Shelf Life and Changes in Phenolic Compounds of Organically Grown Blackberries during Refrigerated Storage

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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.

Keywords: Anthocyanins, blackberries, flavonols, phenolic compounds, postharvest, 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 2,546 ha (6,111 acres) in 2006 to 5,332 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 1,550 and 3,200 ha, respectively) (Strik et al., 2008). Although 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 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 in 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, fruit 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.

2.4. 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 x 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. N₂ 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.

150

151 2.5. HPLC analysis

152 A high performance liquid chromatography system (Elite LaChrom, Hitachi Ltd., Tokyo,
153 Japan) equipped with autosampler, diode array detector, and binary solvent delivery manager
154 was used to analyze anthocyanins and phenolic compounds. A reversed phase C18 column
155 (Synergi 4 μ Hydro-RP 80Å, Phenomenex, Torrance, CA, USA) was used with a column
156 temperature of 30 °C. Five percent formic acid (solvent A) and 100% methanol (solvent B) were
157 used as mobile phases with the flow rate of 16.7 μ L s⁻¹ and the gradient system was 0-5 min, 90%
158 A; 5-15 min, 85% A; 15-20 min, 80% A; 20-25 min, 75% A; 25-45 min, 70% A; 45-47 min, 40%
159 A; 47-60 min, 90% A. Twenty microliter of sample was injected in duplicate. Anthocyanins
160 were detected at 520 nm, and hydroxybenzoic acids, hydroxycinnamic acids, and flavonols were
161 detected at 280, 320, and 360 nm, respectively. Cyanidin 3-glucoside, cyanidin 3-rutinoside,
162 cyanidin 3-xyloside, and pelargonidin 3-glucoside were quantified based on external standards,
163 and unknown anthocyanins were quantified as cyanidin 3-glucoside equivalent. For non-
164 anthocyanin phenolic compounds, standard curves of gallic acid, vanillic acid, quercetin 3-
165 galactoside, and quercetin 3-glucoside were used. Unknown hydroxybenzoic acids,
166 hydroxycinnamic acids, and flavonols were quantified as gallic acid, chlorogenic acid, and
167 quercetin 3-glucoside equivalents, respectively. The sum of individual anthocyanins or phenolic
168 compounds were calculated to obtain total anthocyanin and phenolic contents.

169 2.6. Statistical analysis

The storage experiments were done as a completely randomized design. Replicates consisted of a minimum of three clamshells per cultivar at each harvest date, ripeness stage, and storage treatment per year. For HPLC analysis, replicates consisted of 1-3 clamshells per cultivar at two harvest dates in 2012 and each ripeness stage and storage treatment. Univariate analysis of variance (ANOVA) was done using SAS (SAS 9.2, SAS institute, Cary, NC, USA), and the means were separated using Tukey's honestly significant difference test (HSD) or Fisher's least significant difference (LSD) at the 95% significance level. Pearson correlation coefficients were determined using SAS (SAS 9.2, SAS institute, Cary, NC, USA).

3. Results and discussion

3.1. Fruit quality after storage

Weight loss was 2-4% in all treatments (Fig. 1). In general, berries harvested at the dull black ripeness stage or stored at 20 °C for the last 2 d of storage had a higher weight loss than shiny black berries or fruit stored constantly at 1 °C. This weight loss occurred during the last 2 d of storage at 20 °C, most likely due to higher respiration at 20 °C compared to 1 °C. The higher weight loss of DB compared to SB berries may be due to the loss of firmness and subsequent loss of water and is similar to results reported by Perkins-Veazie et al. (1999) for 'Navaho' and 'Shawnee' blackberries held at 2 or 5 °C for 7, 14, or 21 d followed by 2 d at 20 °C. 'Navaho' berries had a slightly higher weight loss compared to 'Natchez' or 'Ouachita' berries. 'Navaho' is a smaller berry (5-6 g) than 'Natchez' (8-9 g) and the higher surface to volume ratio of smaller berries is associated with higher weight loss.

For all cultivars, SB berries had a lower leakage and decay incidence and a higher overall rating for both storage regimes compared to DB berries (Table 1). Softness was higher in DB berries of all cultivars for both storage treatments. When berries were exposed to room temperature for 2 d, more leakage and decay were observed and overall rating decreased for fruit of all cultivars and at both ripeness stages. Red drupelet incidence in ‘Natchez’ was higher than in the other cultivars and was significantly higher when stored at 20 °C for 2 d in SB berries. The higher incidence of red drupelet and the corresponding higher incidence of soft berries in ‘Natchez’ compared to ‘Navaho’ and ‘Ouachita’ indicate that relative fruit firmness may play a role in red drupelet incidence.

