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Original article

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.

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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

Table 1
Literature reported anthocyanin ranges of strawberry, cherry, blackberry, red raspberry, and black raspberry (in mg/100 g or mg/100 mL) in fruit and fruit products, as determined by HPLC.

Fruit	Fresh form	Dried form	Liquid form	Literature cited
Strawberry	7–66 (n = 31)	104–617 (n = 18)	26 (n = 1)	Aaby et al. [22]; Alonzo-Macias et al. [34], Finn et al. [23]; Jakobek et al. [35]; Patras et al. [36]; Vasco et al. [37]
Cherry	3–230 (n = 31)	6–106 (n = 6)	26–37 (n = 2)	Goncalves et al. [38]; Jakobek et al. [35,39]; Kirakosyan et al. [40]; Simunic et al. [41]; Vasco et al. [37]
Blackberry	28–366 (n = 1306)	975–1039 (n = 5)	12–107 (n = 11)	Fan-Chiang and Wrolstad [42]; Lee [2], and reference therein
Red raspberry	28–116 (n = 27)	267–374 (n = 12)	0.04–110 (n = 15)	Boyles and Wrolstad [8]; De Ancos et al. [27]; Jakobek et al. [35,39]; Mazur et al. [28]; Patras et al. [36]; Remberg et al. [43]
Black raspberry	3–996 (n = 1050)	18–1139 (n = 11)	0.8–2904 (n = 15)	Lee [2] (and reference therein); Dossett et al. [31], Lee [12]

(5.60) < SBFP08 (8.04) < SBFP09 (8.54). SBFP10 (spread) and SBFP11 (preserves) had no detectable anthocyanin. Samples sold as freeze-dried whole fruit had the highest levels of anthocyanins, whether compared in 100 g units or mg per serving (SBFP08–160.82 mg/100 g and SBFP09–170.83 mg/100 g; SBFP08–8.04 mg/serving and SBFP09–8.54 mg/serving).

3.2. Cherry products

As Picariello et al. [24] pointed out, there is a discrepancy in cherry anthocyanin identification within the literature. The cherry dietary supplements tested had a wide range of species and common names listed as ingredients, such as black cherry (*Prunus serotina*), tart cherry (*P. cerasus*), sweet cherry (*P. avium*), or Montmorency tart cherry (*P. cerasus*). Food products rarely use plant species name for fruit ingredients on package labels. The cherry food products listed black cherry, tart cherry, sweet cherry, dark sweet cherry, or Royal Ann cherry as the fruits source. All cherry products contained one to six of the following anthocyanins (depending on cherry type and anthocyanin retention in the final product): cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, pelargonidin-3-sophoroside, cyanidin-3-rutinoside, and pelargonidin-3-rutinoside. The main anthocyanin in tart cherry and black cherry was cyanidin-3-glucosylrutinoside, and cyanidin-3-rutinoside in sweet cherry. In mg/100 g or mg per serving, there was a significant difference between dietary supplements and food products anthocyanin levels, where cherry food products provided higher levels of anthocyanins compared to dietary supplements ($\alpha = 0.05$).

In mg/100 g, 26 out of 30 cherry products' anthocyanins ranged from 0.72 to 88.80 mg/100 g (CHDS12- capsule; Table 2). In increasing order of anthocyanin concentration, the cherry dietary supplements that contained anthocyanins were (mg per serving): CHDS02 (0.02) < CHDS04 (0.07) < CHDS07 (0.11) < CHDS05 (0.15) < CHDS001 (0.16) < CHDS08 (0.23) < CHDS06 (0.24) < CHDS12 (0.38) < CHDS11 (0.42) < CHDS09 (0.53) < CHDS10 (0.55) < CHDS03 (0.65). CHDS03 was a liquid concentrate sample, but had low anthocyanin levels. CHDS15 was also a liquid dietary supplement concentrate and contained no detectable anthocyanin. CHDS13 (see Fig. 2) and CHDS14 (both capsules) contained no detectable anthocyanins either. CHDS16 (capsule) contained presumptively bilberry (*V. myrtillus* L.) anthocyanin and had no cherry anthocyanins (see Fig. 2).

All cherry food products contained anthocyanins. In increasing order of anthocyanin concentration, the cherry food products that contained anthocyanins were (mg per serving): CHFP03 (0.48) < CHFP01 (0.54) < CHFP08 (3.30) < CHFP02 (4.03) < CHFP04 (6.01) < CHFP05 (6.25) < CHFP06 (6.79) < CHFP07 (8.68) < CHFP11 (9.93) < CHFP12 (10.60) < CHFP09 (20.64) < CHFP10 (30.18) < CHFP13 (34.69) < CHFP14 (39.66). The lowest anthocyanin-containing product (CHFP03) was a spread. The samples with the most anthocyanins were juices (CHFP10, CHFP13, and CHFP14). Cherry food products were mostly sold as juices, and the higher levels seen might be due to larger serving size compared to that of a dietary supplement.

