Inquiry: The University of Arkansas Undergraduate Research Journal

Volume 16 Article 5

Spring 2014

Descriptive Sensory Analysis and Composition of Blackberry Genotypes

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DESCRIPTIVE SENSORY ANALYSIS AND COMPOSITION OF BLACKBERRY GENOTYPES

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Abstract

Consumer interest in blackberries has been increasing due in part to reputed health-promoting factors. Appearance, flavor, and texture attributes of blackberry fruits are important to consumers. The objective of this study was to investigate correlations among sensory and composition attributes of blackberry genotypes from the University of Arkansas Division of Agriculture breeding program. Descriptive panelists evaluated attributes of 20 blackberry genotypes. Composition attributes were evaluated for these and two additional genotypes. 'Natchez' had the most pyrenes/berry and the highest levels of total ellagitannins. Selection A-2215 was scored highest for descriptive-evaluated sweetness and had the highest soluble solids content. Total ellagitannins ($r= 0.57$; $p<0.0095$) and ORAC ($r= 0.54$; $p<0.0146$) were moderately correlated to seediness, which may reflect the value of ORAC factors in pyrenes. These initial investigations of the relationship between sensory and composition of blackberry genotypes provide insights that can be used for future blackberry cultivar assessments.

Introduction

Blackberries are a high-value horticultural crop and are grown worldwide for both the fresh market and for processing. Blackberries are classified in the *Rosaceae* family and *Rubus* genus (Finn & Clark, 2012). The blackberry fruit is an aggregate fruit comprised of drupelets surrounding the soft tissue receptacle or torus. The size of a blackberry fruit is determined by the combination of drupelet number and size (Clark, Stafne, Hall, & Finn, 2007). An individual drupelet includes a thin exocarp, a fleshy mesocarp, and a hard, lignified endocarp, also known as a pyrene, which encloses a single seed (Tomlik-Wyremblewska, Zieliński, & Guzicka, 2010). Pyrene physical characteristics are distinctive to each blackberry genotype and have been classified into the following three groups: straight, concave, or convex (Wada, Nonogaki, & Reed, 2010; Wada & Reed, 2010). Slight variation of pyrene shape can occur in the same genotype and the outer layer of the endocarp typically will have characteristic patterns of hollows (Tomlik-Wyremblewska et al., 2010). Pyrene shape and endocarp thickness influence perceived seediness when consumed (Takeda, 1993).

Not until the late 1990s were fresh blackberries readily available in retail markets in the United States (Clark, 2005; Strik, Clark, Finn, & Bañados, 2007). Since then, blackberries have established a more prominent place in the market due to enhanced shipping capability, prolonged shelf life, off-season availability, and double blossom/rosette disease resistance (Clark, 2005; Strik et al., 2007). In 2005, worldwide blackberry area was 20,036 ha and was projected to increase to over 27,000 ha by 2015 (Strik et al., 2007).

The increase in production area can in part be contributed to blackberry breeding programs. Blackberry breeding initiatives can be found on every continent with the exception of Antarctica (Strik et al., 2007). Blackberry breeding programs have existed for more than 100

years in the United States and continually strive to enhance favored qualities and to reduce undesirable traits. The first blackberry breeding program was initiated in 1909 at the Texas Agricultural Experiment Station (Clark & Finn, 2008). The oldest currently active program is located at the United States Department of Agriculture-Agricultural Research Service at Corvallis, OR; it was initiated in 1928 (Clark & Finn, 2008; Finn & Clark, 2012). In 1964, the University of Arkansas System Division of Agriculture (UASDOA) blackberry breeding program was initiated by Dr. James N. Moore (Clark, 1999). This effort, based at the UASDOA Fruit Research Station, Clarksville, AR, prioritized development efforts on attributes including thornlessness, erect growth habit, mechanical harvesting capability, disease resistance, productivity, and environmental and geographic adaptation (Clark, 1999; Clark & Finn, 2008). The fruit improvement objectives included large fruit size, good flavor, firmness, and high fertility (Clark, 1999). The UASDOA breeding program has expanded to focus on primocane-fruiting genotypes that produce fruit on first-year canes in addition to traditional second-year floricanes resulting in the commercial release of Prime-Jim®, Prime-Jan®, and Prime-Ark® 45 (Clark & Finn, 2008). Even though breeding priorities vary, most blackberry breeding programs focus on promoting consumption through improved fruit quality (Finn & Clark, 2012).