Among cultivars, ‘Ouachita’ and ‘Navaho’ differed with ripeness stage. ‘Ouachita’ berries stored constantly at 1 °C and ‘Navaho’ berries exposed to 20 °C for 2 d at DB ripeness stage had higher red drupelet incidence. In general, subjective ratings were lower for leakage, decay, and red drupelet and the overall rating was higher for ‘Navaho’ blackberries. Although DB blackberries were initially only slightly softer than SB fruit of all cultivars, the DB berries had far more decay and leakage than SB fruit after a short duration of storage at room temperature, indicating that even a slight increase in ripeness can have a large effect on postharvest quality when held at room temperature. The increased weight loss and decreased overall rating in blackberries after only 2 d at room temperature underscore the importance of holding blackberries constantly at low temperature to maintain quality.

3.2. Fruit composition

Soluble solids content and titratable acidity generally decreased, while berry pH increased in stored blackberries (Table 2), as reported by others (Perkins-Veazie et al., 1999; Wu et al., 2010).

Soluble solids content and titratable acidity were reduced most in the berries stored at 20 °C for 2 d compared to those held at constant 1 °C. In fruit not stored, the pH of SB berries was higher and titratable acidity was lower than for DB berries for all cultivars. Preferential use of organic acids for respiration may have decreased the titratable acidity, as shown by Wu et al. (2010) in stored blackberries, and should be greater in berries held at 20 °C, corresponding to a higher respiration rate. Soluble solids content consists primarily of sugars, non-volatile acids, and water-soluble polysaccharides. In this study, both soluble solids content and titratable acidity decreased after storage, indicating a loss of both sugars and acids during storage, and the effect was greater when the berries were held for a few days at room temperature.

3.3. Phenolic composition of blackberries as affected by storage regime

Blackberry anthocyanins identified using HPLC were cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, and pelargonidin 3-glucoside. Cyanidin 3-glucoside was the predominant anthocyanin in the blackberry cultivars used in this study, representing 87 to 96% of the total anthocyanin content. The anthocyanin profile differed slightly among the cultivars, with cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-glucoside, and total anthocyanin contents generally higher in ‘Natchez’ than in ‘Ouachita’ fruit (Table 3). Cyanidin 3-xyloside content and ratio to total anthocyanin content were lower in ‘Natchez’ (0.2%) than in ‘Navaho’ and ‘Ouachita’ fruit (4-7%). Total anthocyanin content was highest in ‘Natchez’ fruit at day 0 and increased with storage at 20 °C in all cultivars.

Cyanidin 3-glucoside content generally increased after storage in all three cultivars, especially when berries were stored at 20 °C for 2 d. The anthocyanins cyanidin 3-xyloside and pelargonidin 3-glucoside contents decreased in ‘Natchez’ fruit but increased in fruit of the other

two cultivars with storage. In contrast, ripeness stage had less impact on fruit anthocyanin content. Total anthocyanin content decreased in DB compared to SB fruit of ‘Ouachita’ stored at 1 °C for 15 d and in ‘Navaho’ blackberries held at 20 °C for 2 d. Cyanidin 3-rutinoside content in ‘Natchez’ and ‘Ouachita’ was higher in SB berries compared to DB berries when stored constantly at 1 °C. Cyanidin 3-glucoside was the predominant anthocyanin in ‘Navaho’ in agreement with others (Cho et al., 2004; Fan-Chiang and Wrolstad, 2005; Koca and Karadeniz, 2009) and in ‘Ouachita’ and ‘Natchez’ blackberries. Peonidin, malvidin, and petunidin, which are methylated forms of cyanidin, pelargonidin, and delphinidin, respectively, were not detected in blackberries used in this study, although these have been reported for other blackberry species (Reyes-Carmona et al., 2006). ‘Navaho’ is a parent of ‘Ouachita’; the lower content of cyanidin 3-xyloside in ‘Natchez’ may be from its different genetic background (Clark and Moore, 2005, 2008).