3.3. Blackberry products

Blackberry products contained three to five anthocyanins (depended on whether the anthocyanin was retained in the final product): cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xyloside, cyanidin-3-malonylglucoside, and cyanidin-3-dioxalylglucoside* (*or possibly cyanidin-3-hydroxymethylglutaroylglucoside, [25]). The main blackberry anthocyanin was cyanidin-3-glucoside [2,26]. In mg/100 g, eight of nine blackberry products' anthocyanins ranged from none detected to 263.35 mg/100 g (BBDS02- capsule; Table 2). In mg/100 g and mg per serving, there was no significant difference

Table 2
Anthocyanin content (mg/100 g) of Rosaceae fruit products sold as dietary supplements and foods. Only samples that contained Rosaceae fruit anthocyanins were included in the table. Samples were excluded if they had no detectable anthocyanins or contained non-Rosaceae anthocyanins. Differing letters following the mean for each Rosaceae fruit's sample form indicate a significant difference ($p \leq 0.05$) between dietary supplement and food product pairs.

Rosaceae fruit	Sample form	Total samples evaluated	Number of samples not included in quantification*	Number of non-adulterated samples quantified	Mean (*standard deviation*)	Minimum ACY*	Maximum ACY
Strawberry	Dietary supplements	3	0	3	87.61a (*49.61*)	43.15	141.12
	Food products	11	2	9	40.13a (*71.32*)	2.37	170.83
Cherry	Dietary supplements	16	4	12	37.81b (*32.18*)	3.20	88.80
	Food products	14	0	14	7.23a (*5.83*)	0.72	16.76
Blackberry	Dietary supplements	3	1	2	243.42a (*28.19*)	223.49	263.35
	Food products	6	1	5	135.99a (*95.64*)	27.60	248.91
Red raspberry	Dietary supplements	3	1	2	468.96a (*138.55*)	370.99	566.93
	Food products	9	2	7	70.66a (*169.48*)	3.96	454.99
Black raspberry	Dietary supplements	8	2	6	735.01a (*760.50*)	32.09	1725.43
	Food product	1	0	1	6.11**	–	–

–, Not determined.
*, Not included in the quantification data here due to absence of anthocyanin, too degraded, or suspicious profiles.
**, One sample available and analyzed, so no t-test comparison, mean, and standard deviation to report.

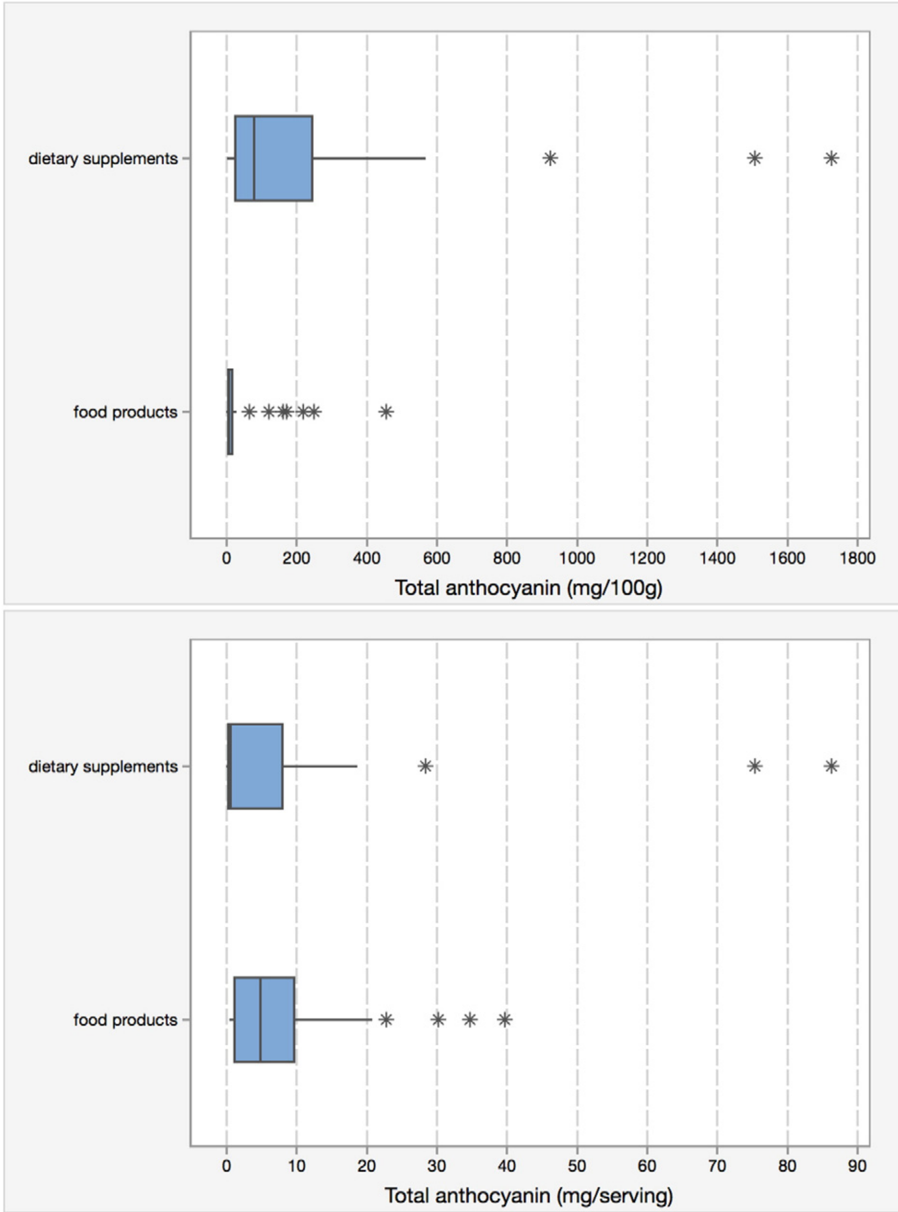

Fig. 1. Plot showing the range of the total anthocyanin concentration in mg/100 g (top) and mg per serving (bottom) of both dietary supplements and food products. In mg/100 g, the dietary supplements tested contained 3.20–1725.43, and food products contained 0.72–454.99. In mg per serving, the dietary supplements tested contained 0.02–86.27, and food products contained 0.48–39.66. Figure excludes samples that contained no detectable, or unlisted sources of, anthocyanins. *indicate outliers.

