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Quantification of Quality Attributes, Functional Compounds and Antioxidant Capacity of Blackberry and Blackberry Wine

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Abstract

This study examined selected quality and chemical properties of extracts and value-added products made from two blackberry cultivars, 'Apache' and 'Ouachita', that are commonly grown in the Midwest region of the United States. The effect of growing year was also investigated. General qualitative tests, total phenolic content (TP), total monomeric anthocyanin content (TMA), and oxygen radical absorbance capacity (ORAC) assays were conducted on blackberry extract, juice and wine samples. Between the two years, whole-berry extract from 2009 had higher concentrations of phenolic compounds, anthocyanins and higher antioxidant capacity than samples from 2008. Between the two cultivars, products from 'Apache' berries had higher concentrations of phenolic compounds, anthocyanins and higher antioxidant capacity than products from 'Ouachita' berries. The TP, TMA, and ORAC in blackberry extract showed strong interactions between year × location, and cultivar × location. This work provided the first multiyear evaluation of TP, TMA, and ORAC of two important blackberry cultivars grown in Midwest region of the Unites States.

Consuming fruits and vegetables can provide health-enhancing antioxidant compounds that help to protect the body from oxidative stress. Plant-derived antioxidants function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, and enzyme inhibitor and synergists (Wang et al., 2000). Among the phenolic antioxidants, flavonoids, particularly anthocyanins, are of interest because of their high occurrence in many fruits and vegetables (Sellappan et al., 2002). Antioxidant capacity has been shown to vary with cultivar (genotypes), growing temperature, growing season, maturity at harvest, and environmental stress (Reyes-Carmona et al., 2005).

Blackberries (*Rubus* subgenus *Rubus* L.) are relatively high in phenolic substances in general, mainly flavonoids, and are particularly high in anthocyanins, which give the red, blue, orange and purple color of berry fruits (Hager et al., 2008; Hassimotto et al., 2008). Anthocyanin and total phenolic level have shown substantial variation among genotypes and among years due to environmental and genetic variation for those traits (Clark et al., 2002).

There is a good deal of research on the health benefits of blackberries related to phenolics and anthocyanins but not much research on the phenolic concentration of different cultivars grown in the Midwest region of the United States. This research is focused on two different cultivars, 'Apache' and 'Ouachita', grown in both Arkansas and Oklahoma. The common characteristics of these cultivars are early ripening and high yield of fruit i.e. two to three kilograms (five to seven pounds) per plant. According to Clark and Moore (2006), 'Apache' berries typically have larger fruit size (8-10 g) than 'Ouachita' berries (6-7 g) but the 'Ouachita'

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berries are firmer than 'Apache' berries, which may lead to a relatively longer shelf life.

The goal of this study was to investigate the influence of cultivar and growing location on basic quality attributes and antioxidant capacity of whole berries as well as further-processed juice and wine products. Samples were collected over two harvest seasons.

Materials and Methods

Berry collection and storage

'Apache' and 'Ouachita' berries were collected from the University of Arkansas Fruit Research Substation in Clarksville, Arkansas (elevation: 273 m; average annual precipitation: 125.5 cm; in-row spacing: 61 cm) and from Toomey's Thornless Blackberry Farm in Broken Arrow, Oklahoma (elevation: 238 m; average annual precipitation: 107.7 cm; in-row spacing: 1.2 m). Harvest of blackberries began in May (Arkansas) or June (Oklahoma) and berries were harvested after they turned fully black. Berries were placed into polyethylene bags and frozen within one hour of harvest for subsequent analyses. Berry samples were stored at -15°C. For analyses and processing, frozen blackberry samples were placed into a cooler at 4°C and allowed to thaw for 24-48 h prior, then removed from the cooler and allowed to come to room temperature (≈ 2 h).