An increase in total anthocyanin content after storage has been reported in strawberries, blueberries, and raspberries (Jin et al., 2011; Kalt et al., 1999). Šamec and Piljac-Žegarac (2011) reported that blackberries (*R. fruticosus*) gained 15-20% in total anthocyanin when stored 4 d at 4 or 25 °C. Wu et al. (2010) reported a loss of total anthocyanins after storage in processing types of blackberry cultivars. In our study, the increase in total anthocyanins after storage may be due to the firmness of these fresh market fruit compared to the softer processing type blackberries used by Wu et al. (2010), which have a high incidence of juice leakage.

Blackberry non-anthocyanin phenolic compounds identified by HPLC were hydroxybenzoic acids and derivatives, hydroxycinnamic acids and derivatives, and flavonols. Hydroxybenzoic acids including gallic and vanillic acids and the flavonols quercetin 3-galactoside and quercetin

257 3-glucoside were identified. Total hydroxybenzoic acid content was generally higher in ‘Navaho’
258 while total hydroxycinnamic acids and flavonol contents were slightly higher in ‘Natchez’ fruit
259 tissue (Table 4). In ‘Natchez’ and ‘Ouachita’, total hydroxybenzoic acids and flavonols
260 represented 36-59 and 26-41% of the total phenolic content, respectively. In ‘Navaho’ tissue, the
261 relative ratio of hydroxybenzoic acids as a portion of total phenolics was 59-72%, compared to
262 19-27% for flavonols. Among hydroxybenzoic acids, vanillic acid content and ratio were
263 significantly lower in ‘Natchez’ (2-6%) than in ‘Ouachita’ and ‘Navaho’ (15-30%) fruit.
264 Quercetin 3-glucoside and quercetin 3-galactoside contents were highest in ‘Natchez’ fruit tissue
265 in all storage conditions while gallic acid content was highest in ‘Navaho’ berries.

266 Total phenolic content and profiles have been investigated in several blackberry species.
267 Quercetin 3-galactoside or quercetin 3-glucoside was found to be the predominant flavonol in
268 blackberries (*Rubus* spp.) depending on cultivar (Cho et al., 2004, 2005). In *R. plicatus*, a
269 European blackberry species, hydroxycinnamic acids were dominant, and gallic acid, vanillic
270 acid, salicylic acid, caffeic acid, *m*- and *p*-coumaric acids, and hydroxycaffeic acid were reported
271 (Zadernowski et al., 2005). Most of these compounds were present as esters or glycosides rather
272 than in free form. Veberic et al. (2014) reported the presence of neochlorogenic acid and *p*-
273 coumaric hexoside in several cultivars of European blackberries. Ellagic acid and flavan 3-ols
274 including (+)-catechin and (-)-epicatechin were reported in ‘Evergreen’ and ‘Marion’
275 blackberries, mostly in the pyrenes (Siriwoharn and Wrolstad, 2004). We were unable to detect
276 these compounds, probably because pyrene tissue was excluded during sifting of freeze dried
277 tissue for analysis.

Phenolic contents differed among ripeness stages for each cultivar and in response to storage regime (Table 4). In ‘Natchez’, vanillic acid, total hydroxycinnamic acids, total flavonols, and total phenolic content were significantly higher in SB compared to DB in freshly harvested berries (Table 4). Total hydroxycinnamic acids and flavonols were lower in DB berries compared to SB berries in freshly harvested ‘Ouachita’. Total phenolic content increased with storage in all cultivars and was greater in berries held at 20 °C for 2 d. Total hydroxybenzoic acid content was the highest in berries stored at 20 °C for 2 d in all cultivars but DB ‘Ouachita’ berries (Table 4). Gallic acid and vanillic acid contents increased in DB ‘Navaho’ berries after storage at 20 °C for 2 d. Total hydroxycinnamic acid content increased significantly with storage only in SB berries. Quercetin 3-galactoside content increased in stored ‘Natchez’ and ‘Navaho’ blackberries. Quercetin 3-glucoside and total flavonol content increased after storage at both ripeness stages in ‘Navaho’ blackberries (Table 4). An increased in flavonol content during frozen storage was reported for ‘Chester Thornless’ blackberry, a firm-fruited semi-erect genotype (Türkben et al., 2010).