Table 3
Anthocyanin content (mg per serving) of Rosaceae fruit products sold as dietary supplements and foods. Only samples that contained Rosaceae fruit anthocyanins were included in the table. Samples were excluded if they had no detectable anthocyanins or contained non-Rosaceae anthocyanins. Differing letters following the mean for each Rosaceae fruit's sample form indicate a significant difference ($p \leq 0.05$) between dietary supplement and food product pairs.

Rosaceae fruit	Sample form	Total samples evaluated	Number of samples not included in quantification*	Number of non-adulterated samples quantified	Mean (*standard deviation*)	Minimum ACY*	Maximum ACY
Strawberry	Dietary supplements	3	0	3	4.38a (*2.48*)	2.16	7.06
	Food products	11	2	9	3.07a (*3.35*)	0.60	8.54
Cherry	Dietary supplements	16	4	12	0.29a (*0.21*)	0.02	0.65
	Food products	14	0	14	12.98b (*12.97*)	0.48	39.66
Blackberry	Dietary supplements	3	1	2	6.25a (*6.97*)	1.32	11.17
	Food products	7	2	5	7.63a (*3.88*)	3.24	12.45
Red raspberry	Dietary supplements	4	2	2	23.45a (*6.93*)	18.55	28.35
	Food products	8	1	7	4.38a (*8.1*)	0.79	22.75
Black raspberry	Dietary supplements	8	2	6	28.98 (*40.41*)	0.15	86.27
	Food product	1	0	1	1.22**	–	–

–, Not determined.
*, Not included in the quantification data here due to absence of anthocyanin, too degraded, or suspicious profiles.
**, One sample available and analyzed, so no t-test comparison, mean, and standard deviation to report.

Table 4
Examples of information provided on dietary supplement products' packaging. The samples shown below contained undeclared sources of anthocyanin or had no detectable anthocyanins. Identification codes: CHDS—cherry dietary supplements, BBDS—blackberry dietary supplements, RRDS—red raspberry dietary supplements, and BRDS—black raspberry dietary supplements.

Sample code	Ingredient listing	Additional label information
CHDS13	Tart cherry (from 300 mg of tart cherry 4:1 extract), gelatin, rice powder, vegetable magnesium stearate.	Capsule form. Tart cherries are rich in antioxidants called anthocyanins and have the highest anti-inflammatory content of any food.* *These statements have not been evaluated by the Food and Drug Administration, This product is not required to diagnose, treat, cure, or prevent any disease.
CHDS14	Tart cherry 10:1 extract, natural gelatin capsules, and maltodextrin.	Professional grade nutraceuticals.
CHDS15	Organic black cherry juice concentrate, citric acid and xanthan gum.	Liquid dietary supplement contains 100% pure certified organic black cherry (*Prunus serotina*) concentrate. No sugar added. Preservative free.
CHDS16*	Tart cherry (*Prunus cerasus*) (fruit concentrate) (guaranteed to contain 6.8 mg [0.8%] anthocyanin, silicon dioxide, vegetable cellulose capsule, cellulose and magnesium stearate	No ingredients other than those listed on this label have been added to this product. 0.8% anthocyanins. 425 mg per capsule.
BBDS03*	Blackberry fruit powder.	This statement have not been evaluated by the Food and Drug Administration. This product is not required to diagnose, treat, cure, or prevent any disease.
RRDS03	Calcium ascorbate, calcium citrate, magnesium citrate, magnesium aspartate, zinc citrate, selenium amino acid chelate, copper citrate, chromium dinicotinate glycinate, sodium molybdate, sodium chloride, potassium aspartate, potassium phosphate, cranberry fruit extract, non-GMO maltodextrin, stevia leaf extract, natural flavors, citric acid, malic acid, beet color, silica, and luo han guo fruit extract.	Raspberry dietary supplement.
BRDS07	Live organic black raspberry (freeze dried 5:1 extract).	Black raspberry extract powder. No fillers, chemicals, or preservatives.
BRDS08*	Black raspberry (*Rubus occidentalis*), gelatin, silica, vegetable stearate.	Capsule, maximum strength. Super antioxidant. Product is a potent nutrient dense superfood that deliver a superior amount of antioxidant in every dose. Black raspberry are also known as one of the best source of anthocyanins and ellagic acid which helps to promote immunity, healthy vision, and healthy blood pressure levels* Supports healthy aging and health vision*, supports health cardiovascular system*, supports healthy inflammation response*. *These statements have not been evaluated by the Food and Drug Administration, This product is not required to diagnose, treat, cure, or prevent any disease.