The processing industry prefers blackberries that have intense flavor and color, low pH, high soluble solids content and titratable acidity levels, low perceived seediness, and small pyrene size (Clark et al., 2007; Clark & Finn, 2008; Hall, Stephens, Stanley, Finn, & Yorgey, 2002). Of the Arkansas-released cultivars, ‘Choctaw’ has the smallest pyrene size (Clark, 1999); ‘Navaho’ and ‘Ouachita’ have higher soluble solids content (Clark et al., 2007; Clark & Finn, 2008); ‘Navaho’ is commonly sold to processors and is popular in United States fresh markets (Wada & Reed, 2010).

Some blackberry genotypes have been evaluated for nutraceutical/antioxidant levels (Cho, Howard, Prior, & Clark, 2004; Clark & Finn, 2008; Clark, Howard, & Talcott, 2002; Connor, Finn, & Alspach, 2005; Siriwoharn, Wrolstad, Finn, & Pereira, 2004). Blackberries are excellent sources of nutraceutical-rich polyphenolic compounds in the human diet (Reyes-Carmona, Yousef, Martinez-Peniche, & Lila, 2005; Sellappan, Akoh, & Krewer, 2002; Wang, Xu, & Jin, 2009; Zheng & Wang, 2003). High levels of anthocyanin, a polyphenolic antioxidant, naturally occur in blackberries (Clark et al., 2007) and account for the dark red pigmentation of the fruit (Wang et al., 2009). Polyphenols, including anthocyanin, proanthocyanids, flavonones, and flavonols, have been shown to have protective effects against some human health diseases (Ness & Poulens, 1997; Prior et al., 1998; Reyes-Carmona et al., 2005; Steinmetz & Potter, 1996; Van der Sluis, Dekker, De Jager, & Jongen, 2001). Antioxidants are able to reduce free radicals in the human body (Liu, 2003; Narayana, Reddy, Chaluvadi, & Krishna, 2001; Reyes-Carmona et al., 2005; Sariburun, Şahin, Demir, Türkben, & Uylaşer, 2010). Potential beneficial effects of polyphenols include anti-inflammatory, antiviral, antimicrobial, and antioxidant activity (Reyes-Carmona et al., 2005). Anti-cancer properties can be attributed to phenolic compounds in berries (Sariburun et al., 2010; Seeram et al., 2006; Seeram, 2008). These potential nutraceutical components can impact the fruit quality and sensory perception.

Fruit qualities such as sweetness, tartness, flavor, and astringency along with color, firmness, and seediness are important to consumers whether the berries are processed or consumed fresh (Clark & Finn, 2008). In general, consumers prefer fresh blackberries that are perceived as less “seedy” (i.e. fewer pyrene number or smaller size). The structure, size, and number of pyrenes in the blackberry may influence mouthfeel of the blackberries when consumed (Clark et al., 2007). Small pyrene size (< 3 mg) is preferred in both fresh-market and

processed blackberry products, and large pyrenes can be objectionable (Moore, Lundergan, & Brown, 1975). Moore et al. (1975) also found that pyrene size in blackberries is highly heritable with partial dominance for small pyrene size. Fresh-market blackberries can feel "seedy" when consumed, depending on the pyrene attributes. Large pyrene size, based on weight, length, or volume, and seediness are also undesirable in processed blackberry products (Takeda, 1993). Yet, the proportion of pyrene weight to total berry weight is more important than pyrene size (Darrow & Sherwood, 1931). Pyrene weight and pyrene number were positively correlated with blackberry fruit weight (Moore, Brown, & Brown, 1974).

