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Colour stability improvement of strawberry beverage by fortification with polyphenolic copigments naturally occurring in rose petals

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Abstract

Heat stability of strawberry anthocyanins was studied depending on the addition of polyphenolic copigments naturally occurring in rose (*Rosa damascena* Mill.) petals. The anthocyanin degradation ideally followed first-order reaction kinetics ($R=0.99$) and the half-life value increased significantly due to the addition of rose petal polyphenolics. Further, CIELCH colour coordinates of thermally treated strawberry beverage were monitored depending on the fortification with polyphenolic copigments. Colour stability increased due to the addition of rose petal polyphenolics, as the total colour difference was smaller for the fortified beverage, especially after prolonged heating (4 h). The results obtained demonstrated that the addition of polyphenolic copigments extracted from distilled rose petals reduces the thermal degradation of strawberry anthocyanins, allowing improved colour stability of the processed strawberries. Moreover, this polyphenolic fortification could be worthwhile not only from technological point of view, but also with respect to the development of functional foods and beverages.

Keywords: Strawberry beverage; Colour stability; Anthocyanins; Copigmentation; *Rosa damascena*

Industrial relevance: This study presents a nature-derived concept to improve the quality of colour-labile strawberry products by fortification with polyphenolic copigments extracted from distilled rose petals. The approach suggested appears to be easily applicable at industrial scale. Additionally, the recovery of rose petal by-products rich in polyphenolics could be recommended, thus adding value to the rose processing industry.

1. Introduction

Anthocyanin pigments are not only important defining the aesthetic value of foods and beverages, but also play a significant role from a nutritional point of view (Stintzing & Carle, 2004). The attractive red colour is one of the visual quality attributes strongly affecting consumer acceptance both of fresh and processed strawberry fruits. Unfortunately, due to the low total content of strawberry anthocyanins (Clifford, 2000) and their inherent heat and light sensitivity (Hayashi, Ohara, & Tsukui, 1996) an accelerated pigment degradation occurs during conventional processing and storage, and retention of strawberry colour has always been a technological challenge.

Intermolecular copigmentation is a colour stabilizing mechanism in which anthocyanins and copigments, mostly polyphenolics, form complexes, thus explaining the hyperchromic and in some cases bathochromic effects. Copigmentation is assumed to be responsible for the colour variations that occur in flowers in a pH range where anthocyanins alone are virtually colourless (Asen, Stewart, & Norris, 1972). The significance of this phenomenon for the colour evolution during aging of red wines has also been suggested (Boulton, 2001). With respect to strawberry beverages, an increase in colour intensity has been observed after addition of phenolic acids acting as copigments (Rein & Heinonen, 2004; Wilska-Jeszka & Korzuchowska, 1996). However, due to the high copigment/pigment ratios required the flavour threshold concentrations could be exceeded, promoting an astringency perception of the phenolic acids (Clifford, 1997). Moreover, phenolic acids are easily susceptible to oxidation with subsequent browning development (Tomás-Barberán & Espín, 2001).

Recently, industrially distilled petals of *Rosa damascena* Mill. were established as a rich source of flavonols (Schieber, Mihalev, Berardini, Mollov, & Carle, 2005), which have been

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demonstrated as highly effective polyphenolic copigments at low copigment/pigment ratio (Gómez-Míguez, González-Manzano, Escribano-Bailón, Heredia, & Santos-Buelga, 2006).

Therefore, the present study evaluated the copigmentation behaviour and heat stability of purified strawberry anthocyanins (PSA) in the presence of rose petal polyphenolics (RPP). Additionally, the colour changes of strawberry beverage were monitored during thermal treatment when polyphenolic copigments naturally occurring in rose petals were added.

2. Materials and methods

2.1. Materials

Strawberries (*Fragaria x ananassa* Duch. cv. 'Siabella', harvest 2005) were obtained from a local producer and were immediately frozen and subsequently stored at −18 °C until used. Approximately 1 kg of frozen fruits were thawed and crushed in a household-type meat mincer (mesh size 8 mm) before pressing to extract juice. For depectinisation of the juice, 100 ppm Rohapect AP1 (AB Enzymes, Darmstadt, Germany) were added and the juice was kept at 50 °C for 1 h. After enzymatic treatment, the juice was filtered and subsequently pasteurized by microwave heating (2450 MHz, 3 min), quickly cooled in an ice-water bath, and stored overnight at 4 °C. Strawberry beverage (12 °Bx) was prepared on the day of analysis by mixing 30% juice, twice distilled water and sucrose. PSA were isolated as described by Oszmianski and Sapis (1988) using 600 g of the same batch of frozen strawberries.