* CHDS16 contained bilberry anthocyanins and BBDS03 contained highbush blueberry anthocyanins, which were not listed as ingredients. BRDS08 contained blackberry anthocyanins, not black raspberry as labeled.

between dietary supplements and food products anthocyanin levels ($\alpha = 0.05$).

Three blackberry dietary supplements were available, and one (BBDS03) was adulterated. BBDS03 was sold as a loose powder and contained possible highbush blueberry anthocyanins, but none from blackberry (typical adulterated chromatograms can be seen in Lee [13]). The two dietary supplements that contained blackberries, and in increasing order of concentration, were (mg per serving): BBDS01 (1.32) < BBDS02 (11.17). Both of those samples were sold in capsule form and labeled as containing ground whole blackberry fruit.

The six blackberry food products, in increasing order of concentration, were (mg per serving): BBFP06 (not detected) < BBFP02 (3.24) < BBFP01 (5.52) < BBFP03 (6.02) < BBFP04 (10.91) < BBFP05 (12.45). The highest was dried whole fruit form (BBFP05), while the lowest was a spread (BBFP06). Although BBFP02, BBFP03, and BBFP04 were also dried whole fruits, indicating dried whole fruit form itself does not ensure the highest levels of anthocyanins and that purchasing to obtain the highest amount of anthocyanins is not necessarily straightforward. This is not surprising since the raw ingredients have a wide range of anthocyanin concentration (see Table 1).

3.4. Red raspberry products

Red raspberry products contained three to eight anthocyanins (depending on anthocyanin retention in the final product): cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-sambubioside, cyanidin-3-glucoside, pelargonidin-3-sophoroside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside. The main red raspberry anthocyanins were cyanidin-3-sophoroside and cyanidin-3-rutinoside, with the ratio of these two compounds differ depending on cultivar and retention after processing [1,8,27,28], although there are more variations with new cultivar releases [2,23,26,29].

In mg/100 g, red raspberry products' anthocyanins ranged from none detected to 566.93 mg/100 g (RRDS02; Table 2). The two red raspberry dietary supplements that contained anthocyanins (mg per serving) were RRDS01 (18.55) and RRDS02 (28.35), both in loose powder form. One red raspberry dietary supplement (RRDS03, loose powder) contained no detectable anthocyanins; although the sample was red, its ingredients did not list red raspberry, but did include beet powder (see Table 4). The nine red raspberry food products in increasing order of anthocyanin concentration were (mg per serving): RRFP08 and RRFP09 (not detected) < RRFP01 (0.79) < RRFP02 (1.11) < RRFP03 (1.14) < RRFP04 (1.27) < RRFP05 (1.77) < RRFP06 (1.85) < RRFP07 (22.75). RRFP07 was sold as dried whole fruit. RRFP08 and RRFP09 were both spreads. In mg/100 g and mg per serving, there was no significant difference between dietary supplement and food product anthocyanin levels ($\alpha = 0.05$).

3.5. Black raspberry products

Possibly due to the timing of sampling period (seasonal product and limited production), less black raspberry dietary supplement products were available for this study compared to previous work [11,12]. Black raspberry products that contained anthocyanins had two to seven anthocyanins (depending on anthocyanin retention in the final product): cyanidin-3-sambubioside, cyanidin-3-xylosylrutinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, and peonidin-3-rutinoside. Most of the time, cyanidin-3-rutinoside was the main black raspberry product anthocyanin.

In mg/100 g, eight of nine black raspberry products' anthocyanins ranged from not detected to 1725.43. BRDS07 (capsule) contained no detectable anthocyanins. BRDS08 (sold as black raspberry loose powder) was purchased on three separate occasions, each with a different lot number, and all three were found adulterated with blackberry