3.4. Correlation between quality attributes and phenolic compounds

Among postharvest quality attributes, softness and incidence of leakage and decay were positively correlated with each other and negatively correlated with overall rating (Table 5). Total hydroxybenzoic acid was negatively correlated with leakage incidence but positively correlated with overall rating. In contrast, a positive correlation was found between total hydroxycinnamic acid content and leakage incidence. Total phenolic content was negatively correlated with leakage and red drupelet incidence.

Soluble solids content and titratable acidity were negatively correlated with decay incidence and positively correlated with overall rating. Fruit acidity had a significant and positive correlation with red drupelet discoloration and overall rating. Soluble solids content was not significantly correlated with any of the phenolic compounds, while titratable acidity was negatively correlated with total anthocyanin, hydroxycinnamic acid, and flavonol contents (Table 5). In contrast, blackberry pH was positively correlated with total anthocyanin and hydroxycinnamic acid contents. Kalt et al. (1999) theorized that the organic acids in small fruits may provide carbon skeletons for biosynthesis of anthocyanins and non-anthocyanin phenolic compounds during storage.

Negative correlations of incidence of leakage and decay and of fruit softness to titratable acidity, plus positive correlations between these attributes and pH suggest that fruit acidity may have an important role in postharvest quality of blackberry fruit during storage. In this study, the negative correlation between leakage and total hydroxybenzoic acid or total phenolic contents, and the positive correlation between leakage and total hydroxycinnamic acid content suggest that the effects of phenolic compounds on postharvest quality of organically grown blackberries may differ among subgroups of phenolic compounds.

5. Conclusion

The present study determined the postharvest quality and fruit composition of organically grown ‘Natchez’, ‘Ouachita’, and ‘Navaho’ blackberries of slightly different ripeness stages. Berries stored constantly at 1 °C showed better postharvest quality compared to those stored at 20 °C for 2 d, and exposure to room temperature profoundly reduced fruit quality in all cultivars and at either ripeness stage. Anthocyanins, dominated by cyanidin 3-glucoside, increased with

321 storage and relative profile was affected by cultivar rather than by ripeness stage. Total
322 hydroxybenzoic acid, flavonol, and phenolic contents generally increased with storage,
323 especially in fruit stored at 20 °C for 2 d.

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Table 1. Subjective ratings of organically grown shiny black and dull black ‘Natchez’, ‘Ouachita’, and ‘Navaho’ blackberries after storage at two temperature regimes for 15 d.

Storage regime ^y	Subjective ratings									
	Leakage		Decay		Red drupelets		Softness		Overall rating ^x	
	SB	DB	SB	DB	SB	DB	SB	DB	SB	DB
Natchez										
CL	41.0±5.9b ^z	76.5±5.4a*	3.5±1.1b	9.2±1.9a	18.3±4.7a	21.4±5.0a	21.6±5.5b	41.8±5.1a	78.0±3.1a*	57.5±2.8b*
RT	77.2±3.0b*	95.3±1.8a*	47.2±4.3b*	68.0±4.7a*	21.7±5.1a*	24.5±5.1a	23.3±3.2b	53.4±4.8a*	50.8±2.8a	27.8±3.4b
Ouachita										
CL	35.2±3.9b	64.0±4.3a	9.8±1.8b	25.1±3.4a	5.9±1.3b	9.4±2.1a	24.0±3.3b	39.3±4.0a	77.0±2.3a*	57.2±3.0b*
RT	61.9±4.1b*	83.3±3.3a*	51.7±4.4b*	62.3±5.1a*	10.0±2.0a*	12.5±2.8a	28.4±3.6b*	53.0±4.5a*	52.7±3.4a	33.8±2.8b
Navaho										
CL	20.6±3.1b	48.2±4.4a	3.3±0.9b	12.1±2.2a	3.6±0.8a	2.4±0.6a	23.2±3.1b	37.4±3.4a	84.3±1.6a*	67.5±2.5b*
RT	44.1±3.4b*	76.1±3.5a*	30.2±3.8b*	52.1±3.7a*	2.1±0.8b	4.7±1.1a*	26.2±3.1b	48.9±3.4a*	66.5±2.6a	41.0±2.9b

^zDifferent letters indicate significant differences between ripeness stages in each cultivar and storage regime, and asterisk (*) indicates difference between storage regimes within each cultivar and ripeness stage, LSD at $p < 0.05$. Values are expressed as means ± standard error.