Although studies on blackberry pyrene characteristics and morphology have been published, there is limited information on descriptive sensory analysis of Arkansas-developed fresh blackberries and the composition attributes that affect sensory scoring. The objective of this study was to investigate the descriptive sensory attributes and composition of blackberry genotypes from the UASDOA blackberry breeding program.

Materials and Methods

Fruit

Blackberry fruits were hand-harvested from the UASDOA Fruit Research Station, Clarksville, AR in 2012. The cultivars and genotypes 'Ouachita', 'Natchez', 'PrimeArk® 45', APF-190, A-2434, A-2312 ('Stella'), and APF-227 were harvested on 29 May; 'Ouachita', A-2108, 'Osage', A-2215, APF-156, APF-185, APF-205, and A-2473 were harvested on 7 June; and 'Ouachita', A-2252, A-2316, A-2418, A-2416, A-2419, 'Navaho', and 'Apache' were harvested on 14 June. After harvest, the berries were transported to the Food Science Department, University of Arkansas, Fayetteville, AR. In addition, blackberries were purchased commercially including 'Tupy' (Naturipe, Salinas, CA; fresh-market blackberries imported from

central Mexico) and commercial frozen blackberries (Great Value, Wal-Mart Stores, Inc. Bentonville, AR; cultivar unknown).

Sensory Descriptive Analysis

Descriptive sensory analysis of the fresh blackberries was performed at the Sensory and Consumer Research Center at the University of Arkansas, Fayetteville, AR. Trained descriptive panelists (n=8) participated in a 3-hour orientation session where the descriptive ballot was developed through consensus. The commercial frozen blackberries were thawed and used as the reference sample. Scores for the reference sample were also determined through consensus.

The fruits were evaluated on the same day they were harvested. 'Apache' and A-2252 were not sensory-evaluated because of limited quantity. Due to scheduling conflicts with the panelists on the multiple harvest dates, only four descriptive panelists (n=4) evaluated all the genotypes in the study.

Panelists (n=4) evaluated each blackberry genotype in duplicate using Spectrum® methods. The Spectrum® method is an objective method for describing the intensity of the blackberry fruit attributes. Descriptive panelists were trained with an absolute scale anchored by a specific reference. They assessed each attribute per genotype and replication at a particular intensity according to the reference points on the universal scale. Serving order was randomized across replication to prevent presentation order bias. The blackberry genotypes were served sequentially in 60 mL (2 oz) cups and were assigned random three-digit blinding codes. The blackberries were served at room temperature. Panelists were instructed to cleanse their palettes with unsalted crackers and water between samples. Expectorant cups were also provided. For each panelist, one paper ballot was completed per genotype for each replication.

The panelists identified and evaluated color/appearance, flavor by mouth, and texture attributes on a 10-point scale ranging from 0 (*less of the attribute*) to 9 (*more of the attribute*) in terms of intensity. The descriptive panel evaluated the color/appearance attribute [overall color intensity from 0 (*light red*) to 9 (*black*)], flavor attributes (overall flavor intensity, sweetness, and sourness), and the texture attribute (overall seediness) of the berries. The commercial frozen blackberries were thawed and used as a reference during each session. During orientation, scores for the reference sample were determined through consensus (overall color intensity = 4, overall flavor intensity = 4, sweetness = 2, sourness = 7, and overall seediness = 7), and the reference samples were used during the evaluation of the genotypes.

Composition Analysis

All evaluations for composition of blackberries were conducted at the Grape and Wine Research Laboratory, Department of Food Science, University of Arkansas, Fayetteville, AR. Three samples of approximately 100 g of berries were collected for each cultivar or genotype, placed in plastic storage bags, and stored at -20°C until analyses.

Berry and pyrene attributes. From the frozen berries, three berries per genotype and replication were used to determine attributes (individual berry weight, berry length, and berry width) and pyrene attributes (number/berry, dry weight/berry, and individual pyrene length, width, and height). The three-berry samples were weighed on a digital scale (Explorer, Ohause Corporation, Switzerland) and the width and height of each blackberry was measured with a certified calibrated digital caliper. Berry volume was calculated as the volume of a cone.

