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Rosaceae products: Anthocyanin quality and comparisons between dietary supplements and foods

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ABSTRACT

Rosaceae (strawberry, cherry, blackberry, red raspberry, and black raspberry) dietary supplements and food products (total n = 74) were purchased and analyzed to determine their anthocyanin concentrations and profiles. Eight of the 33 dietary supplements had no detectable anthocyanins (five samples) or were adulterated with anthocyanins from unlabeled sources (three samples). Five of 41 food products contained no detectable anthocyanins. In mg per serving, the dietary supplements tested contained 0.02–86.27 (average 10.00), and food products contained 0.48–39.66 (average 7.76). Anthocyanin levels between the dietary supplements and food products were not significantly different in mg per serving. Individual anthocyanin profiles can be used to evaluate quality of Rosaceae food products and dietary supplements. These findings show that increasing anthocyanin content and reducing adulteration could improve the quality of Rosaceae products available in the marketplace.

1. Introduction

The US marketplace offers consumers a wide variety of fresh small fruits, and an even greater variety of products made from those fruits. After reports of small fruits being good sources of dietary phenolics [1, 2], some nutraceutical manufacturers offered more small fruit-based supplement products for their potential health benefits, although these benefits have yet to be clearly demonstrated [2,3,4,5]. The number of fruit-based dietary supplements targeted to increase consumers' anthocyanin intake has risen recently. Unfortunately, there has also been increasing concern with many entries into this market due to adulteration, poor-quality source materials, and the lack of clinical trial evidence to substantiate products' claims [2,3,4,6]. While US dietary supplements are not regulated like conventional food and drugs, though the Dietary Supplement Health and Education Act (DSHEA) of 1994 requires the manufacture of them to follow Current Good Manufacturing Practices (CGMP; 21CFR111). For example, dietary supplements are typically allowed for sale until problems warrant investigation (i.e., adverse event reports), unlike drugs which undergo clinical trials to demonstrate efficacy and safety before being approved for sale [6].

When raw whole fresh fruit is used in products, it is often easiest to authenticate the ingredient by a plant taxonomy expert [7]. But, when whole fruit botanical and morphological features cause confusion, anthocyanin profiling (using metabolites for chemotaxonomy) can offer the assurance of authenticity [7]. Since anthocyanin profiles can identify fruit sources, they have long been used for quality control measurements in fruit juices and concentrates [8,9], and as quality indicator compounds in authenticity determinations of food ingredients [8,10]. For example, since each species of black raspberries has a unique anthocyanin profile, material sourced from *Rubus occidentalis* L. (American black raspberry) can be distinguished from that of *R. coreanus* Miq. (Korean black raspberry) [7,11,12]. A recent study using anthocyanins for fruit authenticity found adulteration of popular *Vaccinium* dietary supplements; where some samples contained none of the labeled fruits' anthocyanins, while others had no detectable anthocyanin of any kind [13].

Rosaceae fruit dietary supplements are also prevalent in the US marketplace. Numerous review articles exist on the potential health benefits from strawberry, cherry, blackberry, red raspberry, and black raspberry consumption [2,14,15,16,17]. Although work is still needed on the underlying mechanism behind eating Rosaceae products and a health advantage, along with additional reports to establish favorable forms, optimum dosage, and likely interactions with other natural compounds. Among the many Rosaceae fruit research projects underway in the US are several human clinical trials to determine how Rosaceae fruit may be potentially beneficial to us. These include studies on the influence that strawberries (US clinical trial identifier NCT02557334) or tart cherries (US clinical trial identifier NCT02154100) have on cardiovascular risks, blackberries and cancer processes (US clinical trial identifier NCT01293617), red raspberries on insulin action (US clinical trial identifier NCT02479035), and black raspberries on oral bacteria (US clinical trial identifier NCT02439255) or ulcerative colitis (US clinical trial identifier NCT02267694).

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While sales for these Rosaceae products have increased, so have concerns about of the quality of dietary supplement ingredients. Anthocyanin profiling can be used for quality measurements on Rosaceae dietary supplements, as demonstrated with *Vaccinium* fruit based dietary supplements [13]. The National Institutes of Health-Office of Dietary Supplements (NIH-ODS) and AOAC (Association of Analytical Communities) International have recently made efforts to establish analytical methods that aid the dietary supplement industry producing *Vaccinium* fruit-based ingredients, and these methods can be applied to Rosaceae-based products as well.

Rosaceae (also known as the Rose family) contains a vast array of plants, including weeds, ornamentals, and edible plants. Some of the best known Rosaceae crops are apples, peaches, almonds, and strawberries [18], and in the dietary supplement marketplace, the most commonly encountered Rosaceae products are made from strawberry, cherry, blackberry, red raspberry, and black raspberry ingredients. The main objectives of this work were to demonstrate anthocyanin profiles could be used for quality assessments of typical Rosaceae dietary supplement products, to determine their anthocyanin concentrations, and compare those values to ones obtained from Rosaceae fruit products.