^yCL, constant low (1 °C for 15 d); RT, retail (1 °C for 13 d + 20 °C for 2 d)

^xOverall rating=100%-(average of leakage, decay, and softness)

Table 2. Soluble solids content, titratable acidity, and pH of organically grown ‘Natchez’, ‘Ouachita’, and ‘Navaho’ blackberries after storage for 0 and 15 d.

Storage regime ^y	Soluble solids content (%)		Titratable acidity (%citric acid equivalent)		pH	
	SB	DB	SB	DB	SB	DB
Natchez						
Day 0	10.4±0.2a	10.6±0.2a*	1.02±0.03a*	0.94±0.03a	3.67±0.03c	3.73±0.03b*
CL	9.3±0.2b	9.8±0.1b*	0.95±0.04b	0.92±0.04a	3.75±0.03b	3.74±0.03b
RT	8.6±0.2c	9.1±0.3c*	0.85±0.04c	0.86±0.05b	3.84±0.04a	3.83±0.04a
Ouachita						
Day 0	10.6±0.2a	10.5±0.2a	1.04±0.04a*	0.91±0.04a	3.67±0.03c	3.77±0.03b*
CL	10.0±0.2b	10.1±0.2b	0.99±0.04b*	0.94±0.03a	3.75±0.03b	3.75±0.03c
RT	9.3±0.2c	9.3±0.3c	0.82±0.03c*	0.74±0.04b	3.92±0.04a	3.96±0.05a*
Navaho						
Day 0	10.5±0.3a	10.6±0.3a	1.05±0.04a*	0.89±0.03b	3.65±0.03b	3.73±0.03a*
CL	10.1±0.2ab	10.4±0.2a*	1.05±0.02a*	0.98±0.02a	3.61±0.02b	3.65±0.02b*

RT	9.6±0.2b	9.6±0.2b	0.90±0.02b	0.90±0.03b	3.77±0.02a*	3.73±0.02a
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^zMean separation within column in each ripeness stage and storage condition by HSD at $p < 0.05$. Asterisk (*) indicates a significant difference between ripeness stages in each cultivar and storage regime, LSD at $p < 0.05$. Values are expressed as means $\pm$ standard error.

^yDay 0, unstored; CL, constant low (1 °C for 15 d); RT, retail (1 °C for 13 d + 20 °C for 2 d)

Table 3. Anthocyanin contents (g kg⁻¹ dry weight) in ‘Natchez’, ‘Ouachita’, and ‘Navaho’ blackberries after storage for 0 and 15 d.

Cultivar	Storage	Anthocyanins				
	regime ^y	Cyanidin 3- glucoside	Cyanidin 3- rutinoside	Cyanidin 3- xyloside	Pelargonidin 3- glucoside	Total
Shiny black						
‘Natchez’	Day 0	26.7±1.2b ^z	0.41±0.01b	0.04±0.02a	0.25±0.06a	29.1±1.2b
	CL	25.7±1.3b	0.44±0.01b*	0.00±0.00b	0.18±0.04b	28.2±1.3b
	RT	29.5±1.3a	0.50±0.02a	0.01±0.01b	0.18±0.03ab	32.4±1.3a
‘Ouachita’	Day 0	15.8±1.3b	0.10±0.01a	0.72±0.04b	0.04±0.01b*	17.6±1.3b
	CL	22.2±1.9a*	0.10±0.01a*	1.06±0.16a	0.14±0.01a	24.9±2.2a*
	RT	22.5±1.6a	0.10±0.01a	1.10±0.16a	0.15±0.02a	25.3±1.9a
‘Navaho’	Day 0	16.9±0.7c	0.13±0.01b	1.15±0.07b*	0.06±0.01c*	19.6±0.7c
	CL	25.0±1.1b	0.14±0.01b	1.60±0.08a	0.18±0.02b	28.9±1.3b
	RT	29.1±1.3a*	0.16±0.01a	1.84±0.1a	0.23±0.02a	33.5±1.5a*
Dull black						
‘Natchez’	Day 0	25.0±1.0b	0.36±0.03b	0.03±0.01a	0.28±0.07a	27.1±1.0b