Whole berry extract preparation

Extracts were prepared by pureeing three of 100 g replications randomly taken from the bulk frozen samples. For each puree sample, two subsamples were extracted using an extraction solvent composed of 40% acetone (Fisher Scientific, Fair Lawn, NJ, USA), 40% methanol (Fisher Scientific, Fair Lawn, NJ, USA), 20% deionized water, to which was added 0.1% acetic acid (Spectrum Quality Products, Gardena, CA, USA). According to Dai and Mumper (2010), acetone/water is a good solvent for extracting a broad array of phenolics while methanol/water is better suited to extracting lower weight phenolics. Acetic acid is often added in low concentrations to help dissolve cell membranes and extract and stabilize anthocyanins. The solvent mixture we used was intended to offer good extraction of large and small molecular weight phenolics and to extract and stabilize anthocyanins. Extraction was conducted by weighing 20 g of puree into a 100 mL volumetric flask, filling the flask to volume with the extraction solvent, and agitating the puree/solvent mixtures in a reciprocal shaking water bath (Model 50, Precision Scientific, Winchester, VA, USA) for one hour at 60°C. Cooled extracts were then filtered through a funnel lined with Miracloth 1R filter paper (Calbiochem, La Jolla, CA, USA), filled into 125 mL brown glass bottles which helps to reduce light penetration and aid in nutraceutical stability. The bottles were capped, the caps were wrapped with plastic film, and the sealed bottles were placed in frozen storage at -15°C for subsequent analyses.

Blackberry juice and wine processing

After berries were thawed, four juice samples of ≈ 100 mL each were removed from each of the four replications representing the two growing locations and two cultivars, sealed in 125 mL brown glass bottles, and frozen at -15°C for subsequent analyses. The juice was obtained by pressing thawed berries and filtering through Miracloth filter paper.

Blackberry wines were made by adjusting the soluble solids content of each lot to approximately 20°Brix by adding granulated sucrose as needed. Berries were then inoculated at a rate of 2 $g \cdot kg^{-1}$ must with Fermirouge (DSM, Delft, Netherlands) yeast, which was first rehydrated 15:1 with warm water. Lallzyme C (Scott laboratories, Petaluma, CA, USA) and Lafase® (Scott laboratories, Petaluma, CA, USA) fruit pectinases were then added at a rate of 1 $g \cdot 10\ kg^{-1}$ must. The yeast nutrient Fermaid K (Presque Isle Wine Cellars, North East, PA, USA) was then added at a rate of 1.3 $g \cdot kg^{-1}$ must. Potassium metabisulfite (Presque Isle Wine Cellars,

North East, PA, USA) was also added to the must sufficient to yield a free SO_2 concentration of ≈ 30 ppm. The potassium metabisulfite was prepared by 1 g·½ gal^{-1} which makes 300 ppm of solution. The free SO_2 was measured by quick tests (Accuvin,Corvallis, OR, USA).

The winemaking process varied between the two harvest seasons in that for the first season, the skins and seeds were pressed after fermentation was complete whereas for the second harvest season, the berries were pressed at the start of the winemaking process and fermentation proceeded using the pressed juice. For both years, blackberries were pressed using a 25 L bladder press (Zambelli Enotech, Camisano Vicentino, Italy).

The inoculated berries were then transferred to four 19.0 L glass carboys that had been cleaned and sanitized with 300 ppm potassium metabisulfite solution and the carboys were sealed with rubber stoppers and S-type airlocks filled with a 300 ppm potassium metabisulfite solution. Each carboy was agitated by hand once a day during fermentation in order to mix the contents. The fermentation was completed in ≈ three weeks, as judged by no further evolution of CO_2 gas and negligible amounts of residual sugars. Four samples of ≈ 100 mL were removed from each lot, sealed in 125 mL brown glass bottles, and frozen at -15°C for subsequent analyses.

Additional potassium metabisulfite was added to the wine samples during aging/storage to maintain the SO_2 desired concentration of ≈ 50 ppm. After ≈ five weeks, blackberry wine samples were decanted off of the lees (the sediment that collects at the bottom of the vessel) and four 100 mL samples of each wine were removed from each of the four cultivar-location combinations and frozen and held at -15°C to retard further chemical changes prior to subsequent analyses.

Quality analyses

Soluble solids. Soluble solids (%) were measured using a Leica Auto ABBE refractometer (Buffalo, NY, USA) with sample temperature compensation. Two duplicate readings were taken from each berry juice and wine sample.

pH. The pH of the blackberry juice was measured using an Accumet AB 15 pH meter (Buffalo, NY, USA). Two duplicate readings were taken from each berry juice and wine sample.

Titratable acidity. A 5 mL sample of blackberry juice was diluted to 105 mL using deionized water. The titratable acidity was then measured as g·L^{-1} citric acid using a 809 Titrando automatic titrator (Metrohm ion analysis, Herisau, Switzerland). Two duplicate readings were taken from each berry juice and wine sample.