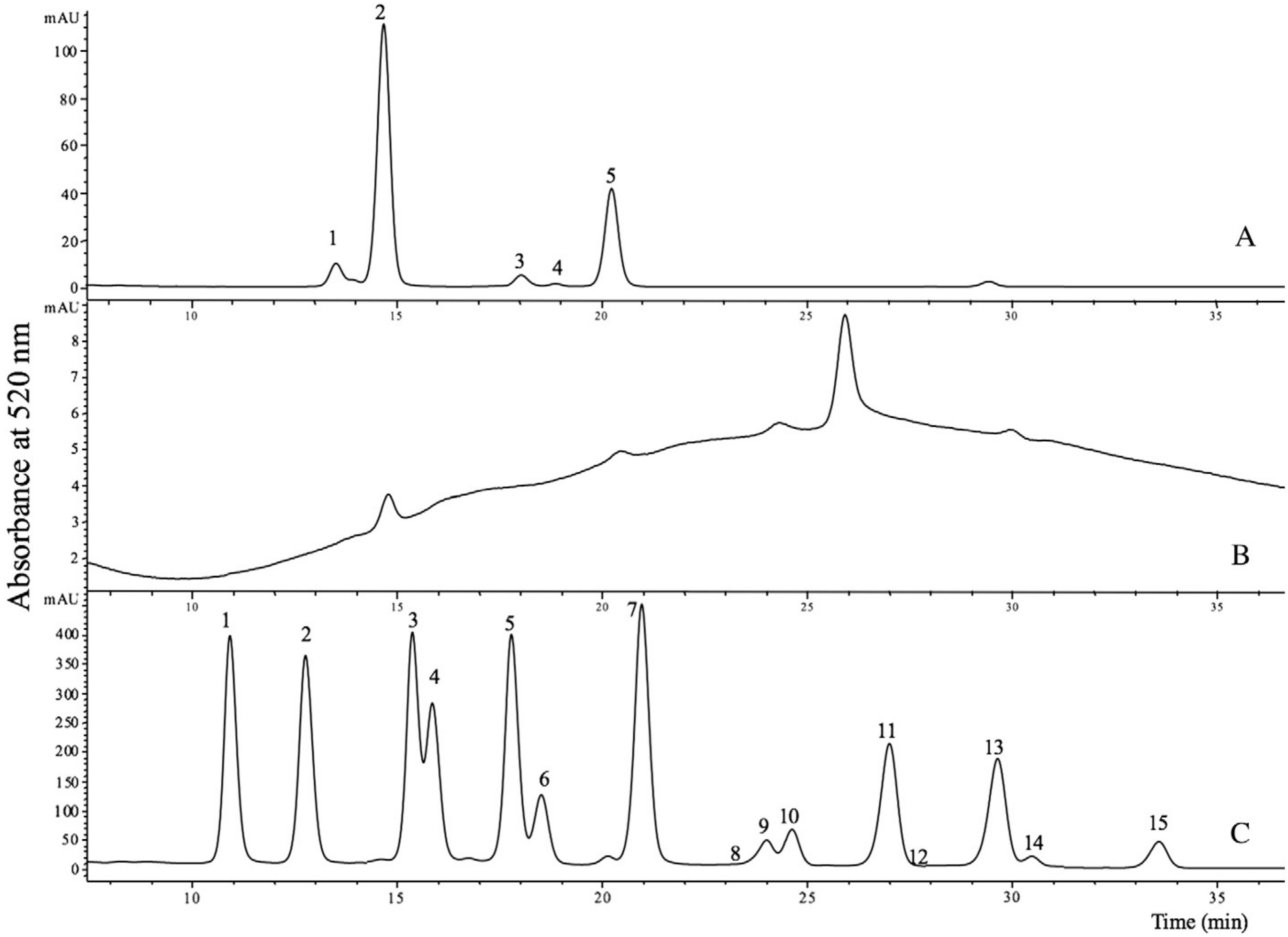

Fig. 2. Three examples of anthocyanin chromatograms from the cherry dietary supplements (CHDS). Sample CHDS12 (A) was packaged as containing sour cherries, and its anthocyanin profile supported that claim. CHDS13 (B) contained no detectable anthocyanins. CHDS16 (C) had the anthocyanin profile of bilberry (*Vaccinium myrtillis* L., [13]), and the profile that of cherry (*Prunus cerasus* L.) as the package claimed. Peak assignments for chromatogram A are 1, cyanidin-3-sophoroside; 2, cyanidin-3-glucosylrutinoside; 3-cyanidin-3-glucoside, 4, pelargonidin-3-sophoroside; and 5, cyanidin-3-rutinoside. Peak assignments for chromatogram C are 1, delphinidin-3-galactoside; 2, delphinidin-3-glucoside; 3, cyanidin-3-galactoside; 4, delphinidin-3-arabinoside, 5, cyanidin-3-glucoside; 6, petunidin-3-galactoside; 7, cyanidin-3-arabinoside; 8, petunidin-3-glucoside; 9, peonidin-3-galactoside; 10, petunidin-3-arabinoside; 11, malvidin-3-galactoside; 12, peonidin-3-glucoside; 13, malvidin-3-glucoside; 14, peonidin-3-arabinoside and 15, malvidin-3-arabinoside.

based on anthocyanin profile. The six black raspberry dietary supplements listed in increasing order of concentration were (mg per serving) BRDS01 (0.15) < BRDS02 (0.24) < BRDS04 (3.12) < BRDS03 (8.75) < BRDS05 (75.34) < BRDS06 (86.27). BRDS05 was a loose powder form and BRDS06 was dried whole fruit. Only one food product (BRFP01, spread) was available and contained 1.22 mg per serving.

3.6. All Rosaceae products

Four of the five food items that had no detectable anthocyanins were spreads (SBFP10, BBFP06, RRFP08, and RRFP09). Fruit spreads do not have as much fruit compared to jams or preserves (21CFR150.160). This emphasizes the importance for consumers to understand that food ingredients are listed in decreasing order, especially for consumers who want to monitor intake of specific items. While there are recent works on limiting excess sugar consumption (USDA 2015–2020 dietary guidelines) and the public understands that fruit naturally contains sugar [30], it is much less recognized that fruit-based dietary supplements also likely contain sugar since their production does not include its removal (e.g., dehydrated fruit juice; see Table 4).

It was not surprising that two of the black raspberry samples (BRDS05 and BRDS06) contained the highest levels of anthocyanins in the Rosaceae fruit samples here, as it is well established that black raspberry fruit contains high amounts of, and a wide range of, anthocyanins compared to other fruit (Table 1) [29,31]. Starting with highly pigmented fruit will result in products that are high in anthocyanins (see Fig. 3 and Lee [11]).