To determine pyrene attributes, a 0.1 mL of Pec5L enzyme (Scott Laboratories, Petaluma, CA) was added to each bag containing the three-berry frozen sample to break down the skin and pulp. Once the berries thawed, they were hand-mashed in the bags. After 1.5 hours

at 21°C, 100 mL of distilled water was added to each bag. The samples were then poured into a strainer. Under running water, the pulp was mashed against the strainer until only pyrenes remained. The pyrenes were placed onto paper towels and dried at ambient temperature (21°C) for 1.5 hours. The pyrenes for each three-berry sample were counted and weighed. The pyrenes were further dried in a laboratory oven (Fischer Scientific, Pittsburg, PA Isotemp®, Model 655F) at 55°C for approximately 24 hours. The pyrenes were removed from the oven and weighed, and then the length, width, and height of six randomly-selected individual pyrenes per genotype and replication were measured with a digital caliper. Pyrene volume was calculated as length x width x thickness. The pyrenes for each genotype and replication were placed in plastic storage bags and stored in a freezer at -20°C for further measurements. Images of the individual pyrenes were taken, after freezing, using a macro lens [Nikon D90, Tokyo, Japan; Nikon AF micro Nikkor 105mm (1:2.8 D)].

Soluble solids, pH, and titratable acidity. Three replicate three-berry samples of each cultivar and genotype were used to determine the soluble solids, pH, and titratable acidity for each genotype. Samples were thawed and placed in cheesecloth to extract the juice from the berries. Titratable acidity and pH were measured by an 877 Titrino Plus (Metrohm AG, Herisau, Switzerland) with an automated titrimeter and electrode standardized to pH 2.0, 4.0, 7.0, and 10.0 buffers. Titratable acidity was determined using 6 mL of juice diluted with 50 mL of deionized, degassed water by titration with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.2; results were expressed as g/L citric acid. Total soluble solids (expressed as %) was measured with a Bausch & Lomb Abbe Mark II refractometer (Scientific Instrument, Keene, NH). Soluble solids/titratable acidity ratio was calculated as soluble solids (%)/(titratable acidity (g/L)/10).

Nutraceutical Analysis

Nutraceutical analysis was conducted on each genotype in triplicate. To obtain sample extracts, samples (25 g) were homogenized with 20 mL of acetone/water/acetic (70:29.5:0.5 v/v/v) with a Euro Turrax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, OH). The samples were filtered through Miracloth (Calbiochem, La Jolla, CA), the filter cakes were isolated, and the extraction was repeated. The filtrates were adjusted to a final volume of 250 mL with extraction solvent.

High Pressure Liquid Chromatography (HPLC) analysis of ellagitannins and flavonols. Sample extracts (3 mL) were dried using a Speed Vac concentrator (ThermoSavant, Holbrook, NY) and re-suspended in 0.5 mL of extraction solvent. The reconstituted samples were passed through 0.45 μm PTFE syringe filters (Varian, Inc., Palo Alto, CA) prior to HPLC analysis. The ellagitannins were analyzed on a Waters Alliance HPLC system (Milford, MA) equipped with a Waters model 996 photodiode array detector and Millenium version 3.2 software (Waters Corp., Milford, MA). Separation was performed using a Phenomenex Aqua 5 μm C18 (250 x 4.6 mm) column (Torrance, CA) with a binary gradient of 2% acetic acid for mobile phase A and 0.5% acetic acid in water/acetonitrile (1:1 v/v) for mobile phase B at a flow rate of 1.0 mL/min. A linear gradient was run from 10 to 55% B (0-50 min), from 55 to 100% B (50-60 min), and from 100 to 10% B (60-65 min). The ellagitannins and flavonols were identified on the basis of comparison of HPLC retention times to our previous HPLC results obtained using the identical HPLC conditions and LC-MS analysis (Hager, Howard, Liyange, Lay, & Prior, 2008; Hager, Prior, & Howard, 2010). The ellagitannin peaks were quantified at 255 nm as ellagic acid equivalents using external calibration curves of ellagic acid, with results expressed as milligram ellagic acid equivalents per 100 g of fresh berry weight. The flavonols

were quantified at 360 nm as rutin with results expressed as equivalents per 100 g of fresh berry weight.

