# Stability of pelargonidin 3-glucoside in model solutions

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## AIM

To elucidate the mechanisms involved in the alteration of the colour produced in strawberry derived products during processing and storage.

## METHODOLOGY

Aqueous solutions of Pg 3-glucoside ($1{,}8x10^{-3}$M in citrate buffer 0,1M at pH 3,5) were prepared and stored:

**(a)** in the presence of oxygen (**GOx**)

**(b)** in the presence of oxygen and sugars (5,7%, glucose:fructose:sacarose, 42:37:21) (**GZ**)

**(c)** in anaerobic conditions (**GsinOx**)

Solutions were stored in darkness at 25ºC for 8 months, and their evolution was followed by UV-vis spectrophotometry, HPLC-DAS and LC-MS.

## RESULTS AND DISCUSSION

A progressive decrease occurred in the levels of the Pg 3-gluc in the solutions. After two months, hardly anthocyanin was detected in the assays carried out in the presence of oxygen, whereas about half of its initial concentration was still present in the solutions maintained in anaerobic conditions.

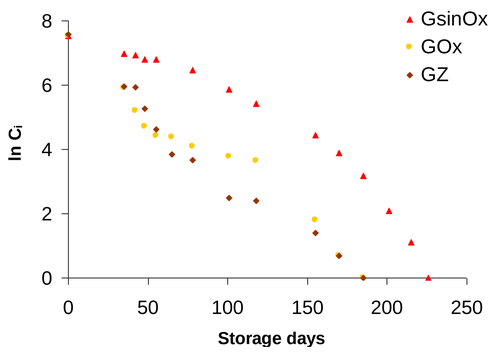


**Figure 1** - Neperian logaritm of Pg 3-gluc remaining concentration *vs* time of storage.

Pg 3-gluc disappearance followed a first order process ($\ln C_i = \ln C_0 - k \cdot t_i$), although a deviation to this model existed when the levels of remaining anthocyanin in the solutions were low. Anthocyanin decrease was considerably faster in the presence of oxygen ($t_{1/2}$ 13,6 days, $k = -5{,}7x10^{-2}$) than in anaerobic storage ($t_{1/2}$ 48 d, $k = -1{,}4\ x10^{-2}$). Sugar only provided a small stabilising effect ($t_{1/2}$ 15,9 d, $k = -5{,}0\ x10^{-2}$).

**Table I** - Linear regression parameters of Pg 3-gluc degradation in model systems GOx, GsinOx y GZ (0 - 55 days).

| *Model System* | *Degradation rate, k (days $^{-1}$)* | $r^2$ | *Half-life time, $t_{1/2}$ (days)* |
|---|---|---|---|
| GOx | $-5{,}7x10^{-2}$ | 0,98 | 13,6 |
| GsinOx | $-1{,}4\ x10^{-2}$ | 0,98 | 48,0 |
| GZ | $-5{,}0\ x10^{-2}$ | 0,98 | 15,9 |

The decrease of Pg 3-gluc was accompanied by the formation of new products and by a change in the colour of the solutions from the initial bright red to a more or less dark orange hue. The appearance of turbidity and further accumulation of a brown precipitate was also observed in the assays carried out in the presence of oxygen, but it was hardly produced in its absence.

