Postharvest Biology and Technology 110 (2015) 257–263

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Shelf life and changes in phenolic compounds of organically grown blackberries during refrigerated storage

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ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form 26 August 2015
Accepted 27 August 2015
Available online xxx

Keywords:
Anthocyanins
Blackberries
Flavonols
Phenolic compounds
Postharvest
Storage

ABSTRACT

Fruit of organically grown fresh market blackberry cultivars ‘Natchez’, ‘Ouachita’, and ‘Navaho’ harvested at shiny black and dull black ripeness stages were stored at 1 °C for 15 d or at 1 °C for 13 d plus 20 °C for 2 d. Berries were subjectively rated and anthocyanins and phenolic compounds were analyzed by high performance liquid chromatography (HPLC) to determine storage effects on berry postharvest quality and phenolic compounds. Berries harvested at the shiny black ripeness stage or those stored constantly at 1 °C had lower leakage, decay, and softness and a higher overall rating. ‘Navaho’ fruit had generally better storability compared to the other cultivars. Total anthocyanin content was generally higher in ‘Natchez’ and ‘Navaho’ than in ‘Ouachita’, and increased with storage at 20 °C for 2 d, while shiny black and dull black fruit were similar in anthocyanin content. Cyanidin 3-glucoside was found to represent 87–96% of the total anthocyanin content, and cyanidin 3-rutinoside, cyanidin 3-xyloside, and pelargonidin 3-glucoside were also detected. Total phenolic content increased during storage for all cultivars, especially when berries were stored at 20 °C for 2 d. Hydroxybenzoic acids were the primary phenolic compounds identified in fruit tissues of the three cultivars. Content and proportion of cyanidin 3-xyloside and vanillic acid in ‘Natchez’ were significantly lower than in ‘Ouachita’ or ‘Navaho’ fruit. Results indicate that the fruit of blackberry fruit of these cultivars, when grown organically, could be held with acceptable results when harvested at the shiny black stage of ripeness and stored at 1 °C. Transferring fruit to room temperature for as little as 2 d, even after cold storage, decreased marketability. In contrast, phenolic composition differed among cultivars and generally increased with storage.

1. Introduction

The organic food industry continues to increase in the US, in part because of consumer perception that organic foods are safer and have better health benefits, nutritional value, and flavor than conventionally grown foods (Bourn and Prescott, 2002; Zhao et al., 2006). The Organic Trade Association (OTA 2011) reported that the US organic industry was valued at about $29 billion in 2010, and fruits and vegetables represented 39.7% of total organic food values. In 2011, the US organic market had $31.5 billion in sales (OTA, 2012). Organic berry production is consistent with this trend, as acreage in the US doubled from 2546 ha (6111 acres) in 2006 to 5332 ha (12,796 acres) in 2011 (USDA, 2013) in response to the expanding demand for organic foods (Zhao et al., 2006).

Domesticated blackberries (*Rubus* sp.) are grown worldwide with concentrated production in Europe and North America (about 15,000 ha) and new production in Asia and Central and South America (about 1550 and 3200 ha, respectively) (Strik et al., 2008). Although a blackberry production has increased steadily over two decades (Strik et al., 2008), only 125 ha (308 acres) of organically grown blackberries and dewberries in the US were reported for 2011 (USDA, 2012).

Blackberries are consumed as fresh or processed products (Türkben et al., 2010). Fresh market blackberries release easily from pedicels when still firm and shiny (shiny black) or when there is a loss of gloss but are still firm (dull black), and can be held several weeks in cold storage (Perkins-Veazie et al., 1996). In contrast, processing cultivars generally have soft berries (Finn and Strik, 2008) that deteriorate within a few days, even in cold storage. The shelf life of fresh market blackberries depends greatly on genotype and cultivar. Fernandez-Salvador et al. (2015) reported that fresh market trailing type blackberries could be held 12–15 d at 5 °C, while Perkins-Veazie et al. (1999) found that some erect-type cultivars can be held up to 21 d at 2 °C. Exposure of blackberries to temperatures above 5 °C quickly shortens shelf life

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http://dx.doi.org/10.1016/j.postharvbio.2015.08.020
0925-5214/

due to increased weight loss, respiration, and decay (Perkins-Veazie et al., 1999).

An extensive review on the health benefits and associated compounds and their bioavailability from blackberry fruit was done by Kaume et al. (2012). Phenolic compounds in blackberries have potential health benefits for prevention of oxidation, cancer, inflammation, and age-related change in cognitive function in cell and animal models (Cuevas-Rodríguez et al., 2010; Pergola et al., 2006; Seeram et al., 2006; Shukitt-Hale et al., 2009). Kaume et al. (2012) reported that while the primary anthocyanins in blackberry fruit are cyanidin derivatives, the sugar moieties vary widely among genotypes and cultivars. Flavonols are most often quercetin, myricetin, and kaempferol and can differ in occurrence with genotype and production environment. The phenolic acids such as hydroxybenzoic and hydroxycinnamic acids and free ellagitannins have been reported for various blackberry species. The phenolic composition of berry fruit can change depending on genotype, cultural conditions, and postharvest conditions (Bradish et al., 2012; Cho et al., 2004, 2005; Perkins-Veazie et al., 1999; Türkben et al., 2010).