Dietary supplements are typically sold at a higher price point compared to foods. Some of the dietary supplement package labels also include promises of certain levels of anthocyanin present per serving (see Table 4). Fruit-based dietary supplements are made from an array of pre-processed fruit base, from powdered dehydrated fruit to dehydrated fruit juice/concentrate and extracts, and it is difficult for consumers to distinguish anthocyanin quality, even visually, since dietary supplements are mostly sold in a non-transparent packaging or masked with red/purple-colored fillers in some cases [13]. There are a small number of companies that are selling whole dehydrated fruit forms. Some of the dietary supplements made with dehydrated juices/concentrates which will not include the dietary fiber benefit of consuming whole fruit [2].

In the case of cherry and blackberry dietary supplements evaluated here, in mg/100 g, the supplements contained lower anthocyanin (see values in Table 1) amounts compared to the fresh fruit concentrations reported in the literature (Table 1). Using dehydrated processing waste (i.e., juice pomace) in dietary supplements might be one reason that dietary supplements have differing anthocyanin peak proportions

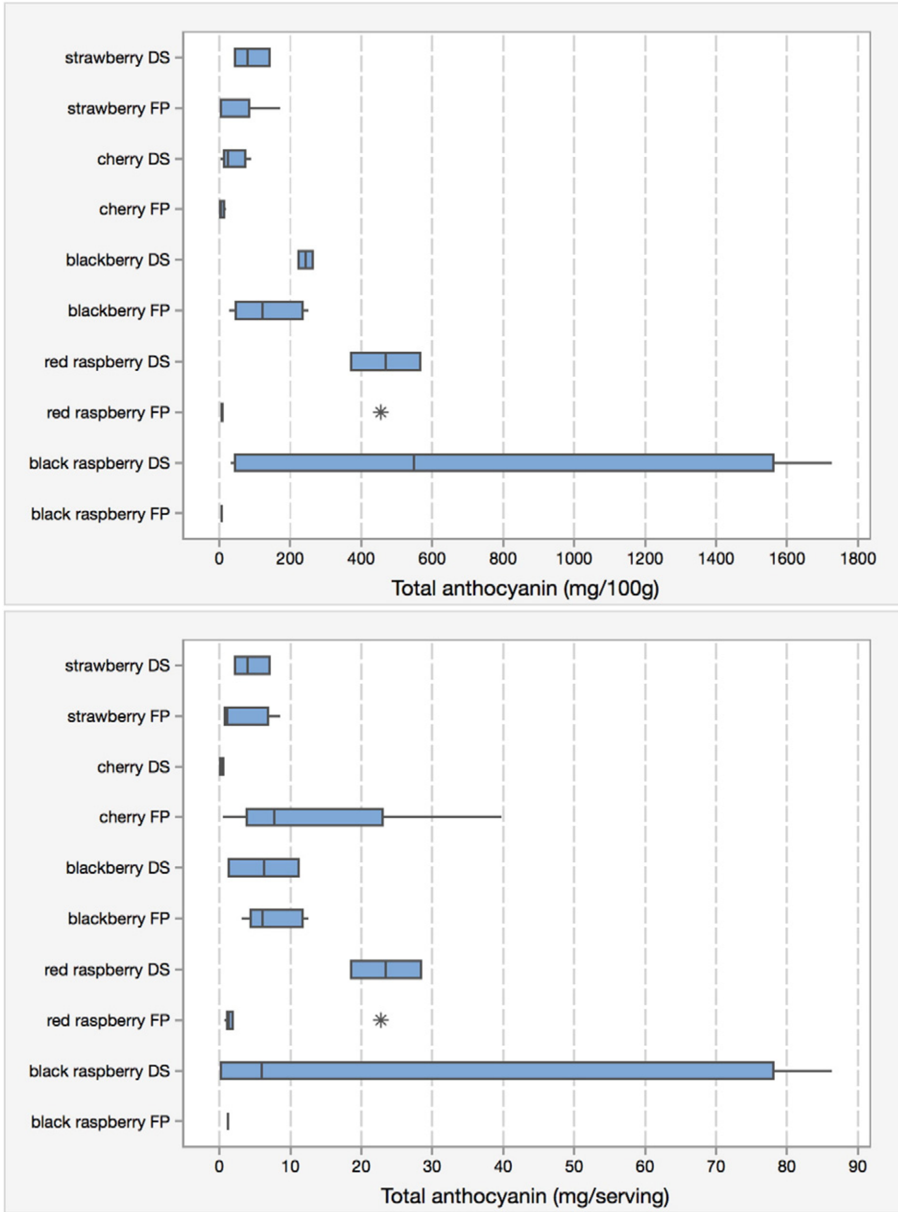

Fig. 3. Total anthocyanin concentration grouped by each berry in mg/100 g (top) and mg per serving (bottom) of both dietary supplements (DS) and food products (FP). Two black raspberry samples contained the highest levels of anthocyanins in the Rosaceae fruit samples examined. Figure excludes samples that contained no detectable, or unlisted sources of, anthocyanins. * indicates outlier.

than what has been reported in the original fruit (anonymous, personal communication), but based solely on ingredient listings of dietary supplements, it is difficult to distinguish fruit ingredient sources. Some examples of altered anthocyanin peak portions in red-pigmented fruit based on fruit fraction and processing can be found in Aaby et al. [21], Lee et al. [32], and Lee and Wrolstad [33]. Additional reasons why dietary supplements might contain lower than expected levels of anthocyanins are dilution by using carrier agents, fillers, and (again) the small serving size (i.e., capsule, tablet) [13].