Chemical analyses

Total phenolic concentration. Total phenolics were measured using the method of Singleton and Rossi (1965). Briefly, 0.5 mL of prepared, blackberry extract or blackberry juice from random bulk samples of frozen berries was added to 1 mL of Folin-Ciocalteu reagent (Fluka Biochemika, Steinheim, Switzerland) and 5 mL of deionized water in a 25 mL volumetric flask. The contents were mixed and allowed to stand for 5-10 min at room temperature (~25°C). Next, 10 mL of a 7% (w/v) sodium carbonate (Spectrum Quality Products, New Brunswick, NJ, USA) solution were added and deionized water was used to fill the flask to volume. The solution was mixed and allowed to stand at room temperature for about 2 h.

Following this, the absorbance was measured at 720 nm using a Beckman DU 520 (Brea, CA, USA) spectrophotometer. Total phenolic content was expressed as mg gallic acid (GAE) ·100 g^{-1} starting puree. Equivalent gallic acid concentration was calculated using a standard curve prepared from gallic acid (Sigma St. Louis, MO, USA). Two duplicate assays were performed on each sample of extract and triplicate assays were performed on each sample of juice and wine.

Total monomeric anthocyanin concentra-

tion. Total monomeric anthocyanins were measured using the pH differential method first described by Giusti and Wrolstad (2000). For this assay, 1 mL of blackberry juice or whole-berry extract from random bulk samples of frozen berries was added to a 25 mL volumetric flask. The flask was then brought to volume with pH 1 potassium phosphate buffer. A sample of 1 mL of juice or extract was then added to another 25 mL volumetric flask, which was brought up to volume with pH 4.5 sodium acetate buffer. These solutions were allowed to equilibrate for 15 min. Then the absorbance of each solution was measured at 520 nm and 700 nm using a Beckman DU 520 (Brea, CA, USA) spectrophotometer. Total anthocyanin content was expressed as cyanidin-3-glucoside (C3G) ·100 g^{-1} of whole berries or 100 mL^{-1} of juice. Anthocyanin content of all samples were measured in duplicate (berry extract) or triplicate (juice and wine).

Antioxidant activity analysis

Oxygen radical absorbance capacity (ORAC) assay. ORAC assays were conducted using the method first developed by Ou et al. (2001). Fluorescence readings were obtained using a Perkin Elmer HTS 7000 Plus Bio Assay reader (Waltham, MA, USA) using fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Juice and extract samples were diluted prior to being assayed with phosphate buffer as needed in order to bring their decay curves into the proper range, approximating the Trolox (Fluka Chemika, Steinheim, Switzerland) decay curve. Prepared plates were placed into the microplate reader immediately after the 2-2`-azobis (2-aminopropane) dihydrochloride (AAPH, Waco Chemicals Inc., Richmond, VA, USA) was added and plate reading was initiated. The total run time for each assay was 70 min at 37°C and the microplate reader was programmed to record fluorescence every two minutes. Results were obtained by calculating the Area Under the fluorescence decay Curve (AUC) for each of the Blank, Trolox, and Sample wells as follows:

$$AUC = f_1/f_0 + \ldots f_i/f_0 + \ldots + f_{34}/f_0 + f_{35}/f_0$$

where:

f_0 = initial fluorescence reading at 0 min and f_i = fluorescence reading at time i.

Subtraction of the area of "Blank" wells allowed us to directly compare the net areas of "Standard" wells and "Sample" wells. By figuring in additional dilution factors and sample weights we calculated the final results in terms of µmoles Trolox equivalent (TE) ·100 g^{-1} of fresh blackberry tissue or 100 mL^{-1} of blackberry juice or wine. All the samples were measured in quadruplicate.

Statistical analyses

Statistical analyses were performed using SAS version 9.4 (SAS institution, Cary, NC, USA). For all analyses such as quality analyses, chemical analyses, and antioxidant analyses, an analysis of variance (ANOVA) for each set of data was conducted using a completely randomized design. Means were separated using Duncan's test with a 95% confidence interval ($p< 0.05$). Pearson correlation coefficients were created to assess the relationships between chemical constituents as measured by the total phenolic content, total monomeric anthocyanin content and ORAC values.