Waste material originating from water–steam distillation of *Rosa damascena* Mill. petals was supplied by Nikolaev Distillery (Paisievo, Bulgaria) and after pressing, the pomace obtained was hot air-dried (60 °C, 6 h). RPP were extracted with 30% aqueous ethanol using finely milled pomace (particle size<0.4 mm) at solvent-to-solid ratio of 20:1 (v/w). After stirring for 1 h at ambient temperature, the extraction mixture was filtered and the organic solvent was evaporated under vacuum (40 °C). The extract obtained was kept at −12 °C.

The copigments sinapic acid, chlorogenic acid, and caffeic acid (all purum grade) were from Fluka (Buchs, Switzerland). The reagents used to prepare the buffer system (citric acid monohydrate, di-sodium hydrogen phosphate dodecahydrate) were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Sample preparation

RPP were added to strawberry beverage at 1:5 total anthocyanins (expressed as pelargonidin 3-glucoside) to total polyphenolics (expressed as gallic acid) molar ratio. The pigment concentration of the fortified beverage was approximately 1×10^{-4} M and the pH value was set to 3.40±0.01, which was the natural pH of the strawberry juice.

Model solutions of PSA (1×10^{-4} M), with and without copigments addition, were obtained using McIlvaine buffer (0.1 M, pH 3.4) prepared according to Elving, Markowitz, and Rosenthal (1956).

2.3. Heat stability test

For thermal stability, samples (7 mL) in hermetically sealed glass tubes were placed in a water bath at 85 °C. After heating, the samples were cooled in an ice-water bath for 1 min and then transferred in a water bath at 25 °C.

Half-life value was calculated as recently proposed for betacyanins (Herbach, Stintzing, & Carle, 2006).

2.4. Spectrophotometric measurements and colour analysis

All measurements were performed with a PU 8800 UV/Vis spectrophotometer equipped with a UV/Vis and a colour (Issue 2) software (all from Pye Unicam, Cambridge, UK), using 1 cm path length glass cuvettes. After 30 min of equilibration at 25 °C, visible spectra from 450 to 600 nm with a 2 nm bandwidth and from 380 to 770 nm with a 10 nm bandwidth were recorded for the copigmentation reactions and colourimetric calculations, respectively. Before the colour measurements, exactly 10 min after heating, the samples were diluted with McIlvaine buffer (pH 3.4) to reach maximum absorbance values of 0.40±0.01. CIELCH colour coordinates were determined using illuminant D_{65} and 10° observer angle. The total colour difference of heated samples as compared to the respective not heated solutions was calculated as proposed by Gonnet (1998).

2.5. Chemical analyses

Total monomeric anthocyanins content was determined by the pH-differential method (Giusti & Wrolstad, 2001) and the results were calculated using a molar absorptivity of 27,300 L/mol cm and a molecular weight of 433.2 g/mol for pelargonidin 3-glucoside (Aaby, Skrede, & Wrolstad, 2005).

Total phenolics in the rose petal extract were assessed according to the Folin–Ciocalteu's reagent procedure (Singleton & Rossi, 1965) and the content was calculated as gallic acid equivalents.

2.6. Statistical analysis

The results reported in the present study are the mean values of at least two determinations and the coefficients of variation, expressed as the percentage ratio between the standard deviations and the mean values, were found to be <3% in all cases. Linear regression analysis was performed using the statistical package from Microsoft Excel.

3. Results and discussion

Since the PSA concentration in the model solutions was constant, the spectral variations caused by the copigmentation depended on the RPP concentration. As shown in Fig. 1, the magnitude of the bathochromic ($\Delta\lambda_{max}$) and hyperchromic ($\Delta A\%$) effects increased with the copigment concentration. For the solution with the highest pigment/copigment molar ratio (1:10) $\Delta\lambda_{max}$ and $\Delta A\%$ amounted to 6 nm and 10%, respectively.