HPLC analysis of anthocyanins. Sample extracts (3 mL) were dried using a Speed Vac concentrator (ThermoSavant, Holbrook, NY) and re-suspended in 2 mL of 3% formic acid. The anthocyanin analysis by HPLC was performed based on previous methods (Cho et al., 2004; Hager, Prior, & Howard, 2008) with a 250 × 4.6 mm Symmetry C18 column (Waters Corp., Milford, MA). The mobile phase consisted of a binary gradient of 5% formic acid (A) and 100% methanol (B). The flow rate was 1.0 mL/min with a linear gradient from 2 to 60% B over 60 min. The anthocyanin peaks were quantified at 510 nm using a photodiode array detector. All anthocyanins (cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, cyanidin 3-malonylglucoside, and cyanidin 3-dioxalylglucoside) were quantified as cyanidin 3-glucoside equivalents with total monomeric anthocyanins results expressed as milligrams per 100 g of original berry.

Total phenolics. Total phenolics were measured using the Folin-Ciocalteu assay (Slinkard & Singleton, 1977) with a gallic acid standard and a consistent standard curve based on serial dilutions. Absorbencies were measured at 760 nm, and results were expressed as gallic acid equivalents (GAE).

Oxygen Radical Absorbance Capacity (ORAC). ORAC values were determined on a dual pump BMG Fluostar Optima plate reader (Durham, NC). Results were generated by evaluating the area under the curve for test samples as compared to a Trolox standard and developing a standard curve based on dilutions (final concentrations 6.25, 12.5, 25, 50 μM) of Trolox. ORAC values were calculated using regression to TE concentration.

Design & analyses. The experiment was designed as a completely randomized design by harvest date. The composition attributes were evaluated with three replicated samples for each genotype. Analyses were conducted using JMP® (version 8.0; SAS Institute Inc., Cary, NC). Tukey's HSD (Honestly Significant Difference) was used for mean separation. Pearson's correlation was used to test the relationship between/within descriptive intensity scores and composition attributes.

Results and Discussion

The analyses of descriptive sensory attributes and the composition of blackberry genotypes from the UASDOA breeding program demonstrated significant variation among the genotypes. This information is useful for both release of cultivars and for future breeding decisions.

Descriptive Sensory Analysis

The descriptive characteristics of 20 blackberry genotypes for overall color intensity, flavor intensity, sweetness, sourness, and overall seediness varied significantly (Table 1). The visual attributes, scores for overall color intensity ranged from 6.8 (APF-156) to 8.3 (A-2316 and A-2434). The scores were highest for those genotypes that had the blackest color at evaluation; ratings were less for those that had red color on berries at evaluation.

Table 1
The scored descriptive sensory attributes (appearance, flavor, and texture) for blackberry genotypes evaluated on a 10-point scale (0=less of the attribute and 9=more of the attribute in terms of intensity), Clarksville, AR 2012.