Results and Discussion

Overall, a statistically significant interaction was seen between cultivar and growing location and between harvest year and growing location. However, the effects of growing location were not consistent and no clear relationship between growing location and other main variables was observed. Therefore, the results presented below were averaged over both growing locations.

Table 1. Average (n = 2) soluble solids concentrations, pH values and titratable acidity of blackberry juice and wine samples over two years.

Cultivar	Year	pH		Soluble solids	Titratable acidity[z]	
		Juice	Wine	(%)	Juice	Wine
Apache	2008	3.14	3.25	10.3	13.73	13.46 Z [y]
Apache	2009	3.3	3.47	10.9	9.59	11.65 Y
Ouachita	2008	3.27	3.31	9.6	16.48 Z	15.95
Ouachita	2009	3.64	3.57	9.2	11.53 Y	13.08

[z] Titratable acidity expressed as g·L^{-1} of citric acid.
[y] Letters denote statistically significant differences between the two years within cultivar ($p<0.05$).

Quality analyses

Soluble solids. Soluble solids values are presented in Table 1. The berries evaluated in this study fell within the typical value, roughly 10%, and had slightly higher soluble solids contents than those reported by Wang et al. (2008) and slightly lower than those reported by Siriwoharn et al. (2004). No differences were seen in soluble solids content between the two cultivars or between the two growing years ($p>0.05$).

The 'Apache' and 'Ouachita' berries were originally developed at the University of Arkansas. Conversely, Clark and Moore (2006) also noted that 'Ouachita' berries had consistently higher soluble solid contents than 'Apache' berries.

pH and titratable acidity. The pH values of blackberry juice and wine samples are presented in Table 1. The pH values observed ranged from 3.1 to 3.6. No statistically significant differences between cultivars or growing years were seen in pH values for juice or wine samples ($p>0.05$).

Observed titratable acidity values ranged from 11-15 g·L^{-1} citric acid, somewhat higher than the values reported by Wang et al. (2008) and Siriwoharn et al. (2004). The relatively high titratable acidity values found in this study may have been due to variation in cultivars and/or to growing conditions. According to Acosta-Montoya et al. (2010), titratable acidity decreases significantly during ripening and ripening is affected by the amount of light intensity; a greater light intensity accelerates the ripening process. According to data collected by the "Mesonet", a state-wide network of automated environmental monitoring stations, implemented by Oklahoma State University and the University of Oklahoma - the summer of 2008 had more rainfall and less day/night temperature variation than summer 2009, especially in May and June <http://www.mesonet.org/index.php/weather/station_monthly_summaries>. Since the weather condition in in 2009 had less rainfall and more day/night temperature variation than 2008, it may have caused more plant stress. The average day/night temperature difference recorded in June of 2009 was 25°C (high 36.7°C, low 11.7°C) while in June of 2008 the difference was 18.3°C (high 33.3°C, low 15°C). The average day/night temperature difference recorded in July of 2009 was 23.3°C (high 38.3°C, low 15°C) while in July of 2008 the difference was 21.7°C (high 37.8°C, low 16.1°C) . Also, the rainfall in June and July of 2009 was lower than in the same months in 2008: 20.5 cm in June 2008 vs. 5.7 cm in June 2009; 11.9 cm in July 2008 vs. 8.3 cm in July 2009.

In 2008, titratable acidity values were numerically higher for juice samples than for wine samples whereas in 2009 the reverse was true. Ordinarily we would expect titratable acidity levels to be relatively stable or to decline slightly from juice to wine as acids may precipitate in the form of salts during fermentation and storage. We observed this decrease in 2008, but not in 2009. We did not discover a clear explanation for the increase

in titratable acidity observed in 2009.

Overall, titratable acidity values for 'Ouachita' were numerically higher than values for 'Apache'. Comparing the two growing years 'Ouachita' juice from 2008 was significantly more acidic than juice from 2009 and 'Apache' wine from 2008 was significantly more acidic than wine from 2009.