While differences in phenolic compounds have been well studied among genotypes and cultivars, few studies have been done with postharvest changes in fresh blackberries. In frozen fruit, blackberries quick frozen (−35 °C for 5 h) or maintained frozen (−22 °C for 6 months) were lower in most phenolic compounds than fresh berries, with differences dependent on cultivar (Türkben et al., 2010). Blackberries of unknown cultivars grown in Croatia were found to be similar in total phenolic content and increased in total anthocyanin content after 14 d of storage at 4 °C (Šamec and Piljac-Žegarac, 2011). No reports were found on phenolic composition and their changes during storage for organically produced blackberry fruit. The objective of this study was to determine the shelf life of organically produced fresh market blackberries and changes in phenolic compounds with storage regimes.

2. Materials and methods

2.1. Chemicals

Purified standards of anthocyanins (cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, and pelargonidin 3-glucoside) were purchased from ChromaDex (Irvine, CA, USA) and phenolic compounds (gallic acid, vanillic acid, quercetin 3-glucoside, and quercetin 3-galactoside) were from Sigma (St. Louis, MO, USA). The HPLC grade methanol and formic acid were purchased from Fisher Scientific (Hampton, NH, USA) and Sigma (St. Louis, MO, USA), respectively.

2.2. Plant materials and storage conditions

Freshly harvested organic fruit of 'Natchez', 'Ouachita', and 'Navaho' blackberries were purchased from a commercial farm in Bunn, NC (USDA hardiness zone 7a) in June and July of 2011 and 2012. The blackberry field was established in 2008 in a soil of Vance sandy loam and Varina loamy sand, and plants were organically grown following the US National Organic Standards (USDA, 2014). In 2011, each cultivar was harvested three to four times, corresponding to the early, mid, and late-season periods for each cultivar (a total harvest period of about 6 weeks) into 250 g vented plastic boxes with hinged lids (clamshells). In 2012, each cultivar was harvested three times. Fruit at commercial ripe stage were held at 5–10 °C during transport to the laboratory in Kannapolis, NC (about 3 h). All berries were sorted into two groups, consisting of shiny black (SB) (firm and with gloss) and dull black (DB) (about one-day riper than SB, still acceptable for fresh market sales). Berry samples representing day 0 (no storage) were immediately frozen at −20 °C and then held at −80 °C until analysis. Remaining berries were divided into groups for storage, maintaining ripeness stage and cultivar integrity and minimum three replicates, each of 150 g per vented clamshell (No. 1560, Southern Containers, Wilson, NC). One group was stored at 1 °C for 15 d and the other group was stored at 1 °C for 13 d and at 20 °C for the next 2 d. Clamshells were placed in cardboard masters and covered with plastic to keep relative humidity above 90%.

2.3. Subjective ratings

Clamshells were weighed weekly to follow weight loss. After storage, fruits were warmed to room temperature prior to subjective ratings, as cold fruit can feel softer than room temperature fruit. Subjective rating was done by the same person throughout experiment to avoid variation among individuals. All berries were subjectively rated for the presence or absence of leak and decay incidence. Presence of red drupelets was positive if three or more drupelets in a group on the berry were bright red in color and not leaky. Softness was evaluated by a 5 point subjective scale where 1 was very firm, 3 was marginally marketable, and 4 and 5 fruit were in stages of disintegration. Percent leakage, decay incidence, red drupelet, and soft berries (sum of those rated 4 or 5) were calculated as the relative ratio of number of fruits with each characteristic to the total number of fruits. An overall rating was determined by subtracting the average of percentages of leaky, decayed, and soft fruit from 100% (Clark and Perkins-Veazie, 2011).

2.4. Soluble solids content and titratable acidity

Soluble solids of purees were measured using a digital refractometer (P100, Atago USA Inc., Bellevue, WA, USA) and expressed as percent soluble solids content. Titratable acidity was measured using a titrometer (Model 862 Compact Titrosampler, Metrohm AG, Herisau, Switzerland) and calculated as% citric acid equivalent, the predominant nonvolatile organic acid in this blackberry genotype (Fan-Chiang and Wrolstad, 2010).

2.5. Extraction for HPLC analysis

Berries harvested in 2012, free of visible mold or leak, were used for analysis of anthocyanins and non-anthocyanin phenolic compounds. All samples were held at −80 °C and then freeze-dried using a VirTis LyoTroll (SP Scientific, Warminster, PA, USA), with berries gradually warmed from −40 °C to 20 °C as water sublimated. Freeze-dried blackberries were ground using a mortar and pestle, and pyrenes (seeds) were removed by filtering powder through mesh sieves. Blackberry fruit tissue powders were kept at −80 °C until analysis.

Blackberry fruit powders were extracted following the method of Bradish et al. (2012) with slight modification. Briefly, 20 mg of blackberry powder were extracted with 1.5 mL of acidified methanol (formic acid:methanol:deionized water, 1:60:39, v/v/v). Samples were vortexed and centrifuged at 10,600 × g for 20 min at 4 °C. All samples were re-extracted, with 98% of phenolic content recovered in two extractions. Supernatants were combined and filtered through 0.2 μm syringe filter (PTFE, 17 mm) into HPLC vials. N_2 was added to the headspace to inhibit oxidation, and the vials were capped with screw top lids. All samples were kept at −80 °C until analysis.

2.6. HPLC analysis

A high performance liquid chromatography system (Elite LaChrom, Hitachi Ltd., Tokyo, Japan) equipped with autosampler,