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Original Research Article

# Stability of anthocyanins in berry juices stored at different temperatures

Jarkko Hellström [a,*], Pirjo Mattila [a], Reijo Karjalainen [a,b]

[a] MTT Agrifood Research Finland, Biotechnology and Food Research, Myllytie 1, FI-31600 Jokioinen, Finland
[b] University of Eastern Finland, Department of Biosciences, Box 1627, 70211 Kuopio, Finland

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ABSTRACT

The bright purple-red colour caused by anthocyanins is characteristic of berry products. The colour becomes easily distorted as anthocyanins are susceptible to various degradation reactions. In this study, we monitored the stability of structurally different anthocyanins in several berry juices during storage at different temperatures. The anthocyanin stability was found to be affected by a number of factors including the type of anthocyanin, the origin of the juice, and especially the storage temperature. In all studied juices, half-life ($t_{½}$) of anthocyanins was much shorter at room temperature than at cold storage. Anthocyanins were more stable in chokeberry juice ($t_{½}$ = 6.7 weeks at 21 °C and 23.8 weeks at 9 °C, 32.5 weeks at 4 °C for total anthocyanins) than in blackcurrant ($t_{½}$ = 3.0 weeks at 21 °C, 11.5 at 9 °C and 20.3 weeks at 4 °C) and crowberry juice ($t_{½}$ = 2.2 weeks at 21 °C, 7.3 at 9 °C and 12.3 weeks at 4 °C). It was also evident that the long shelf life often applied for the commercial juice drinks is detrimental to the anthocyanins. Only 11–15% of the original anthocyanin content was detected in two commercial juice drinks at their expiry date, after storage of 35–49 weeks at room temperature.



## 1. Introduction

Anthocyanins are important quality factors of berry juices. As natural pigments anthocyanins are responsible for the attractive red, purple, and blue colours in many fruits, berries and vegetables. As a subclass of polyphenols, dietary anthocyanins have indisputable role in the prevention of diverse widespread degenerative diseases. In fruits and berries anthocyanins are considered to contribute to the health promoting features by their antioxidant, anti-carcinogenic, anti-inflammatory, and anti-angiogenic properties although the related mechanisms remain obscure (Castañeda-Ovando et al., 2009; Cooke et al., 2005; Kong et al., 2003; Prior and Wu, 2006; Rossi et al., 2003; Wang and Stoner, 2008). The anthocyanin concentration correlates well with the darkness of the berry colour. Among the berries consumed in Finland the highest anthocyanin contents were found in bilberry (*Vaccinium myrtillus* L.), chokeberry (*Aronia mitchurinii*) and crowberry (*Empetrum nigrum*) followed by Saskatoon berry (*Amelanchiar alnifolia*), blueberry (*Vaccinium corymbosum* L.) and blackcurrant (*Ribes nigrum*) (Koponen et al., 2007).

Anthocyanins are highly unstable and very susceptible to degradation. The colour stability of anthocyanins is affected by several factors such as pH, their own chemical structure, concentration, storage temperature, light, oxygen, and the presence of enzymes, flavonoids, proteins and metal ions (Rein, 2005). The manufacturing processes of berry products lead also to deterioration and alter the colour (Hager et al., 2008). The storage may further degrade anthocyanins but by the choice of storing conditions the anthocyanin stability in food products can be affected substantially (Gimenez et al., 2001; Morais et al., 2002; Rubinskiene et al., 2005). The stability can be improved by increasing anthocyanin concentration (self association), removal of oxygen and inactivation of enzymes (Rein, 2005). Furthermore, in berry products the stability of anthocyanins is often enhanced by naturally existing phenolic co-pigments, such as phenolic acids and flavanols (González-Manzano et al., 2009; Rein, 2005). However, some commonly used additives in berry juices such as sugars and ascorbic acid, as well as their degradation products, may decrease anthocyanin stability (De Rosso and Mercadante, 2007; Marti et al., 2002; Meschter, 1953; Nikkah et al., 2007).