Chemical analyses

Total phenolic concentrations. Total phenolic concentrations in whole blackberries are shown in Table 2. The berries were frozen after harvest in order to minimize chemical changes between the time of harvest and the beginning of wine processing, but we cannot exclude the possibility that some nutraceutical content was lost during frozen storage. However, sample to sample comparisons are valid as all samples were stored identically. Concentrations ranged from 5771 to 7783 mg GAE·100 g^{-1} of whole blackberry. We found higher total phenolic content than previously reported (Deighton et al., 2000; Moyer et al., 2002; Pantelidis et al., 2007; Sellappan et al., 2002; Wang et al., 2008). Part of the reason for the higher concentrations measured in this study may be that the use of frozen blackberries allowed for a more complete extraction of phenolic compounds. Asami et al. (2003) reported that ice crystals formed during the process of freezing berries can rupture plant cell structure and lead to more complete solvent saturation of tissues and extraction of phenolic compounds. We had slightly lower values for total phenolic concentrations than those reported for 'Evergreen' and 'Marion' blackberries by Siriwoharn et al. (2004), but this is not necessarily surprising given that our study evaluated a different species of blackberry grown under very different conditions.

Berries grown in 2008 had lower concentrations of total phenolics than those grown in 2009 and 'Apache' berries had higher concentrations of total phenolics than 'Ouachita' berries. Also, a statistically significant interaction was observed between growing year and cultivar. According to Acosta-Montoya et al. (2010), phenolic synthesis in plants acts as a defense mechanism. So, wild blackberries typically have higher total phenolic concentrations than cultivated blackberries due to greater exposure to stress such as extreme temperatures, drought, high humidity, pests, and fungal disease. In the South central region of the United States, 'Ouachita' berries ripen in mid-June while 'Apache' berries ripen in early July (Clark and Moore, 2006). Thus, the difference we observed between 'Apache' and 'Ouachita' berries overall may be partially explained by the difference in ripening times. 'Apache' berries ripened in somewhat hotter and drier conditions and 'Ouachita' berries and this stress may have led to the higher concentrations of phenolic compounds observed in the 'Apache' berries. In addition, as noted above, weather conditions were more stressful

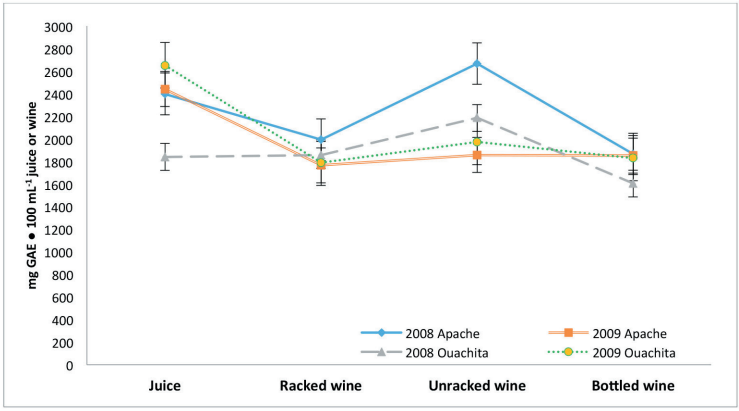

Fig. 1. Mean (n = 3) total phenolic concentration of 'Apache' and Ouachita blackberry juices and wines at various stages of proccessing. Error bars represent the standard error of the means.

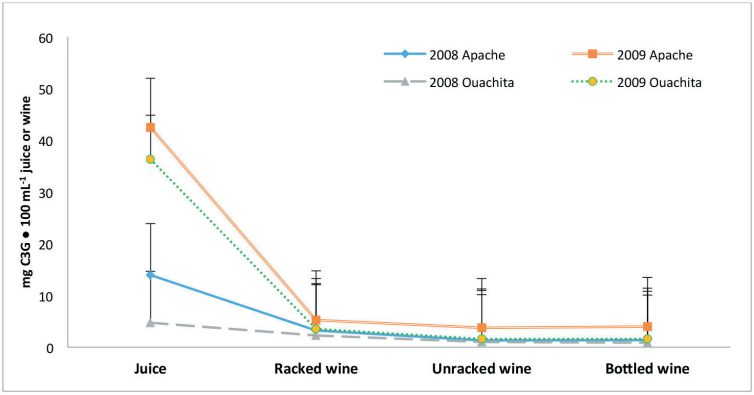

Fig. 2. Mean (n = 3) total anthocyanin concentration of 'Apache' and 'Ouachita' blackberry juices and wines at various stages of processing. Error bars represent the standard error of the mans.

in 2009 than in 2008, particularly as the summer advanced. This may explain the overall higher concentrations of phenolic compounds observed in 2009.

