Stabilization of cranberry anthocyanins in nutraceutical capsules

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Stabilization of cranberry anthocyanins in nutraceutical capsules

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Abstract

Anthocyanins can be considered spy-substances, useful in obtaining information regarding the shelf-life of food supplements containing cranberry juice or cranberry-derived extracts. The level of total anthocyanins, as evaluated by high-performance liquid chromatography-diode-array detector and analytically expressed as cyanidin aglycone, represents a 'quality index' useful for routine estimation of shelf-life. The objective of this work was to study the stability of anthocyanins in a commercial cranberry (Vaccinium macrocarpon) dried extract compared with the stability of the same extract contained in a food supplement enriched with α-tocopheryl succinate and ascorbic acid. The values obtained after exposure of the samples to natural light and to alternating hot and cold temperatures show considerable sensitivity of the commercial cranberry dried extract to the agents used for the same evaluation (time, temperature and light) and emphasize a positive effect of the enrichment of the derived preparation with α-tocopheryl succinate and ascorbic acid.

Keywords: Stabilization, anthocyanins, cranberry, food supplements, α-tocopheryl succinate, ascorbic acid

Introduction

Cranberries are the small red fruits of the perennial Vaccinium macrocarpon, and are known to contain many biologically active components; therefore, they can be thought of as one of the earliest functional foods. Some papers concerning cranberry phytochemicals and their health benefits are cited in the bibliography (Beachey 1981; Avorn et al. 1994; Howell et al. 1998, 2001; Weiss et al. 1998; Wilson et al. 1998; Foo et al. 2000a,b; Henig and Leahy 2000; Leahy et al. 2001; Porter et al. 2001; Ferreira and Slade 2002; Yan et al. 2002; Cunningham et al. 2004). The molecules present in cranberries that are believed to be responsible for the positive health effects are proanthocyanidins (PAC) and anthocyanins. The PAC are polymers of flavan-3-ols linked together by either a single (B-type) or a double (A-type) interflavan bond. To date, the PAC identified in cranberries are mostly oligomers and polymers of epicatechin and epigallocatechin containing one or more A-type interflavan bonds. Anthocyanins are the result of glycosylation of the anthocyanidin aglycone at position 3 in the case of cranberry anthocyanins (Foo et al. 2000a, 2000b; Porter et al., 2001; Cunningham et al. 2004).
The quantification of PAC poses many analytical challenges due to their polymeric nature and the lack of analytical standards. Additionally, structural heterogeneity based on differing monomer units complicates the isolation and quantification of individual compounds. It is difficult to base stability studies of cranberry juices and extracts on analytical control of PAC, and therefore it would be better to carry out both quality control and stability analysis on anthocyanins. These compounds can be considered as spy-substances, useful in providing information regarding the shelf-life of food supplements containing cranberry juice or cranberry-derived extracts.

Figure 1 shows the chemical structure of cranberry anthocyanins. They are composed of cyanidin and peonidin aglycons linked to the sugars galactose, glucose and arabinose.

It is important to consider that only the fruits of Vaccinium spp. (cranberry, blueberry, bilberry, cowberry and big whortleberry) contain a similar anthocyanin composition (Mazza and Miniati 1993). This paper reports the results of experiments carried out to verify the stability of anthocyanins in a commercial cranberry dried extract, and to verify the stability of anthocyanins in a food supplement containing the same extract enriched with other antioxidants (ascorbic acid and α-tocopheryl succinate). The idea that these substances have protective properties is not new. There are reports concerning the mutual protecting power of anthocyanins and ascorbic acid (Shrikhande and Francis 1974; Mazza and Miniati 1993), and it has also been reported that cyanidin-3-galactoside as well as tocopherols show antioxidant activity (Zhou and Singh 2004).

Materials and methods

Reagents

The reagent used for the extraction of the samples was high-performance liquid chromatography (HPLC)-grade methanol obtained from Merck (Darmstadt, Germany). Analytical-grade water and 85% phosphoric acid used in the preparation of the
mobile phase were purchased from Merck and Riedel-de Haën (Sigma Aldrich, Seelze, Germany), respectively. HPLC-grade acetonitrile was purchased from Sigma Aldrich (Steinheim, Germany).

**Apparatus**

HPLC analysis of anthocyanins was performed on a Shimadzu instrument (Shimadzu, Milan, Italy) equipped with two pumps (10AD vp), a diode array detector (SPD-M10A vp), a system controller (SCL-10A vp) and a Rheodyne 20 μl injection loop (Cotati, CA, USA). The HPLC pumps and diode array system were controlled by computer using a CLASS VP version 5.032 Workstation program (Shimadzu, Milan, Italy). The analytical column employed was a Chromspher 5 C18 (25 cm × 4.6 cm², particle size 2 μm) manufactured by Chrompack (Alltech Associates, Laarne, Belgium).

**Chromatographic conditions**

The mobile phase consisted of (A) water/phosphoric acid (96:4 v/v) and (B) acetonitrile. The operating conditions were as follows: 0–10 min isocratic elution 6% B; linear gradient from 6% B to 20% B, 10–50 min; and isocratic elution 20% B, 50–60 min. The flow rate was 1 ml/min. The injection volume was 20 μl and the wavelength used for the detection was 530 nm.

**Samples**

The commercial cranberry dried extract (Cran Max®; Cape Cod Biolab Corporation, Venice, FL, USA) was derived from mature, fresh or frozen, cleaned cranberry fruit powder, cranberry concentrate, cranberry seed and cranberry seed oil, cellulose gum and lecithin. It is gently dried using a proprietary process designed to maintain high recoveries of the bioactive ingredients and it is then milled to acceptable mesh for various applications.