HPLC separation of anthocyanins is not the only way to determine authenticity of ingredients. There are other ways of measuring authenticity of anthocyanin depending on the purpose. A simple acid and base addition to the samples will allow the analyst to distinguish between anthocyanins and other red pigments, since anthocyanins will undergo a color change and others will not [9,13]. Anthocyanins are not the sole quality indicator compound in fruit dietary supplements and food products [1,5,30], although it is an easier class of phenolics to distinguish among various fruit genera and species [5].

4. Conclusion

There was no significant difference in anthocyanin concentration between dietary supplements and food products in mg per serving. Over 20% of the Rosaceae dietary supplements evaluated contained no detectable anthocyanins, or had unlabeled anthocyanins despite promising specific sources of anthocyanins on the packaging. Systems to improve Rosaceae dietary supplements' quality are needed from source material to final products. Anthocyanin profiling by HPLC has been commonly used in food ingredient quality control and assurance and could be used for quality assurance of dietary supplement ingredients and products as well. We still do not know the optimum consumption method for high-anthocyanin-containing fruit; or if greatest benefits are achieved simply starting with the highest pigmented cultivar, harvest at peak anthocyanin concentration, then maximize retention through processing and storage until it reaches consumer is the answer. Regardless, natural compounds will continue to be inherently more variable than synthesized compounds, although based on these findings, the most effective way to increase dietary phenolics is to choose fresh fruit sources whenever possible.