Total phenolic concentrations for juice and wine samples at various stages of processing are shown in Fig. 1. Overall, the whole blackberries had phenolic concentrations three to four times higher than the juice and wine samples. As phenolics are concentrated in the skin and seeds of the berry (Siriwoharn et al., 2004), it is not surprising to see concentrations decline when these components are removed. Overall, we did not observe significant decreases in the concentration of total phenolics in the process of making wine from juice; few differences were observed between cultivars or between growing years. It appears that, at least within certain ranges, differences in the starting concentrations of total phenolics in the fresh berries may not necessarily be reflected in the final concentrations observed in value-added products such as juices and wines.

Total monomeric anthocyanin concentrations. Total monomeric anthocyanin concentrations in whole blackberries are shown in Table 2. Concentrations ranged from 230 to 550 mg C3G·100 g^{-1} of whole blackberry. The anthocyanin concentrations we measured are higher than those reported for some other cultivars (Moyer et al., 2002; Pantelidis et al., 2007; Sellappan et al., 2002; Siriwoharn et al., 2004; Wang et al., 2008). However, our results were within the range of values (1-1186 mg C3G·100 g^{-1}) reported by Deighton et al. (2000). Anthocyanin concentrations followed the same pattern as overall phenolic concentrations: berries grown in 2008 had lower concentrations than those grown in 2009 and 'Apache' berries had higher concentrations than 'Ouachita' berries.

Total monomeric anthocyanin concentrations for juice and wine samples at various stages of processing are shown in Fig. 2. Overall there was an ≈ 13- to 45-fold decline in anthocyanin concentrations between whole berries and juices. This was certainly due at least in part to separating the anthocyanin-rich skins from the juice (Dai et al., 2009).

Significant differences were seen between years in the concentration of total monomeric anthocyanins in the blackberry juice at the beginning of fermentation. This may have been due to the higher concentrations of total phenolics and anthocyanins observed in the whole berries in 2009. However, it is important to note that some of the differences observed may also have been due to the different winemaking techniques employed for each year: whole berries were fermented and then pressed in 2008 whereas pressed juice was fermented in 2009. The reason for this variation in winemaking technique was that the first year's technique was judged to be impractical due to the fact that the must material was difficult to transfer from vessel to

Table 2. Average (n = 3) total phenolic concentration, total monomeric anthocyanin concentration and oxygen radical absorbance capacity (ORARC) of whole blackberries over the two years.

Cultivar	Year	Total phenolic (mg GAE·100 g^{-1} of fresh blackberry)	Total anthocyanin (mg C3G·100 g^{-1} of fresh blackberry)	Oxygen radical absorbance capacity (mg TE·100 g^{-1} of fresh blackberry)
Apache	2008	7255.3 Y [z] z [y]	482.6 Yz	18530 Yz
Apache	2009	7783.0 Zz	550.1 Zz	19929 Zz
Ouachita	2008	5771.3 Yy	229.5 Yy	15753 Yy
Ouachita	2009	6984.1 Zy	300.9 Zy	18811 Zy

[z] Upper case letters denote significant differences between the two years within cultivar ($p<0.05$).
[y] Lower case letters denote significant differences between the two cultivars within a year ($p<0.05$).

vessel and the large amount of solids present in the must appeared to have a negative impact on fermentation efficiency.

Overall, two- to 10-fold reductions in anthocyanin concentrations were observed during the process of wine making (Fig. 2) and no significant differences were seen after fermentation regardless of fermentation technique or starting concentrations of anthocyanins in the whole berries ($p>0.05$).

Oxidative degradation and/or polymerization of the anthocyanins could have contributed to the observed decline in anthocyanin concentrations (Cespedes et al., 2008; Thomas et al., 2005). Regardless of the exact mechanism, monomeric anthocyanins are known to be lost during the process of fermenting and aging in wine; our results reflect that (Acosta-Montoya et al., 2010).

Antioxidant activity analysis

Oxygen radical absorbance capacity (ORAC) assay. The ORAC values measured for whole blackberries are shown in Table 2. Values ranged from 15,753 to 19,929 mg TE·100 g^{-1} of whole blackberry. Our results showed ORAC values two- to three-fold higher than ORAC values observed in some other studies of blackberries (Moyer et al., 2002; Siriwoharn et al., 2004; Wang et al., 2008), possibly due to cultivar, growing conditions, or both. Interestingly, ORAC values for whole berries also closely mirrored the pattern seen for total phenolic and anthocyanin concentrations: berries grown in 2008 had lower ORAC values than those grown in 2009 and 'Apache' berries had higher ORAC values than 'Ouachita' berries.

