P263. STABILITY OF PELARGONIDIN 3-GLUCOSIDE IN MODEL SOLUTIONS

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The behaviour of pelargonidin 3-glucoside (Pg 3-gluc) in aqueous solution (citrate buffer, pH 3.5) with and without addition of sugar (5.7%, glucose:fructose:sucrose mixture, 42:37:21) during eight months of storage at 25ºC in the presence and absence of oxygen was followed by UV-visible spectroscopy, HPLC-DAD and LC-MS (Lopes da Silva et al., 2002). The study is part of a project aiming to elucidate the mechanisms involved in the alteration of the colour produced in strawberry derived products during processing and storage.

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. 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. Oxygen was the most influential factor on the stability of Pg 3-gluc. The decrease of the anthocyanin was considerably faster in the presence of oxygen ($t_{1/2}$ 13.6 days, $k = -5.7 \times 10^{-2}$) than in anaerobic storage ($t_{1/2}$ 48 d, $k = -1.4 \times 10^{-2}$). Sugar only provided a small stabilising effect on the anthocyanin ($t_{1/2}$ 15.9 d, $k = -5.0 \times 10^{-2}$).

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.

Anthocyanin breakdown together with the slow formation of the orange/brown pigments were the principal processes observed in the assays carried out in aerobic conditions. 2,4,6-Trihidroxybenzaldehyde (THB) and p-hidroxybenzoic acid (HBA) were identified as major products from the anthocyanin breakdown by LC-MS. These compounds result from the fragments corresponding to the pelargonidin A and B rings, respectively. The formation of THB and HBA allows corroborating the mechanism of thermal degradation proposed by Furtado et al. (1993) and Piffaut et al. (1994). Transient levels of pelargonidin aglycone were also detected in the solutions as an intermediary product before the heterocycle breakdown, confirming that the separation of the glucose residue would be a first step in the anthocyanin structural degradation.

The transient presence of several bad defined peaks, corresponding both to colourless compounds and pigments showing maximum absorption between 330 and 450 nm, and a progressive increase in the baseline background absorption were also observed in the HPLC chromatograms. The transient nature of the peaks pointed out that they are intermediary products towards the formation of the orange/brown pigments that finally precipitate. Newly-formed pigments remaining in the solutions would explain their orange hue once the anthocyanin is lost. No good mass spectra could be obtained to contribute to the identification of such pigments and their colourless intermediaries, due to their irregular elution and background absorption existing in the chromatographic baseline. Mass information was only obtained for one of these peaks (λmax 340 nm), which showed a positive molecular ion at m/z 865 yielding two main MS^2 fragments at m/z 703 and 541 (successive lost of two glucose moieties). Such characteristics are coherent with a dimer containing two anthocyanin units, which could be either C-C bonded (i.e., flaven-flaven dimer) or doubly- linked similar to A-type proanthocyanidins (flavan-flaven dimer).

Hardly anthocyanin breakdown and reduced formation of brown pigments were observed in the assays carried out in anaerobic conditions. In those solutions the formation of a major product (λmax 292 nm) showing a positive molecular ion at m/z 257 was detected. Fragmentation pattern of this compound suggested that it lacked glucose and possessed three hydroxyl groups (MS^2 fragment at m/z 209, -48 mass units). A possible structure for this compound would be a flavan resulting from pelargonidin reduction and

bearing hydroxyl groups at 5, 7 and 4' (or 3) positions. Lower levels of this compound were also observed in the solutions stored in aerobic conditions.

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