Conflicts of interest

None

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

[1] J. Lee, M. Dossett, C.E. Finn, *Rubus* fruit phenolic research: the good, the bad, and the confusing, Food Chem. 130 (2012) 785–796.
[2] J. Lee, Blackberry fruit quality components, composition, and potential health benefits, in: R.C. Funt, H.K. Hall (Eds.), Blackberries and Their Hybrids (CABI, Oxfordshire, United Kingdom), 2017 (In press).
[3] European Food Safety Authority, Scientific opinion on the re-evaluation of anthocyanins (E163) as a food additive, EFSA J. 11 (2013) 3145.
[4] I. Fernandes, A. Faria, V. de Freitas, C. Calhau, N. Mateus, Mutiple-approach studies to assess anthocyanin bioavailability, Phytochem. Rev. 14 (2015) 899–919.
[5] J. Lee, Proanthocyanidin A2 purification and levels found in American cranberry (*Vaccinium macrocarpon* Ait.) products, J. Funct. Foods 5 (2013) 144–153.
[6] J. Lee, Further research on the biological activities and the safety of raspberry ketone are needed, NFS J. 2 (2016) 15–18.
[7] J. Lee, M. Dossett, C.E. Finn, Mistaken identity: clarification of *Rubus coreanus* Miquel (bokbunja), Molecules 19 (2014) 10524–10533.
[8] M.J. Boyles, R.E. Wrolstad, Anthocyanin composition of red raspberry juice: influences of cultivar, processing, and environmental factors, J. Food Sci. 58 (1993) 1135–1141.
[9] R.E. Wrolstad, R.W. Durst, J. Lee, Tracking color and pigment changes in anthocyanin products, Trends Food Sci. Technol. 16 (2005) 423–428.
[10] V. Hong, R.E. Wrolstad, Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection, J. Agric. Food Chem. 38 (1990) 698–708.
[11] J. Lee, Marketplace analysis demonstrates quality control standards needed for black raspberry dietary supplements, Plant Foods Hum. Nutr. 69 (2014) 161–167.
[12] J. Lee, Analysis of bokbunja products show they contain *Rubus occidentalis* L. fruit, J. Funct. Foods 12 (2015) 144–149.
[13] J. Lee, Anthocyanin analyses of *Vaccinium* fruit dietary supplements, Food Sci. Nutr. (2016), http://dx.doi.org/10.1002/fsn3.339 (in press).
[14] A. Basu, A. Nguyen, N.M. Betts, T.J. Lyons, Strawberry as a functional food: an evidence-based *review*, Crit. Rev. Food Sci. Nutr. 54 (2014) 790–806.
[15] B.M. Burton-Freeman, A.K. Sandhu, I. Edirisinghe, Red raspberries and their bioactive polyphenols: cardiometabolic and neuronal health links, Adv. Nutr. 7 (2016) 44–65.
[16] C. Garcia-Muñoz, V. Vaillant, Metabolic fate of ellagitannins: implications for health, and research perspectives for innovative functional foods, Crit. Rev. Food Sci. Nutr. 54 (2014) 1584–1598.
[17] L.M. McCune, C. Kubota, N.R. Stendell-Hollis, C.A. Thomson, Cherries and health: a review, Crit. Rev. Food Sci. Nutr. 51 (2011) 1–12.
[18] S. Jung, M. Staton, T. Lee, A. Blenda, R. Svancara, A. Abbott, D. Main, GDR (Genome Database for Rosaceae): integrated web-database for Rosaceae genomics and genetics data, Nucleic Acids Res. 36 (2008) D1034–D1040.
[19] J. Lee, C.D. Rennaker, Influence of extraction methodology on grape composition values, Food Chem. 126 (2011) 295–300.
[20] J. Lee, C.E. Finn, Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars, J. Sci. Food Agric. 87 (2007) 2665–2675.
[21] K. Aaby, G. Skrede, R.E. Wrolstad, Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*), J. Agric. Food Chem. 53 (2005) 4032–4040.
[22] K. Aaby, S. Mazur, A. Nes, G. Skrede, Phenolic compounds in strawberry (*Fragaria* x *ananassa* Duch.) fruits composition in 27 cultivars and changes during ripening, Food Chem. 132 (2012) 86–97.
[23] C.E. Finn, P.P. Moore, B.M. Yorgey, J. Lee, B.C. Strik, C. Kempler, R.R. Martin, 'Charm' strawberry, Hortscience 48 (2013) 1184–1188.
[24] G. Picariello, V. De Vito, P. Ferranti, M. Paolucci, M.G. Volpe, Species- and cultivar-dependent traits of *Prunus avium* and *Prunus cerasus* polyphenols, J. Food Compos. Anal. 45 (2016) 50–57.
[25] M. Jordheim, K.H. Enerstvedt, O.M. Andersen, Identification of cyanidin 3-*O*-ß-(6″-hydroxy-3-methylglutaroyl)glucoside) and other anthocyanins from wild and cultivated blackberries, J. Agric. Food Chem. 59 (2011) 7436–7440.
[26] C.E. Finn, B.C. Strik, B.M. Yorgey, M.E. Peterson, J. Lee, R.R. Martin, H.K. Hall, 'Columbia Star' thornless trailing blackberry, Hortscience 49 (2014) 1108–1112.
[27] B. De Ancos, E. Ibanez, G. Reglero, M.P. Cano, Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit, J. Agric. Food Chem. 48 (2000) 873–879.
[28] S.P. Mazur, A. Nes, A. Wold, S.F. Remberg, K. Aaby, Quality and chemical composition of ten red raspberry (*Rubus idaeus* L.) genotypes during three harvest seasons, Food Chem. 160 (2014) 233–240.
[29] J. Scalzo, A. Currie, J. Stephens, T. McGhie, P. Alspach, The anthocyanin composition of different *Vaccinium*, *Ribes*, and *Rubus* genotypes, Biofactors 34 (2008) 13–21.
[30] J. Lee, Sorbitol, *Rubus* fruit, and misconception, Food Chem. 166 (2015) 616–622.
[31] M. Dossett, J. Lee, C.E. Finn, Variation of anthocyanins and total phenolics in black raspberry populations, J. Funct. Foods 2 (2010) 292–297.
[32] J. Lee, R.W. Durst, R.E. Wrolstad, Impact of juice processing on blueberry anthocyanins and polyphenolics: comparison of two pretreatments, J. Food Sci. 67 (2002) 1660–1667.
[33] J. Lee, R.E. Wrolstad, Extraction of anthocyanins and polyphenolics from blueberry processing waste, J. Food Sci. 69 (2004) C564–C573.
[34] M. Alonzo-Macias, A. Cardador-Martinex, S. Mounir, G. Montejano-Gaitan, K. Allaf, Comparative study of the effects of drying methods on antioxidant activity of dried strawberry (*Fragaria* var. Camarosa), J. Food Res. 2 (2013) 92–207.
[35] L. Jakobek, M. Seruga, M. Medvidovic-Kosanovic, I. Novak, Anthocyanin content and antioxidant activity of various red fruit juices, Deut. Lebensm. -Rubdsch. 103 (2007) 58–64.
[36] A. Patras, N.P. Brunton, S. Da Pieve, F. Butler, Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purees, Innovative Food Sci. Emerg. Technol. 10 (2009) 308–313.
[37] C. Vasco, K. Riihinen, J. Ruales, A. Kamal-Eldin, Phenolic compounds in Rosaceae fruits from Ecuador, J. Agric. Food Chem. 57 (2009) 1204–1212.
[38] B. Goncalves, A.-K. Landbo, D. Knudsen, A.P. Silva, J. Moutinho-Pereira, E. Rosa, A.S. Meyer, Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.), J. Agric. Food Chem. 52 (2004) 523–530.
[39] L. Jakobek, M. Seruga, B. Seruga, I. Novak, M. Medvidovic-Kosanovic, Phenolic compound composition and antioxidant activity of fruits of *Rubus* and *Prunus* species from Croatia, Int. J. Food Sci. Technol. 44 (2009) 860–868.
[40] A. Kirakosyan, E.M. Seymour, D.E.U. Llanes, P.B. Kaufman, S.F. Boiling, Chemical profile and antioxidant capacities of tart cherry products, Food Chem. 115 (2008) 20–25.
[41] V. Simunic, S. Kovac, D. Gaso-Sokac, W. Pfannhauser, M. Murkovic, Determination of anthocyanins in four Croatian cultivars of sour cherries (*Prunus cerasus*), Eur. Food Res. Technol. 220 (2005) 575–578.
[42] H. Fan-Chiang, R.E. Wrolstad, Anthocyanin pigment composition of blackberries, J. Food Sci. 70 (2005) C198–C202.
[43] S.F. Remberg, A. Sonstebyb, K. Aaby, O.M. Heide, Influence of postflowering temperature on fruit size and chemical composition of Glen Ample Raspberry (*Rubus idaeus* L.), J. Agric. Food Chem. 58 (2010) 9120–9128.