Beverage companies are currently making lots of efforts to formulate fortified juices which contain anthocyanins and other bioactive compounds from different berries and fruits. Commercial juices are often thermally processed which can significantly affect the stability of anthocyanins and thereby reduce the absorption of these important polyphenols (Hollands et al., 2008). Although the stability of anthocyanins has been widely studied more knowledge is still needed about the effects of storage times, temperatures,

* Corresponding author. Tel.: +358 29 5317207; fax: +358 3 4188 3244.
*E-mail address:* jarkko.hellstrom@mtt.fi (J. Hellström).

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matrixes and structural features of the compounds derived from different berries and fruits to be able to formulate juices with optimal health promoting impact and to find optimal storage conditions especially for commercial juices. Our aim was to find out if storage temperature and the juice matrix affect the stability of anthocyanins. Another goal was to look into the behaviour of anthocyanins in commercial juice drinks during their shelf life. To this end we monitored the fates of anthocyanins in different commercial and laboratory made pure and blended berry juices as a function of their storage time at different temperatures.

## 2. Materials and methods

### 2.1. Samples

The analyzed material contained both laboratory-made and commercially available juice drinks. Blackcurrant and crowberry juices were diluted of concentrates purchased from a Finnish industrial producer while chokeberry juice was squeezed from freeze-stored chokeberries in the laboratory. Three ready-to-drink juices (2–3 Bx) were prepared from juice concentrates by dilution with water, i.e. blackcurrant juice concentrate (65 Bx); dilution 1:30, crowberry juice concentrate (60 Bx); dilution 1:30, and chokeberry juice (15 Bx); dilution 1:5. Furthermore, a blended juice drink was prepared by mixing together the above mentioned ready-to-drink juices in equal proportions. Benzoic acid was added in all juice drinks as a preservative (100 mg/L). Juices were poured into 50 ml test tubes and the caps were tightened. Some residual air (2–3 ml) remained in the tubes. Tubes were stored in the darkness at three temperatures (4 °C, 9 °C, and 21 °C) and their anthocyanin contents were determined at chosen intervals during storage of 11 weeks (4 °C and 9 °C) and 22 weeks (4 °C).

One smoothie (100 ml plastic bottles) and two juice drinks (1 L tetrapacks) were provided as freshly prepared by a Finnish commercial juice producer. The smoothie contained grape, strawberry, bilberry, apple, lingonberry, chokeberry, acerola, and boysenberry with juice content of 100%. One juice drink was made from bilberry, chokeberry and grape, and its juice content was 15%. The other juice drink contained blackcurrant and lingonberry with 10% juice content. Smoothie was stored at refrigerator (4 °C) and the two juice drinks at room temperature (21 °C) as recommended on the product labels. The degradation of anthocyanins in each beverage was followed during the storage until the expiry date. For the smoothie the storage time was 10 weeks, for the bilberry–chokeberry–grape drink 35 weeks and for the lingonberry–blackcurrant juice drink 49 weeks.

In every test sets three individual samples (tubes, bottles, tetrapacks) were taken at each of the chosen time points and the samples were analyzed thrice.

At the beginning of the study the initial pH-value (at temperature 21 °C) of ready-to-drink juices was measured with the following readings: blackcurrant juice, pH 3.27; crowberry juice, pH 3.51; chokeberry juice, pH 3.33; mixed-berry juice, pH 3.37; smoothie, pH 3.54; bilberry–chokeberry–grape juice drink, pH 2.93; blackcurrant–lingonberry juice drink, pH 2.86.

### 2.2. Chemicals

Cyanidin 3-*O*-rutinoside (>97%), delphinidin 3-*O*-rutinoside (>97%), cyanidin 3-*O*-glucoside (>97%), and delphinidin 3-*O*-glucoside (>97%) were purchased from Polyphenols Laboratories AS, Sandnes, Norway. Chlorogenic acid (>95%) was from Sigma–Aldrich Co., St. Louis, MO, USA. All chromatographic solvents were of HPLC grade and the purity of the other reagents used was p.a. or comparable.

