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Influence of rutin and ascorbic acid in colour, plum anthocyanins and antioxidant capacity stability in model juices

J.A. Hernández-Herrero, M.J. Frutos *

Department of Agro-Food Technology, Universidad Miguel Hernández, Ctra. Beniel, km. 3.2, CP 03.312 Orihuela (Alicante), Spain

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ABSTRACT

Model juices at pH 3.7 were prepared with different combinations of ascorbic acid, rutin (quercetin 3-rutinoside) and concentrated anthocyanin extract of plums (cv. Black Gold). The anthocyanins in the concentrated extract were cyanidin 3-glucoside and cyanidin 3-rutinoside, in a proportion of 76% and 24% respectively. The model juices were stored during 17 weeks in darkness at 20 °C. The colour stability was improved by the presence of rutin and strongly damaged by the ascorbic acid. The fortification of anthocyanin model juices with ascorbic acid originated the degradation of most of anthocyanins. However, anthocyanins improved ascorbic acid stability during storage. The copigmentation of anthocyanin and rutin showed a beneficial effect on colour stability from the 5 weeks of storage. In model juices prepared exclusively with purified plum extract a high correlation (R^2 = 0.881) between anthocyanins and antioxidant capacity was found.

1. Introduction

Nowadays, consumers prefer natural food additives against the synthetic ones, including food colourings. Therefore, it is interesting to evaluate their behaviour in different food matrix. Anthocyanins are a natural colouring in the red range that have a high antioxidant capacity, that contributes to beneficial effects in reducing the risk of heart disease, cancer and stroke (Heo, Kim, Chung, & Kim, 2007; Huang, Davidge, & Wu, 2013; Hui et al., 2010). Plums may be good sources of food colourings, due to the high level of anthocyanins and other phenolic compounds (Kim, Chun, Kim, Moon, & Lee, 2003). Previous studies have reported that plums show higher colour levels and stability compared to other plant sources as grape, bilberry, eggplant, strawberry and red raspberry (Hernández-Herrero & Frutos, 2011, 2014a).

Anthocyanins are unstable to different environmental conditions, as pH, light, vitamin C, oxygen and water activity among others (Malien-Aubert, Dangles, & Amiot, 2001; Sun, Bai, Zhang, Liao, & Hu, 2011; Wrolstad, Durst, & Lee, 2005). The most limiting factor is pH and for this reason, anthocyanin based food colourings are been exclusively used in low pH foods, as juices, yoghurts and soft drinks (Aryana, Barnes, Emmick, Mcgrew, & Moser, 2006; De Rosso & Mercadante, 2007; Hernández-Herrero & Frutos, 2014b). Ascorbic acid is other limiting factor, and can be found in many food products, including fruit juices, either naturally or as an antioxidant additive (Elliott, 1999), preventing browning and improving the nutritional value (Freedman & Francis, 1984). In previous studies, the detrimental effect of ascorbic in anthocyanin stability has been studied in anthocyanin standards solutions (García-Viguera & Bridle, 1999) and fortified juices (Pacheco-palencia, Hawken, & Talcott, 2007). Therefore, the presence of ascorbic acid could also reduce the number of foods where anthocyanins can be applied as food colouring. It has been previously reported that the colour of anthocyanins can be stabilised by the presence of other phenolic compounds through intermolecular copigmentation (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galan-Vidal, 2009; Cavalcanti, Santos, & Meireles, 2011; He et al., 2012; Malien-Aubert et al., 2001). Flavonols as quercetin-3-rutinoside (rutin) are the most efficient copigments (Mazza & Miniati, 1993).

Owing to the importance of ascorbic acid and of polyphenols as rutin in colour and antioxidant capacity of anthocyanins, the main aim of this study was to investigate, in model juices coloured with plum concentrated anthocyanin extract, the influence of anthocyanin–ascorbic acid and anthocyanin–rutin interactions on the stability of those parameters.

2. Materials and methods

2.1. Plant material

Plums (*Prunus salicina* L.) cv. Black Gold, were purchased in local supermarkets from Orihuela (Alicante, Spain). The plant materials were stored under refrigerated conditions at the market.

* Corresponding author. Tel.: +34 966749744; fax: +34 966749677.
E-mail address: mj.frutos@umh.es (M.J. Frutos).

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2.2. Extract preparation

The samples were ground in a Waring blender, and then 14 g of the homogenate were extracted with 0.15% HCl in acetone for 4 h in the dark at 4 °C, in a proportion of 1:4 (plant material-solvent). The filter cake residue was re-extracted in the same proportion with aqueous acetone (30:70 v/v) acidified with 0.15% of HCl. Filtrates were combined and vacuum concentrated at 35 °C. The concentrate was resuspended with 20 mL of acidified water with HCl at 0.15% and lyophilised (Christ. Mod. ALPHA 2–4). The lyophilised extracts were stored at −84 °C prior to analysis. Extractions were repeated on three independent samples.

2.3. Purification of phenolic compounds

The extract was purified by an extraction in solid phase. The extract (approx. 1 g) was resuspended with 10 mL of acidified water with HCl at 0.15%. The C-18 Sep-Pack cartridge (Agilent Technologies) was activated passing through 10 mL of acidified methanol (0.15% of HCl), followed by 10 mL of acidified water (0.15% of HCl). The resuspended extract (10 mL) was also passed through and the phenolic compounds were absorbed onto cartridge. Sugars, organic acids and other water-soluble compounds were washed with 20 mL of acidified water (0.15% of HCl). The phenolic compounds were recovered with 20 mL acidified methanol (0.15% of HCl). The methanolic extract was vacuum concentrated at 35 °C to dryness leaving a red residue.