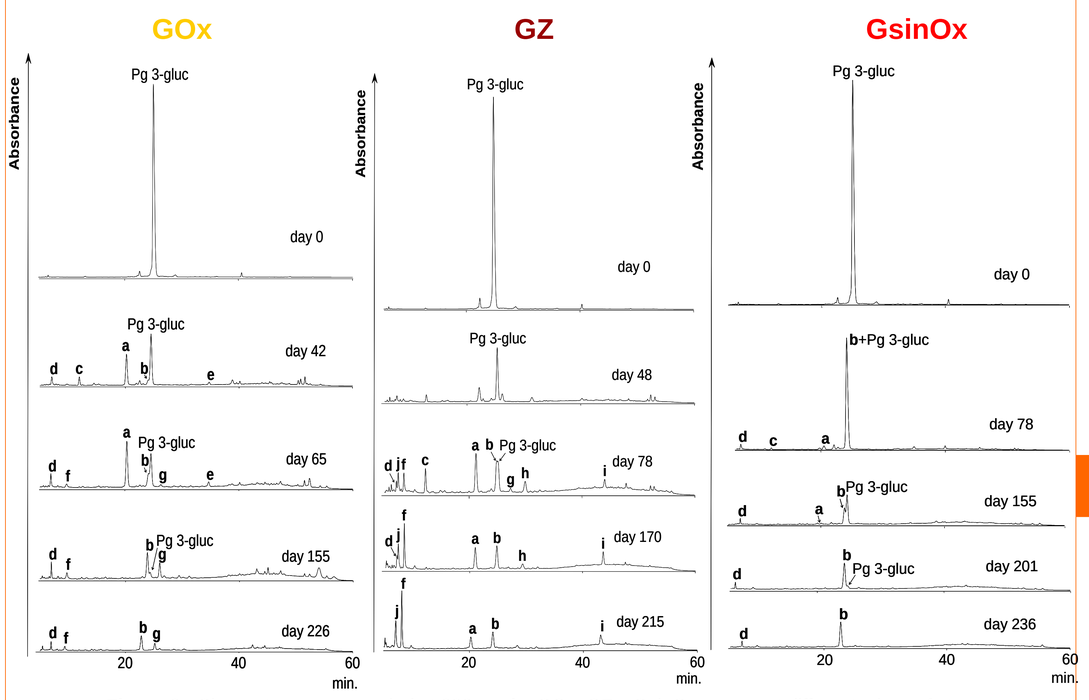


**Figure 2 -** Chromatograms recorded at 280 nm for GOx, GZ y GsinOx assays at differents days of storage.

**There are at least three processes involved in anthocyanin disapearance and formation of new compounds in the model solutions**

**Table II** – Wavelength of maximum absorption ($\lambda_{max.}$), molecular ion and $MS^2$ y $MS^3$ fragments of products detected in the model solutions.

| *Product* | *$\lambda_{máx.}$ (nm)* | *Molecular ion (m/z)* | *Fragmentation (m/z) $MS^2 \rightarrow MS^3$* |
|---|---|---|---|
| **a (THB)** | 291 | 155.3 | 155.3 → 155.3; 137.2 |
| **b** | 292 | 257.2 | 257.2 → 257.3 |
| **c (HBA)** | 254 | n.d. | |
| **Pg (pelargonidin)** | 513 | 271 | |
| **d** | 290 | 487.2 | 457.3<br>325.1<br>317.3 |
| **e** | 340 | 865.1 | 703.1 → 541.2 |
| **f** | 293 | 487.2 | 365.5<br>325.0 |
| **g** | 259, 340 | 466.9 | 329.1 → 285.6; 301.3; 261.1 |
| **h** | 282 | n.d. | |
| **i** | 274; 400 | n.d. | |
| **j** | 285 | 143.3 | 143.3 → 143.3 |
| **k** | 287 | 365.3 | 203.1 → 203.1<br>365.0<br>202.2<br>305.1 |

### 1) Anthocyanin degradation leading to colourless compounds

Main compounds formed were **a** (2,4,6-trihydroxybenzaldehyde) and **c** (p-hydroxybenzloic acid).
This process was hardly observed in the absence of oxygen.

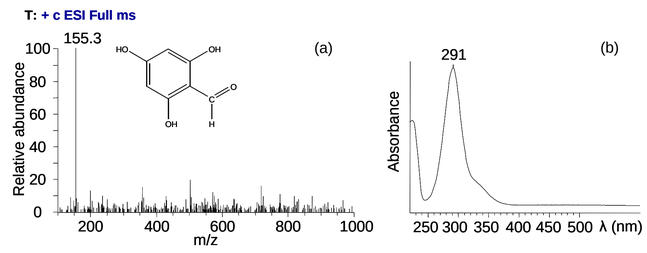


**Figure 3 –** Mass spectrum (a) and UV-Vis spectrum (b) of compound **a** (2,4,6-trihydroxybenzaldehyde).

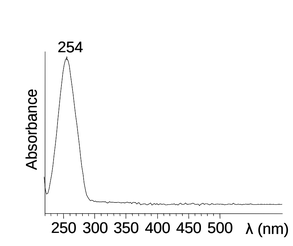


**Figure 4** – UV-Vis spectrum of compound **c** ( *p*-hydroxybenzoic acid).

### 2) Structural reorganizations on the anthocyanin basic structure

Main compound formed was **b**, tentatively identified as a product of the anthocyanin reduction.
It is the main process for anthocyanin disappearance in the absence of oxygen.