The ORAC values measured for juice and wine samples at various stages of processing are shown in Fig. 3. Overall, the ORAC values seen in juice and wine samples were ≈ 11-12 times lower than those seen in whole

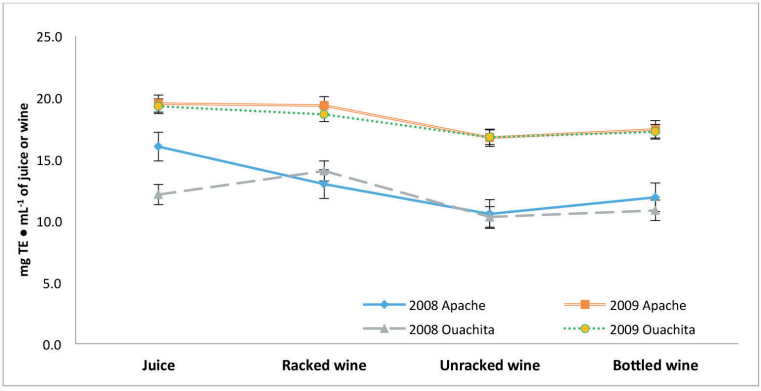

Fig. 3. Mean (n = 4) oxygen radical absorbance capacity assay values of 'Apache' and 'Ouachita' blackberry juices and wines are various stages of processing. Error bars represent the standard error of the means.

Table 3. Correlation among ORAC, total anthocyanin concentrations, and total phenolic concentrations in whole berry extracts, blackberry juice and wine samples

Blackberry product	Correlation coefficient
Whole Berry	
Phenolics:ORAC value	0.7575* [z]
Anthocyanins:ORAC value	0.0600
Blackberry Juice	
Phenolics:ORAC value	0.6142*
Anthocyanins:ORAC value	0.7644*
Bottled Blackberry Wine	
Phenolics:ORAC value	0.2878
Anthocyanins:ORAC value	0.8455*

[z] * denotes a statistically significant correlation ($P<0.05$).

berries. Unlike the results seen for anthocyanin concentrations and more like the results seen for total phenolic concentrations, we did not see a significant decrease in ORAC values during wine fermentation and initial ageing ($p>0.05$). Also, while the differences in ORAC values originally observed between cultivars did not persist in the juices or wines, the differences observed between growing years did; juices and wines made in 2009 had higher ORAC values at all stages of processing than juices and wines made in 2008. This may have been due to the different winemaking technique employed in 2009 or it may have been due to other environmental factors.

Correlations between ORAC values and phenolic compounds

Overall, correlations between monomeric anthocyanin concentration and ORAC values and between total phenolic concentrations and ORAC values for whole blackberries, juice, and bottled wine are presented in Table 3. For whole berries, a significant positive correlation between total phenolics and ORAC values was observed, while no correlation between ORAC values and anthocyanin concentration was noted. For juices, both correlations were significant whereas for wine, only the correlation between anthocyanin concentrations and ORAC values was significant. From this we can conclude that total phenolic concentration tracks well with ORAC values in whole berries but that other chemical constituents more closely reflect ORAC values in juices and particularly in wines. Interestingly, even though a strong positive correlation was seen between anthocyanin content and ORAC values in bottled wines, the fact that anthocyanin concentrations generally dropped much more than ORAC values during the winemaking process suggests that other chemical compounds are influencing the ORAC values in the finished wine apart from the phenolics and anthocyanins measured by our assays in this study (Cespedes et al., 2008).

In conclusion, this study showed that the whole berries had higher concentrations of total phenolics and total monomeric anthocyanins as well as higher ORAC values than juices and wines made from those berries. Overall, 'Apache' berries grown in 2009 had higher concentrations of total phenolics, total monomeric anthocyanin content and higher ORAC values than any other berries tested. In general, the differences seen in these attributes in the whole berries did not carry over into the value-added products, juice and wine, made from these berries. The exception to this was the effect of growing year on measured ORAC values.