	CL	24.8±0.6b	0.39±0.02ab	0.00±0.00b	0.17±0.03b	27.2±0.6b
	RT	28.0±0.8a	0.42±0.03a	0.01±0.01ab	0.19±0.06ab	30.5±0.8a
	Day 0	16.0±0.6b	0.10±0.01a	0.81±0.05b	0.07±0.02b	17.9±0.7b
‘Ouachita’	CL	19.2±0.7a	0.09±0.01a	0.96±0.10a	0.12±0.01a	21.6±0.8a
	RT	19.8±0.9a	0.09±0.01a	0.90±0.15ab	0.14±0.02a	22.1±1.0a
	Day 0	18.8±0.6c	0.14±0.01a	1.46±0.05b	0.11±0.02b	21.7±0.6c
‘Navaho’	CL	23.4±1.0b	0.14±0.01a	1.58±0.06ab	0.17±0.01a	27.0±1.2b
	RT	25.5±0.5a	0.15±0.01a	1.72±0.06a	0.20±0.02a	29.4±0.7a

^zData are presented as mean ± standard error. Mean separation within column in each cultivar by HSD and asterisk (*) shows the difference between ripeness stages in each cultivar by LSD at $p < 0.05$. Values are expressed as means ± standard error.

^yDay0, 0 day after storage; CL, constant low (1 °C for 15 d); RT, retail (1 °C for 13 d + 20 °C for 2 d).

Table 4. Phenolic contents in (mg kg⁻¹, dry weight basis) ‘Natchez’, ‘Ouachita’, and ‘Navaho’ blackberries after storage for 0 and 15 d.

Cultivar	Storage regime ^y	Phenolic compounds ^x							
		HBA			HCA		Flavonol		Total phenolic
		GA	VA	Total	Total	Q-gal	Q-glu	Total	
Shiny black									
‘Natchez’	Day 0	130.8±31.6ab ^z	245.6±26.6a*	2522.2±634.2b	704.4±38.3b*	250.3±32.4b	215.3±28.6b	1733.3±96.9b*	4959.9±662.2b*
	CL	80.7 ±19.8b	251.3±30.0a	2807.9±288.3b	711.5±49.8b*	356.8±34.5ab*	278.5±30.8ab*	1913.4±124.0ab*	5432.8±386.9b*
	RT	158.9±31.4a	283.0±17.1a	3698.3±284.8a	852.2±79.8a*	421.1±40.9a	327.4±30.0a	2267.8±175.5a	6818.3±479.2a
‘Ouachita’	Day 0	112.5±21.1a*	859.7±55.5a	1683.7±27.8b	486.6±38.3b*	141.3±18.6b*	78.8±10.7a*	900.5±46.0b*	3070.8±340.4b
	CL	54.8±17.0a	910.6±39.8a*	2433.9±497.0a*	545.6±88.2a	202.6±25.2a*	87.4±9.1a*	1079.3±125.3a*	4058.8±703.6a*
	RT	75.9±35.0a	880.9±88.3a	2673.5±691.0a	570.5±101.6a	213.7±46.3a	81.9±13.3a	1132.5±170.1a	4376.6±951.2a
‘Navaho’	Day 0	126.6±14.4b*	961.6±71.8b*	2702.2±249.1b*	442.5±29.7b	224.8±33.6b*	91.5±13.8b*	1045.9±77.7b*	4190.6±291.2b*
	CL	206.7±45.3ab	1110.5±131.1ab	4729.3±424.3a*	570.1±46.5a*	344.5±45.6a*	126.2±17.4a*	1397.0±133.8a*	6696.4±584.6a*

