stability of cy3glc. There seems to be some degree of interaction between ascorbic acid/anthocyanin and pH/Oxygen.

REFERENCES

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