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Short communications

Analysis of bokbunja products show they contain *Rubus occidentalis* L. fruit

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ABSTRACT

This is the first report of species adulteration in a collection of commercially available bokbunja (*Rubus coreanus* Miquel) products (n = 17) sold in Korea and the US (all originated from Korea). Twelve samples contained *R. occidentalis* L. fruit; the two species are clearly distinguishable by their anthocyanin profiles. Seven of 17 products were labeled in English to contain *R. coreanus* fruit; five of these samples contained *R. occidentalis* and two contained black carrot anthocyanins. The ten other products described contents as "bokbunja." For two of the 17 products, species classification could not be made due to low anthocyanin present, while three of the 17 were adulterated with black carrot anthocyanins and contained no fruit anthocyanins. Anthocyanins ranged from 0.8 to 56.9 mg/100 mL, a 71-fold difference, for all samples. We are hopeful that this research will aid bokbunja growers, processors, and researchers in creating, reporting, and marketing accurately labeled improved foods.

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1. Introduction

Bokbunja refers to Korean black raspberry (*Rubus coreanus* Miquel) fruit and derived products, and is native to Korea, Japan, and China. The recent expansion in the worldwide functional food movement (Menrad, 2003) has made many bokbunja products become available in the International marketplace, and many more so in South Korea where bokbunja products originate. However, based on the black raspberry plants observed during visits to commercial plantings, and the images used on contemporary bokbunja labels, we suspected many products were predominately made from American black raspberry (*R. occidentalis* L.), native to eastern North America (Lee, 2014a; Lee, Dossett, & Finn, 2014; M. Dossett personal observation). It is unclear how this mix-up between species occurred. While *R. coreanus* is native to Korea (Miquel, 1867), at some point *R. occidentalis* was introduced to Korea, though the exact date is unknown (Lee et al., 2014). There are challenges to growing *R. coreanus* on a commercial scale; compared to *R. occidentalis* it is thornier and produces smaller softer fruits that have less intense flavor (Lee et al., 2014). It is possible *R. occidentalis* was imported for agriculture because of those issues, and over time the two species both became identified as *R. coreanus*, since native black raspberry plants still grew wild and few were aware that any under cultivation were not Korean black raspberry (Lee et al., 2014; Miquel, 1867).

The effects of misidentified bokbunja products packaged under an incorrect species name have reached the scientific community (as summarized before in Lee, 2014a; Lee et al.,

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2014). To date, much of the research conducted on bokbunja has been carried out on Korean grown *R. occidentalis* L., potentially without researchers' knowledge (see references listed in Lee, 2014a; Lee et al., 2014). This confusion has been further compounded with genomic work being conducted into the health beneficial properties of *R. coreanus*, and when based on a presumptive identification of source material, was more likely carried out on *R. occidentalis* (see summary in Lee, 2014a; Lee et al., 2014). Proper identification of plants or source materials could prevent more problems in the literature. Using phenolics in botanical classification and food authenticity is not a new concept and has long aided the work of scientists, food authenticity inspectors, and quality control technicians (Lee, 2013, 2014a; Lee et al., 2014; Wrolstad, Durst, & Lee, 2005).

As summarized before (Lee et al., 2014), this issue was initially brought to our attention by the images depicting fruit on bokbunja product labels were of *R. occidentalis* L. and evidence that the majority of the Korean commercial growers are growing *R. occidentalis* L. (Lee et al., 2014; M. Dossett personal observation). The fruit and plant features that differentiate *R. coreanus* from *R. occidentalis* were well summarized before (Lee, 2014a; Lee, Dossett, & Finn, 2013; Lee et al., 2014). Fruits from these two species can be easily distinguished by their anthocyanin profile as well (Lee, 2014a; Lee et al., 2013, 2014). Any detection of cyanidin-3-sambubioside and/or cyanidin-3-xylosylrutinoside indicates the presence of *R. occidentalis* fruit (Lee, 2014a; Lee et al., 2013, 2014). *Rubus occidentalis* fruit contains up to seven individual anthocyanins, while only three of those anthocyanins can be found in *R. coreanus* fruit; in order of elution they are (*R. coreanus* anthocyanins in bold): cyanidin-3-sambubioside, cyanidin-3-xylosylrutinoside, **cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside,** pelargonidin-3-rutinoside, and peonidin-3-rutinoside (Lee, 2014a; Lee et al., 2013, 2014). Based on our analyses of over 1000 cultivars and genotypes, *R. occidentalis* does not diverge from this profile (Dossett, Lee, & Finn, 2008, 2010, 2011; Lee et al., 2013), indicating that anthocyanin profiles can be used for detecting adulteration or demonstrating authenticity of bokbunja products (Lee, 2014a; Lee et al., 2014). The objectives of this work were to collect and analyze bokbunja products in the marketplace, then determine if species adulteration is occurring based on the anthocyanin profiles.