The samples of cranberry food supplement (Istituto Ganassini S.p.A., Milan, Italy) are composed of animal or vegetable capsules containing the same commercial cranberry dried extract, enriched with α-tocopheryl succinate (approximately 2.5 mg per capsule) and Acerola (*Malpighia punicifolia*) dried extract, corresponding to approximately 7.5 mg ascorbic acid per capsule.

Different degradation treatments were carried out to verify the stability of anthocyanins. Initially, the cranberry dried extract was subjected to treatment with natural light, exposing about 3 g powder placed in a thin layer on various open Petri plates to the natural light for 30 days. Subsequently, the sample was also subjected to thermal treatment, exposing the sample to alternating hot and cold temperatures (40°C for 1 day followed by 4°C for 1 day) for 30 days. Every 5 days the sample was analyzed to verify the anthocyanin content. The cranberry food supplement was also subjected to the same conditions and analyzed in the same manner.

**Sample extraction**

*Cranberry dried extract.* One gram of dried extract powder was weighed into a 50 ml flask. Then, 10 ml methanol was added and the mixture was stirred for 40 min. The sample was then placed in an ultrasonic bath for 10 min. The mixture was
Figure 2. HPLC chromatogram of cranberry dried extract anthocyanins: (a) Detection on the extract not treated, (b) after 30 days of exposure to natural light, and (c) after 30 days of thermal treatment. Peak identification: 1, cyanidin-3-galactoside; 2, cyanidin-3-glucoside; 3, cyanidin-3-arabinoside; 4, peonidin-3-galactoside; 5, peonidin-3-glucoside; 6, peonidin-3-arabinoside.
then transferred into a 20 ml centrifugal tube. Phase separation was achieved by centrifugation at 5000 r.p.m. for 10 min. The supernatant was recovered and filtered. Finally, a sample of the filtrate was placed in a vial and injected into the high-performance liquid chromatography-diode-array detector (HPLC-DAD).

**Cranberry food supplement.** A 0.5 g sample of powder drawn out from the capsules was weighed into a 100 ml volumetric flask. After addition of 5 ml methanol, the mixture was stirred for 40 min followed by 10 min in an ultrasonic bath. The mixture was then centrifuged. The supernatant was recovered and filtered. One milliliter of the supernatant was drawn out and diluted to 10 ml with methanol. Finally, the sample was placed in a vial and injected into the HPLC-DAD.

**Results and discussion**

The anthocyanin composition of cranberry is well characterized. Figures 2 and 3 show examples of the HPLC response obtained with the method previously described. Peak assignments were made by comparison with results reported in the literature (Hong and Wrolstad 1990; Mazza and Miniati 1993; Prior et al. 2001; Sapers and Hargrave 1987). Four major anthocyanin pigments can be identified (cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside and peonidin-3-arabinoside), as well as two minor anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside).
Reproducibility was assessed by measuring the peak areas of the six anthocyanins. For the highly concentrated samples, the data were obtained by the injection of 10 extracts obtained from the cranberry dried extract, which was not subjected to any degradation treatment. The samples of lower concentration were obtained by injection of 10 extracts obtained from the cranberry dried extract subjected to exposure to natural light for 30 days. Relative standard deviation (R.S.D.) percentage data, calculated by adding up the areas corresponding to the six anthocyanins, ranged from 7.8% to 3.1% for the high (336.5 mg/kg) and low (33.3 mg/kg) concentrations, respectively. The evaluation of the anthocyanin stability was carried out every 5 days until the 30th day of treatment. The quantification of total anthocyanins was carried out at a wavelength of 530 nm using the calibration curve of cyanidin. The calibration curve used to quantify total anthocyanins, expressed as cyanidin, was prepared as previously reported (Bononi et al. 2006). The correlation coefficient was confirmed to be 0.996. The quantitative calculation was processed as follows:

\[ C_{ac} = C_e \times D \times V/W \]

where \( C_{ac} \) is the total concentration of anthocyanins present in the sample (mg/kg), \( C_e \) is the total concentration of anthocyanins obtained from the cyanidin calibration curve (mg/l), \( D \) is the dilution factor (1–10), \( V \) is the volume (ml) of methanol used for the samples extraction, and \( W \) is the weight (g) of the samples.

Table I reports the total concentration of anthocyanins in the samples not subjected to any treatment and after degradation produced from the thermal treatment and from the exposure to the light.

The values obtained after storage of the dried extract show a considerable sensitivity toward the agents’ temperature and light. In particular, the alternating of hot and cold temperatures causes almost a total loss of anthocyanins after 30 days. Also, the treatment with natural light produces almost a total degradation in the same period of 30 days.

Data obtained after the storage of the food supplement in capsules demonstrate the positive effect of enrichment with \( \alpha \)-tocopheryl succinate and ascorbic acid on the stability of the cranberry dried extracts. The presence of alpha tocopheril succinate and ascorbic acid limit the anthocyanin decay to 10–15% for treatment with natural light.
light and to 21–30% for thermal treatment. There is no significant difference in the behavior of the product with regards to the type of capsules used (animal or vegetable gel capsules). It can be concluded from the present experiments that α-tocopheryl succinate and ascorbic acid can be reasonably utilized to increase the stability of food supplements containing cranberry juice or derived extracts.

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References