Genotype	Overall color intensity	Overall flavor intensity	Sweetness	Sourness	Overall seediness
A-2108	7.9 abc[z]	6.4 a	5.6 ab	3.4 bcd	6.4 a
A-2215	7.9 abc	5.5 ab	6.5 a	2.5 d	4.8 a
A-2312 (Stella)	8.1 a	6.5 a	5.3 ab	3.0 cd	4.8 a
A-2316	8.3 a	5.0 ab	4.5 ab	6.0 abc	6.3 a
A-2416	7.1 abc	5.3 ab	6.1 ab	5.3 abcd	4.4 a
A-2418	7.6 abc	5.1 ab	5.4 ab	5.0 abcd	7.3 a
A-2419	7.3 abc	4.9 ab	4.1 ab	7.0 a	5.5 a
A-2434	8.3 a	5.8 ab	5.6 ab	4.4 abcd	5.9 a
A-2473	7.4 abc	5.4 ab	5.5 ab	4.0 abcd	6.3 a
APF-156	6.8 c	3.8 b	2.8 b	6.3 abc	5.8 a
APF-185	7.3 abc	5.1 ab	6.0 ab	3.5 bcd	5.9 a
APF-190	7.6 abc	4.5 ab	5.6 ab	3.3 cd	5.0 a
APF-205	6.9 bc	4.8 ab	4.3 ab	4.6 abcd	4.8 a
APF-227	7.5 abc	5.9 ab	3.8 ab	6.6 ab	5.4 a
Natchez	7.4 abc	5.1 ab	5.3 ab	4.6 abcd	6.8 a
Navaho	7.4 abc	5.1 ab	3.9 ab	5.9 abc	6.3 a
Osage	8.0 ab	4.4 ab	4.8 ab	3.9 abcd	5.0 a
Ouachita	7.6 abc	5.3 ab	4.5 ab	5.0 abcd	5.6 a
Prime-Ark® 45	7.9 abc	6.0 ab	4.8 ab	4.5 abcd	6.6 a
Tupy	7.6 abc	4.3 ab	3.5 ab	5.3 abcd	4.4 a

[z] Genotypes were evaluated in duplicate (n=2) by four trained panelists. Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD.

Less variation between panelist's intensity ratings indicated that the flavor attributes for sweetness and sourness were more easily and repeatedly determined by the panelists (data not shown). In terms of flavor attributes, A-2312 ('Stella') (6.5) had the highest score for overall flavor intensity and APF-156 (3.8) the lowest score. Flavor intensity for A-2312 ('Stella') and A-2108 was scored significantly higher than APF-156 but not different among other genotypes. For the sweetness attribute, A-2215 (6.5) was scored highest and significantly different from APF-

156 (2.8), which was scored lowest. Selections A-2419 (7.0) and APF-227 (6.6) were scored highest for perceived sourness and A-2215 (2.5), A-2312 ('Stella') (3.0), and APF-190 (3.3) were scored lowest. Sourness scores for A-2419 and APF-227 were significantly higher than A-2215, A-2312 ('Stella'), and APF-190.

For the texture attributes, A-2418 (7.3), 'Natchez' (6.8), and 'Prime-Ark® 45' (6.6) had the highest scores for overall seediness (presence of seediness most notable) and 'Tupy' (4.4) and A-2416 (4.4) had the lowest. Selections APF-205, A-2312 ('Stella'), and A-2215 were scored 4.8, the second lowest. Genotypes were not significantly different for overall seediness.

Composition Analysis

Basic composition. Because flavor is affected by soluble solids content and acidity parameters (Clark et al., 2007), these factors were evaluated. Selection A-2215 (13.0%) and 'Osage' (12.4%) had the highest and APF-156 (7.8%) the lowest soluble solids content, respectively (Table 2). An ideal soluble solids content for blackberry is 10% or higher (Clark et al., 2007); it is noteworthy that only five genotypes had soluble solids contents below 10%. Juice pH ranged from 3.0 (APF-227) to 4.0 ('Osage'). Titratable acidity ranged from 4.3 g/L ('Osage') to 15.5 g/L (APF-227), a four-fold difference. Wang, Galleta, and Maas (1997) reported that a soluble solids/titratable acidity ratio of 10 was the preferred flavor experience for strawberry, and that this could be obtained with high levels of soluble solids and titratable acidity or moderate soluble solids content and low titratable acidity levels (Lewers, Wang, & Vinyard, 2010). Assuming that the findings of Wang et al. (1997) for strawberries are similar for blackberries, a soluble solids/titratable acidity ratio of 10 or greater would improve flavor perception (Lewers et al., 2010). Fourteen of the 22 genotypes evaluated had soluble solids/titratable ratios of 10 or greater. 'Osage' (30.2), A-2252 (22.9), and A-2215 (20.9) had the highest ratios and APF-156

had the lowest ratio (5.7). The measurements for soluble solids, titratable acidity and the soluble solids/titratable acidity ratios are valuable in the assessment of a selection in consideration for public release.