2.4. Concentrated anthocyanin extract (CAE) preparation

The red residue was resuspended with 30 mL of dietil ether and centrifugated at 15,000×*g* for 15 min at 4 °C. The red precipitate contained the anthocyanins due to its insolubility in this solvent. The red precipitate obtained was liquid nitrogen powdered, obtaining approx. 20 mg of CAE powder. This extract was used for the preparation of model juices.

2.5. Preparation and storage of model juices (MJ)

The plum CAE powder, ascorbic acid (AA) and rutin (R) standard were dissolved in buffer solution (KH Ftalato-HCl 0.1 M) at pH 3.7 containing 0.1% (w/v) of sorbic acid (Sigma–Aldrich Co., St. Louis, MO). Therefore, the following MJs were prepared: concentrated anthocyanin extract (CAE) juice, concentrated anthocyanin extract plus ascorbic acid (CAE + AA) juice, concentrated anthocyanin extract plus rutin (CAE + R) juice, ascorbic acid (AA) juice and rutin (R) juice (Table 1).

The 65 mL of each MJ were distributed as follows: for colour measurement, 45 mL were introduced in the chroma-meter tubes, and sealed with parafilm; and 18 mL were kept in 9 screw-cap vials of 2 mL for the analysis of the anthocyanin, rutin and ascorbic acid contents, and antioxidant capacity. MJs were stored during 17 weeks in darkness at 20 °C. The sample vials were frozen at −85 °C after their respective storage period. The analyses of colour, rutin, anthocyanin and antioxidant capacity were performed at 0, 1, 3, 5, 7, 9, 11, 14 and 17 weeks of storage.

Table 1
Composition (mg/100 mL) of the different plum model juices elaborated with concentrated anthocyanin extract (CAE), concentrated anthocyanin extract plus ascorbic acid (CAE + AA), concentrated anthocyanin extract plus rutin (CAE + R), ascorbic acid (AA) and rutin (R).

Model juices	CAE	Ascorbic acid	Rutin
CAE	20	–	–
CAE + AA	20	60	–
CAE + R	20	–	15
AA	–	60	–
R	–	–	15

2.6. Determination of colour stability

Colour measurements were determined using an A5 Chroma-Meter Minolta CR300 (Minolta Co. Ltd., Osaka, Japan), with a liquid tube holder colour space CIELAB. The colour coordinates of the model juices were computed in the CIELAB scale in a CIE D65/10° illuminant/observer condition. Colour results were expressed as tristimulus parameters (L^*, a^*, b^*, H^*, C^*). Hue angle ($H^* = \tan^{-1} b^*/a^*$) indicates sample colour (0° or 360° = red, 90° = yellow, 180° = green, 270° = blue), and chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$) indicates colour purity or saturation (high values are more vivid). a^* and b^*chromaticity coordinates indicate colour directions green ($-a^*$)/red ($+a^*$) and blue ($-b^*$)/yellow ($+b^*$) (Baker, Bridle, & Timberlake, 1986). For each sample 10 measurements were made.

2.7. Anthocyanin and rutin analysis

The identification of anthocyanins and quercetin 3-rutinoside in MJ has been performed according to the following high performance liquid chromatography (HPLC) method:

The analysis was performed using a Hewlett–Packard HP 1100 liquid chromatograph equipped with an Agilent Technologies G1315A photodiode array detector with a reverse phase column C18 Waters Spherisorb ODS-1 (250 mm × 4.6 mm, 5 μm). A mobile phase gradient was used for elution: A, acetonitrile–water (1:1) with 0.5% trifluoroacetic acid; B, acidified water with 0.5% of trifluoroacetic acid. The elution profile was 10% (A) at 0 min, 35% (A) at 10 min, 50% (A) at 20 min, 80% (A) at 21 min, 80% (A) at 23 min and 10% (A) at 25 min. The flow rate was 1 mL/min with an injection volume of 20 μL. The changes in absorbance were measured in the visible–ultraviolet (vis–UV) diode array. The anthocyanins were analysed at 520 nm and rutin at 360 nm. The UV–visible spectra of the separated compounds were recorded from 250 to 600 nm. Anthocyanins were quantified as cyanidin 3-glucoside and cyanidin 3-rutinoside and rutin was quantified as quercetin 3-rutinoside (Polyphenols Laboratories, AS, Sandness, Norway). The standards were dissolved in methanol acidified with 0.15% of HCl.

2.8. Determination of antioxidant capacity stability

The method used for the antioxidant capacity of the plum MJs was the vitamin C equivalent antioxidant capacity (VCEAC) that was expressed as mg of vitamin C equivalents (VCE)/100 mL of solution. The 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation was generated by the enzymatic system formed by the hydrogen peroxide (H_2O_2) and the horseradish peroxidase (HRP). The change in absorbance was measured using an UV–vis spectrophotometer Hewlett–Packard mod. 8453, at a wavelength of 414 nm. The reaction was made mixing 1.5 mM ABTS, 1.18 mM H_2O_2 and 0.25 μM HRP in a glycin–HCl 50 mM (pH 4.5) buffer in a total volume of 2 mL. The antioxidant capacity was quantified using a standard curve of ascorbic acid (0, 1, 2, 3, 4 and 5 nM) (Cano, Hernández-Ruíz, García-Cánovas, Acosta, & Arnao, 1998).

The estimated contribution of the anthocyanins to the total antioxidant capacity was calculated according to the Eq. (1):

$$\text{VCEAC}\,(\text{mg VCE}/100\,\text{mL}) = \sum[\text{CC}(\text{mg}/100\,\text{mL}) \cdot \text{Ra} \cdot \text{PC}(\%) \cdot 100^{-1}] \quad (1)$$

where CC: cyanin content in the CAE juice (1.3 mg/100 mL) (Fig. 3), Ra: ratio VCE/cyanin for the two cyanins present (1.4 for cyanidin