2. Materials and methods

2.1. Dietary supplement and food sample information and extraction

Strawberry (coded SBDS01–SBDS03 and SBFP01–SBFP11 for dietary supplements and food products, respectively), cherry (coded CHDS01–CHDS16 and CHFP01–CHFP14), blackberry (coded BBDS01–BBDS03 and BBFP01–BBFP06), red raspberry (coded RRDS01–RRDS03 and RRFP01–RRFP09), and black raspberry (coded BRDS01–BRDS08 and BRFP01) samples were selected for analyses since corresponding dietary supplements and food products could be purchased. At the time of study, other Rosaceae fruit (e.g., peach, apple, or plum) products were unavailable as corresponding dietary supplements. All Rosaceae supplements (n = 33) and processed food products (n = 41) were purchased from local marketplaces (Boise, ID and Nampa, ID, USA) or Amazon Marketplace (Seattle, WA, USA) during June to December 2015. The dietary supplements were bought as dried whole fruit, bulk loose powder, capsules, tablets, or extracts. Processed Rosaceae food products were packaged as juice, juice concentrate, dried whole fruit, loose powder, jam, preserve, spread, and pie filling forms. Capsulized samples were removed from their shells, and only the capsule contents were extracted. Samples that were in the form of tablets and whole dried fruit were powdered using an IKA Tube Mill control (IKA Works, Inc., Wilmington, NC, USA), equipped with 40 mL disposable grinding chambers. All powder forms were extracted with high purity water as described in Lee and Rennaker [19]. All samples were purchased and analyzed well within their expiration or best use by date.

2.2. Reagents, chemicals, and standards

All chemicals, reagents, and standards used in this study were analytical or HPLC grade from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Cyanidin-3-glucoside was obtained from Polyphenols Laboratories AS (Sandnes, Norway).

2.3. HPLC (high-performance liquid chromatography) condition for individual anthocyanin separation

Individual anthocyanins were eluted as described before in Lee and Finn [20], with the minor modification summarized in Lee [12]-guard column (of the same phase as analytical column) at the inlet of the longer analytical column (Synergi Hydro-RP 80 Å, 250 × 2 mm, 4 μm; Phenomenex, Inc., Torrance, CA, USA). Eluting peaks were monitored at 520 and 280 nm. Anthocyanin peak identifications were conducted by HPLC-DAD-MS (Agilent HPLC 1100; Agilent Technologies Inc., Palo Alto, CA, USA) using obtained mass-to-charge ratio, retention time comparison, UV–VIS spectra, and prior reported identifications [10; references listed in Table 1]. Anthocyanins were expressed as cyanidin-3-glucoside in mg/100 g and mg per serving. For dietary supplement samples, 1 capsule, 1 tablet, 5 mL for extracts, or 1 teaspoon for loose powder (5 g) was used for expression in per serving. For food products, 20 g for jam, preserves, and spread samples, 75 g for pie fillings, 236 mL for juice samples, and 1 teaspoon (5 g) for loose powder or dried fruit was used for expression in per serving. For conciseness, per serving will be used throughout the following text.

2.4. Statistical analysis

Statistical analysis was conducted using Minitab Express for Macintosh version 1.4.0 (Minitab, Inc., State College, PA, USA). Two sample t-test was performed for comparing dietary supplements and food products anthocyanin concentration at $\alpha = 0.05$.

3. Results and discussion

Rosaceae dietary supplements anthocyanin concentration ranged from 3.20 to 1725.43 in mg/100 g (Table 2 and Fig. 1) and 0.02 to 86.27 mg per serving (Table 3 and Fig. 1) (n = 25). Rosaceae food products anthocyanin concentration ranged from 0.72 to 454.99 in mg/100 g (Table 2 and Fig. 1) and 0.48 to 39.66 mg per serving (Table 3 and Fig. 1) (n = 36). There was a significant difference in anthocyanin concentration between dietary supplements and food products when results were expressed in mg/100 g ($p = 0.03$). Dietary supplements' anthocyanin concentration range was higher in mg/100 g, though this might be due to the fact that most of the dietary supplements were dry, while the food items tested were mostly in wet forms (Fig. 1). In mg per serving, there was no significant difference ($p = 0.64$) between the two groups. Some samples of each group (n = 5 dietary supplements and n = 5 food products; examples of ingredient listing in Table 4) had no measureable anthocyanins. Adulteration was detected in three dietary supplement samples (BBDS03, BRDS07, and CHDS16; more information below and examples of ingredient listing in Table 4), where the products contained anthocyanins from sources other than the labeled ingredients. For example, one cherry dietary supplement (CHDS16; see Table 4 and further discussion below) label claims 6.8 mg of anthocyanins per serving and a guarantee that the product contains no unlisted ingredients. For study clarity, dietary supplement samples without measurable anthocyanins were considered as products with inaccurate ingredient labeling. Over 20% of dietary supplements did not contain labeled ingredients.

3.1. Strawberry products

Strawberry products contained two to five anthocyanins (depending on anthocyanin retention in the final product): cyanidin-3-glucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, cyanidin-glucoside-malonate, and pelargonidin-3-glucoside-malonate. The main anthocyanin (in samples with detectable amounts) was pelargonidin-3-glucoside, as reported before [21,22,23]. In mg per 100 g and serving, there was no significant difference between dietary supplements and food products anthocyanin levels ($\alpha = 0.05$).

In mg/100 g, all strawberry products' anthocyanins ranged from none detected to 170.83 mg/100 g (SBFP09- freeze-dried whole fruit; Table 2). All three strawberry dietary supplements contained strawberry anthocyanins, and in increasing order of concentration were (mg per serving): SBDS01 (2.16) < SBDS02 (3.93) < SBDS03 (7.06). All three strawberry dietary supplements were made from dehydrated juice or extract according to label information.

Nine of the eleven strawberry food products contained measureable levels of anthocyanin, and in increasing order of concentration were (mg per serving): SBFP02 (0.60) < SBFP03 (0.70) < SBFP04 (0.75) < SBFP05 (0.84) < SBFP06 (1.09) < SBFP07 (1.45) < SBFP01