Table 2
Composition of blackberry genotypes, Clarksville, AR

Genotype	Soluble solids (%)	pH	Titratable acidity (g/L) [z]	Soluble solids/ titratable acidity ratio[y]
A-2108	11.4 abc[x]	3.4 bcd	8.8 abcdef	13.3 bcd
A-2215	13.0 a	3.7 ab	6.3 def	20.9 abc
A-2252	11.7 ab	3.7 abc	5.6 ef	22.9 ab
A-2312 (Stella)	11.7 ab	3.5 abcd	6.7 cdef	18.5 abcd
A-2316	9.7 abc	3.1 d	14.4 ab	6.8 d
A-2416	11.3 abc	3.2 bcd	10.8 abcdef	10.9 bcd
A-2418	9.9 abc	3.1 d	13.8 ab	7.6 d
A-2419	10.3 abc	3.2 bcd	12.3 abcde	9.2 cd
A-2434	10.8 abc	3.4 bcd	8.8 abcdef	12.7 bcd
A-2473	10.9 abc	3.4 bcd	11.5 abcde	10.5 bcd
APF-156	7.8 c	3.1 cd	13.9 ab	5.7 d
APF-185	11.4 abc	3.3 bcd	8.9 abcdef	13.0 bcd
APF-190	9.7 abc	3.6 abcd	5.9 def	17.3 abcd
APF-205	9.2 bc	3.3 bcd	10.2 abcdef	9.1 cd
APF-227	11.7 ab	3.0 d	15.5 a	7.7 d
Apache	11.7 ab	3.2 bcd	12.5 abcd	9.7 bcd
Natchez	11.1 abc	3.3 bcd	9.0 abcdef	12.5 bcd
Navaho	11.5 ab	3.2 bcd	13.2 abc	8.9 cd
Osage	12.4 ab	4.0 a	4.3 f	30.2 a
Ouachita	11.5 ab	3.3 bcd	8.7 bcdef	14.5 bcd
Prime-Ark® 45	12.0 ab	3.4 bcd	8.5 bcdef	14.3 bcd
Tupy	12.0 ab	3.4 bcd	11.3 abcde	11.1 bcd

[z] Expressed as g-equivalents of citric acid.
[y] Calculated as soluble solids content/[titratable acidity (g/L)/10].
[x] Genotypes were evaluated in triplicate (n=3). Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD.

Berry attributes. The size and shape of the berries varied by genotype (Table 3). Average berry weight among genotypes varied from 5.1 g (A-2252) to 9.6 g (A-2434). Estimated berry volume ranged from 2395 mm^3 (A-2252) to 4532 mm^3 (A-2108).

Table 3
Berry and pyrene attributes of blackberry genotypes, Clarksville, AR, 2012.