### 2.3. Methods

Anthocyanins in laboratory-made blackcurrant and crowberry juices as well in the Finnish commercial juice drinks and smoothie were analyzed by Agilent 1100 HPLC device equipped with a diode array detector (DAD). The samples were diluted with 5% HCOOH (aq) when necessary and then filtered through a 45 μm membrane filter into HPLC vial. Anthocyanins were separated on a 150 mm × 4.6 mm i.d., 5 μm, Gemini C18 column with a C-18 guard column. The temperature of the column oven was set at 35 °C. The mobile phase consisted of 5% HCOOH and acetonitrile and the flow rate was 1 ml/min. Elution was started with 5% acetonitrile, isocratically 5 min followed by a linear gradient to 13% in 5 min, then a linear gradient to 18% in 10 min, a linear gradient to 80% in 2 min, isocratically 3 min, min and back to the starting point in 2 min. Post time was 3 min before the next elution. The injection volume was 10 μL. All anthocyanins were quantified at detection wavelength of 518 nm using an external standard of cyanidin 3-galactoside, cyanidin 3-*O*-rutinoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, and cyanidin 3-arabinoside. Anthocyanins which did not have own external standard were quantified using the most similar anthocyanin available, e.g. cyanidin 3-xyloside was quantified as cyanidin 3-arabinoside. Anthocyanins not derived from cyanidin or delphinidin were quantified as cyanidin 3-glucoside. Three replicates were analyzed from the samples. Additionally, the samples were analyzed by Thermo Finnigan Surveyor HPLC connected to a Finnigan MAT ion trap mass spectrometry. An ESI interface in positive ionization mode was used under full scan ($m/z$ 100–800). The ESI operation conditions were as follows: spray voltage at 4.5 kV, capillary temperature at 280 °C, and a sheath gas ($N_2$) at a flow rate 10 U (arbitrary units). Capillary voltage was set at 25.4 V.

It was possible to determine two caffeoyl-quinic acids, namely chlorogenic and neochlorogenic, in the same HPLC run with the anthocyanins. Caffeoyl-quinic acids were detected at wavelength 329 nm and quantified against an external standard of chlorogenic acid. Caffeoyl-quinic acid analyses were performed only in juices including chokeberry in which they are among the major phenolics.

Degradation rates of anthocyanins were fitted to the first-order reaction kinetics and after logarithmic transformation differences between the rates were evaluated with a linear regression model. Statistical analyses were performed in version 9.3 of SAS/STAT software.

## 3. Results and discussion

### 3.1. Anthocyanins in laboratory-made berry juices

In the laboratory-made juices the total anthocyanin content varied from 44.8 to 128 mg/100 ml being highest in crowberry juice and lowest in blackcurrant juice (Table 1). In chokeberry juice four anthocyanins were identified (Fig. 1A, Table 1), namely cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, and cyanidin 3-xyloside in agreement with previous studies (Oszmianski and Wojdylo, 2005; Slimestad et al., 2005; Wu et al., 2004; Zheng and Wang, 2003). Chokeberry juice contained also notable amounts of chlorogenic acid (9.9 ± 0.1 mg/100 ml) and neochlorogenic acid (13.8 ± 0.1 mg/100 ml).

The anthocyanin profile in blackcurrant juice (Fig. 1B, Table 1) was dominated by rutinosides of delphinidin and cyanidin followed by glucosides of the same anthocyanidins. These results agree well with those in previous studies on blackcurrant juices (Landbo and Meyer, 2004; Mattila et al., 2011). Rutinosides of petunidin and peonidin were tentatively identified as minor anthocyanins in accordance with the earlier reports of blackcurrant anthocyanins (Borges et al., 2010; Ogawa