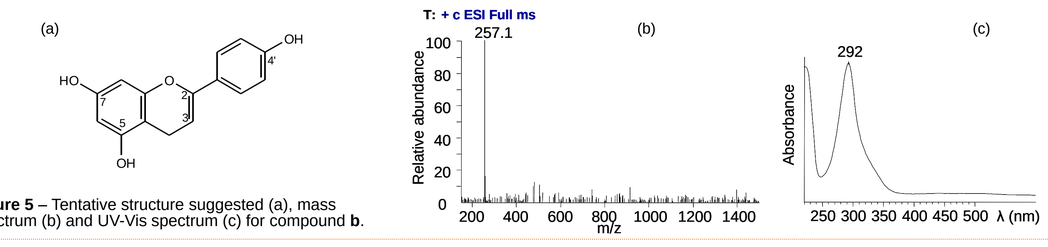


**Figure 5** – Tentative structure suggested (a), mass spectrum (b) and UV-Vis spectrum (c) for compound **b**.

### 3) Condensation/polymerization reactions

(compounds **d**, **e** and **g**)

Lead both to colourless products and brown-yellowish pigments.

Responsable for haze formation in models solutions.

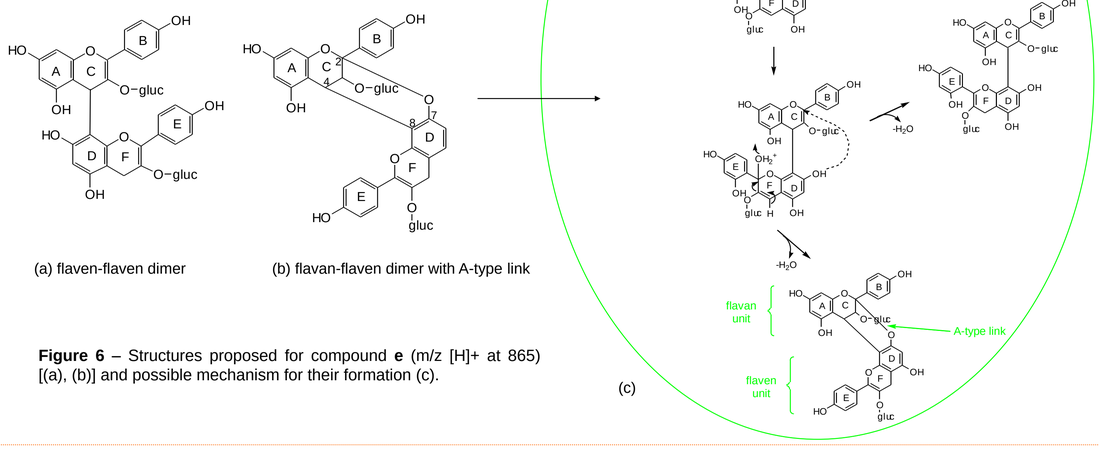


**Figure 6** – Structures proposed for compound **e** (m/z [H]+ at 865) [(a), (b)] and possible mechanism for their formation (c).

## CONCLUSIONS

1 - Anthocyanin breakdown together with the slow formation of orange/brown pigments were the principal processes involved in anthocyanin disappearance in aerobic conditions. 2,4,6-trihidroxybenzaldehyde (THB) and *p*-hidroxybenzoic acid (HBA) were identified as major products from the Pg 3-gluc degradation; trasient levels of Pg aglycone were also detected as an intermediary product before heterocylce breakdown, confirming that the separation of glucose residue would be a first step of antocyanin structural degradation.

2 - The formation of several bad defined peaks, corresponding both to colourless compounds and pigments showing maximum absorption between 330 and 450 nm was also observed. Most of these compounds had a transient nature and rapidly evolved towards the formation of polymeric pigments that finally precipitate.

3- One of these compounds (**e,** $\lambda_{max}$ 340 nm) was tentatively identified as a dimer containing two anthocyanin units, which could be either C-C bonded (flaven-flaven dimer) or doubly linked similar to A-type proanthocyanidins (flavan-flaven dimer).

4 - In anaerobic conditions, hardly anthocyanin breakdown occurred and reduced formation of brown pigments was also observed. The major product formed (**b**, $\lambda_{max}$ 292 nm) showed a positive molecular ion at m/z 257 whose fragmentation pattern suggests a possible flaven structure resulting from Pg reduction.

## ACKNOWLEDGEMENTS

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