	RT	256.1±61.5a	1126.0±101.6a*	4780.3±455.2a	568.5±25.2a*	351.4±49.3a*	130.0±19.4a*	1432.6±127.4a*	6781.3±571.8a*
Dull black									
‘Natchez’	Day 0	72.4±21.5b	176.4±21.1a	1521.2±131.8c	566.8±39.0a	203.5±27.3b	166.3±28.4a	1415.3±125.4a	3503.3±291.3b
	CL	59.3±11.0b	212.0±30.1a	2140.9±292.9b	582.8±15.3a	222.5±21.2ab	173.5±23.5a	1407.6±86.6a	4131.3±327.0b
	RT	148.6±39.3a	185.5±48.0a	3007.5±512.9a	663.6±55.8a	262.1±8.5a	175.0±20.9a	1634.6±89.1a	5305.7±636.9a
‘Ouachita’	Day 0	86.2±20.3a	672.4±31.1ab	1400.2±116.2a	381.8±13.1a	84.6±11.5b	41.0±8.6b	636.1±47.7c	2418.2±165.5b
	CL	68.6±11.1ab	600.4±80.8b	840.6±73.3b	507.8±38.9a	133.5±13.0b	57.2±6.4ab	880.5±59.8b	2229.0±91.5b
	RT	51.8±31.4b	753.8±153.8a	1386.7±537.1a	547.6±27.0a	201.8±58.8a	87.3±33.5a	1090.2±157.1a	3024.5±712.9a
‘Navaho’	Day 0	90.2±8.6b	754.3±43.7b	1839.8±98.0b	423.5±11.4b	142.9±14.9b	56.0±6.1b	818.0±37.2b	3081.4±121.6b
	CL	104.5±27.3b	843.2±69.2ab	3318.1±296.1a	431.3±19.3ab	220.1±26.3a	77.9±10.9ab	998.5±80.7ab	4747.9±377.2a
	RT	224.3±26.8a	931.5±109.0a	3753.4±331.8a	469.4±13.6a	259.1±31.3a	92.9±13.1a	1121.3±80.8a	5344.2±416.9a

^zData are presented as mean ± standard error. Mean separation within column in each cultivar by HSD and asterisk (*) shows the difference between ripeness stages in each cultivar by LSD at $p < 0.05$. Values are expressed as means ± standard error.

^yDay0, 0 day after storage; CL, constant low (1 °C for 15 d); RT, retail (1 °C for 13 d + 20 °C for 2 d).

^xHBAs, hydroxybenzoic acids; HCAs, hydroxycinnamic acids; GA, gallic acid; VA, vanillic acid; Q-gal, quercetin 3-galactoside; Q-glu, quercetin 3-glucoside.

Table 5. Pearson's correlation coefficients for (A) subjective ratings and composition, (B) subjective ratings and phenolic compounds, and (C) composition and phenolic compounds in organically grown 'Natchez', 'Ouachita', and 'Navaho' blackberries.

Variable	Leakage	Decay	RDI ^z	Soft	Overall	SSC	TA	pH	TAN	TBA	TCA	TF
Decay	0.55** ^x											
RDI	0.13	0.06										
Soft	0.38**	0.51**	-0.08									
Overall	-0.85**	-0.84**	-0.07	-0.71**								
SSC	-0.22	-0.36**	-0.08	-0.02	0.26*							
TA	-0.47**	-0.45**	0.40**	-0.41**	0.55**	-0.14						
pH	0.47**	0.53**	-0.30*	0.46**	-0.60**	0.03	-0.87**					
TAN	0.01	0.12	-0.38**	0.12	-0.09	0.02	-0.40**	0.35**				
TBA	-0.38**	-0.10	-0.29*	-0.10	0.27*	0.14	-0.09	-0.01	0.76**			
TCA	0.25*	0.19	-0.08	0.12	-0.24	-0.15	-0.36**	0.39**	0.66**	0.44**		
TF	0.09	0.03	-0.03	0.08	-0.08	0.09	-0.28*	0.23	0.61**	0.53**	0.79**	
TP	-0.27*	-0.06	-0.25*	-0.06	0.18	0.12	-0.16	0.08	0.81**	0.97**	0.62**	0.72**

^zRDI is red drupelet incidence (%); Soft is fruit softness, percent of berries rated 4 or 5 where 1 was very firm, 3 was marginally marketable, and 5 was disintegrating; Overall is overall rating, 100%-(average of leakage, decay, and softness); SSC, soluble solids content; TA is titratable acidity, TAN is total anthocyanin; TBA is total hydroxybenzoic acid; TCA is total hydroxycinnamic acid; TF is total flavonol; TP is total phenolic content.

^xAsterisks * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

Fig. 1. Weight loss after 15 d of storage. SB, shiny black ripeness stage; DB, dull black ripeness stage; CL, constant low (15 d at 1 °C); RT, retail (13 d at 1 °C + 2 d at 20 °C); Non-patterned bars represent the weight loss after 13 d of storage and patterned bars represent the weight loss during the last 2 d of storage. Asterisk (*) indicates a significance between storage regimes for weight loss during 15 d of storage in each cultivar and ripeness stage separated by Fisher's LSD at $p < 0.05$. Weight loss during the last 2 d of storage was significantly higher in DB berries or in berries held at 20 °C for 2 d.