2. Materials and methods

2.1. Samples

All available non-fermented bokbunja (BBJ) beverages (explanation in Results and Discussion) were purchased in the Pacific Northwest (Oregon and Washington) of the United States of America (USA) and Seoul, South Korea from March to April of 2014 as summarized in Table 1. Samples purchased in Korea were air mailed to our USA laboratory and arrived in four days. All samples were labeled to contain bokbunja juice, or extracts, originating from Korea in the ingredient listing. No samples were expired, all were well within their recommended sale dates, and all were marked as room stable if unopened. Seven of the 17 samples listed the species name of *R. coreanus* Miq. in the ingredient list. No samples used *R. occidentalis* on their label. Samples (BBJ01 through BBJ17; Table 1) were logged, prepared, and analyzed immediately upon arrival to the laboratory, with the remaining unused portions stored at –75 °C.

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 purchased from Polyphenols Laboratories AS (Sandnes, Norway).

For aiding in identification, authentic *R. occidentalis* and *R. coreanus* fruits were obtained from the USDA berry fruit breeding research program (Corvallis, OR, USA; Lee et al., 2014). Fresh whole black carrots were obtained from a local store (unknown cultivar; Boise, ID, USA).

2.3. Sample preparation

All obtained samples were in liquid form, either as juice, crude liquid extract, or dietary supplements (extract or juice added to vinegar). Quantifications were done by analyzing samples as-is. Eleven samples that were low in pigment were concentrated using a C_{18} cartridge (Waters corporation, Milford, MA, USA), following the procedure as described in Lee and Finn (2007) for anthocyanin identification. Black carrot outer skins were extracted by high purity water (Lee & Finn, 2007). All analyses were conducted in duplicates. All samples were filtered through a Millex-FH syringe filter (0.45 μm, Bedford, MA, USA) prior to HPLC analysis.

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

Anthocyanins were separated out by HPLC/DAD with identification by HPLC/DAD/MS-MS as described in Lee and Finn (2007), Lee et al. (2013), and Lee (2014a, 2014b). Separation condition was as described in Lee and Finn (2007), but with a longer analytical column (Synergi Hydro-RP 80 Å, 250 mm × 2 mm, 4 μm; Phenomenex, Inc., Torrance, CA, USA) of the same make, and a front mounted guard column (same make; described in Lee et al., 2013). The separation condition did not deviate from previously reported methods (Lee et al., 2013; Lee & Finn, 2007) and will not be described here. Individual anthocyanins were identified by retention time, UV-Visible spectra, mass spectra, and previously identified anthocyanin profiles from authenticated samples (Dossett et al., 2008, 2010, 2011; Lee, 2014a; Lee et al., 2013; Montilla, Arzaba, Hillebrand, & Winterhalter, 2011; Obon, Diaz-Garcia, & Castellar, 2011). Analyses were conducted in duplicates. Anthocyanins were expressed as cyanidin-3-glucoside. If samples contained cyanidin-3-xylosylrutinoside and cyanidin-3-glucoside, these co-eluting peaks were split at each apex, where it was not co-eluting, and multiplied by two to obtain total peak area prior to calculations.

3. Results and discussion

The relevant sample package information is summarized in Table 1. All fruit images used on these bokbunja products were