It is important to note that the quantification of potential antioxidant compounds in the berries and their products was limited to total phenolics and total monomeric anthocyanins. Thus, it may be beneficial for future research to attempt to identify and quantify other antioxidant compounds in whole blackberries, in blackberry juices, and particularly in blackberry wines. Further, our results indicate that a good deal of antioxidant activity is lost in processing. Therefore, blackberry pomace created as a byproduct of juice and/or wine processing may be a good source of antioxidants and have possible applications in the manufacture of functional foods and/or nutritional supplements.

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Literature Cited

Acosta-Montoya, O., F. Vaillant, S. Cozzano, C. Mertz, A.M. Perez, and M.V. Castro. 2010. Phenolic content and antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus* schltdl.) during three edible maturity stages. Food Chem. 119:1497-1501.

Asami D.K., Y.J. Hong, D.M. Barrett, and A.E. Mitchell. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. J. Agr. Food Chem. 51:1237-1241.

Cespedes, C.L., M. El-Hafidi, N. Pavon, and J. Alarcon. 2008. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* (Elaeocarpaceae), Maqui. Food Chem. 107:820-829.

Clark, J. and L. Talcott. 2002. Antioxidant activity of blackberry genotypes. Acta. Hort. 777:475-480.

Clark, J.R. and J.N. Moore. 2006. Blackberry plant named 'Ouachita'. University of Arkansas, Fayetteville, AR, U.S. patent 17:162 P3.

Dai J., A. Gupte, L. Gates, and R.J. Mumper. 2009. A comprehensive study of anthocyanin containing extracts from selected blackberry cultivars: extraction methods, stability, anticancer properties and mechanisms. Food Chem. Toxicol. 47:837-847.

Dai, J. and R. Mumper. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Mol. 15:7313-7352.

Deighton, N., R. Brennan, C. Finn, and H. Davies. 2000. Antioxidant properties of domesticated and wild Rubus species. J. Sci. Food Agr. 80:1307-1313.

Giusti, M. and R. Wrolstad. Characterization and measurement of anthocyanins by UV-visible spectroscopy. 25 May 2015. <http://www.does.org/masterli/facsample.htm>.

Hager, T., L. Howard, and R. Prior. 2008. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blackberry products. J. Agr. Food Chem. 56:689-695.

Hassimotto, N., R. Mota, B. Cordenunsi, and F. Lajolo. 2008. Physico-chemical characterization and bioactive compounds of blackberry fruit (*Rubus* sp.) grown in Brazil. Cienc. Technol. Aliment. 28:702-708.

Mesonet. 2015. Station monthly summaries. The University of Oklahoma, Norman, OK. 20 May 2015. <http://www.mesonet.org/index.php/weather/station_monthly_summaries>.

Moyer, R., K. Hummer, C. Finn, B. Frei, and R. Wrolstad. 2002. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus* and *Ribes*. J. Agr. Food Chem. 50:519-525.

Ou, B., M. Hampsch-Woodill, and R. Prior. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J. Agr. Food Chem. 49:4619-4626.

Pantelidis, G., M. Vasilakakis, G. Manganaris, and G. Diamantidis. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, gooseberries and Cornelian cherries. Food Chem. 102:777-783.

Reyes-Carmona, J., G. Yousef, R. Martinez-Peniche, and M. Lila. 2005. Antioxidant capacity of fruit extracts of blackberry (*Rubus* sp.) produced in different climatic regions. J. Food Sci. 70:497-503.

Sellappan, S., C. Akoh, and G. Krewer. 2002. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J. Agr. Food Chem. 50:2432-2438.

Singleton, V. and J. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Amer. J. Enol. and Viticult. 16:144-158.

Siriwoharn, T., R. Wrolstad, C. Finn, and C. Pereira. 2004. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. Hybrids) anthocyanins, polyphenolics, and antioxidant properties. J. Agr. Food Chem. 52:8021-8036.

Thomas R.H., F.M. Woods, W.A. Dozier, R.C. Ebel, M. Nesbitt, B. Wilkins, and D.G. Himelrick. 2005. Cultivar variation in physicochemical and antioxidant activity of Alabama-grown blackberries. Small Fruit Rev. 4(2):57-71.

Wang, S. and H. Lin. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J. Agr. Food Chem. 48:140-146.

Wang, W., S. Xu, and M. Jin. 2008. Effects of different maceration enzymes on yield, clarity, and anthocyanin and other polyphenol contents in blackberry juice. Intl. J. Food Sci. and Tech.:1-8.