Genotype	Berry weight (g)	Berry volume (mm^3)[z]	Pyrenes/ berry	Pyrene weight (mg)/berry	Pyrene weight/ berry weight (%)	Pyrene volume (mm^3)[y]
A-2108	8.6 abcd[x]	4532 a	74 defghi	324 cdef	3.8 bcdef	8.7 abcde
A-2215	7.6 abcdef	3614 abc	67 fghi	226 fgh	3.0 ef	8.1 bcdefg
A-2252	5.1 f	2395 c	61 ghi	179 gh	3.6 cdef	7.7 bcdefg
A-2312 (Stella)	8.0 abcde	3474 abc	92 cdef	330 cde	4.2 abcdef	8.9 abcd
A-2316	6.2 cdef	3066 abc	88 cdef	257 defgh	4.1 abcdef	7.0 defg
A-2416	6.5 cdef	3168 abc	73 efghi	183 gh	2.8 f	6.2 g
A-2418	7.5 abcdef	3453 abc	91 cdef	356 bcd	4.8 abc	9.4 abc
A-2419	7.8 abcdef	4027 abc	99 bcd	271 defg	3.5 cdef	6.5 fg
A-2434	9.6 a	4457 ab	110 abc	452 ab	4.7 abcd	10.7 a
A-2473	6.9 abcdef	3306 abc	94 cde	317 cdef	4.6 abcd	7.6 cdefg
APF-156	8.8 abc	4310 ab	125 a	380 bc	4.3 abcde	7.7 bcdefg
APF-185	6.8 bcdef	3532 abc	70 efghi	245 efgh	3.6 cdef	8.7 abcde
APF-190	8.5 abcd	3788 abc	84 defg	276 defg	3.3 def	8.4 bcdef
APF-205	7.7 abcdef	3635 abc	122 ab	313 cdef	4.1 abcdef	6.7 efg
APF-227	7.0 abcdef	3352 abc	91 cdef	301 cdef	4.3 abcde	8.5 bcdef
Apache	7.2 abcdef	3944 abc	74 defghi	298 cdef	4.1 abcdef	9.7 ab
Natchez	9.4 ab	4253 ab	131 a	491 a	5.2 ab	8.9 abcd
Navaho	5.3 ef	2804 bc	53 i	179 gh	3.4 cdef	8.0 bcdefg
Osage	6.9 abcdef	3725 abc	73 efghi	273 defg	4.1 abcdef	8.4 bcdef
Ouachita	6.3 cdef	3155 abc	78 defgh	259 defgh	4.1 abcdef	7.2 defg
Prime-Ark® 45	5.8 def	2768 bc	85 cdefg	318 cdef	5.4 a	8.1 bcdefg
Tupy	6.0 def	2846 abc	53 hi	160 h	2.7 f	8.1 bcdefg

[z] Volume calculated as a cone.
[y] Volume calculated as length x width x height.
[x] Genotypes were evaluated in triplicate (n=3). Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD.

Pyrene attributes. 'Natchez' contained the greatest number of pyrenes (131/berry) and 'Navaho' and 'Tupy' the least (53/berry) (Table 3). Because small pyrene size (< 3 mg) is preferred in both fresh-market and processed blackberry products (Moore et al., 1975), this was assessed. Genotypes that had an individual pyrene weight of 3.0 mg or less included 'Tupy' (3.0 mg), APF-156 (3.0 mg), A-2416 (2.5 mg), APF-205 (2.6 mg), A-2252 (3.0 mg), A-2316 (2.9 mg), and A-2419 (2.7 mg). Genotypes A-2434, A-2108, and 'Apache' had individual pyrene weights of 4.0 mg or higher. Pyrene weight/berry varied from 160 mg ('Tupy') to 491 mg ('Natchez'). Darrow and Sherwood (1931) found the proportion of pyrene weight/berry weight more important than pyrene size. APF-190 had, on average, 84 pyrenes/berry, a pyrene volume of 8.4 mm^3, and a ratio of 3.3% whereas 'Prime-Ark® 45' had a significantly higher ratio of 5.4% and 85 pyrenes/berry that had an individual volume of 8.1 mm^3. Of all genotypes, 'Prime-Ark® 45' (5.4%) and 'Natchez' (5.2%) had the highest values for pyrene weight per berry to berry weight while 'Tupy' (2.7%) and A-2416 (2.8%) had the least. Even though some genotypes had a high pyrene weight per berry, these did not necessarily have the most pyrenes/berry. For example, 'Natchez', 'Prime-Ark® 45', and A-2416 had 131, 85, and 73 pyrenes/berry, respectively. Pyrene volume ranged from 6.2 mm^3 (A-2416) to 10.7 mm^3 (A-2434) (Table 3).

The images of individual pyrenes from the genotypes evaluated (Figure 1) were visually placed into three groups as classified by Wada and Reed (2010) as straight, slightly concave, and convex, based on the shape of the raphe, the lower edge of the pyrene. The following genotypes were classified as straight: A-2316, A-2418, 'Apache', APF-205, 'Osage', 'Ouachita', and 'Prime-Ark® 45'. Selections A-2416 and APF-156 were slightly concave. Selections A-2215, A-2312 ('Stella'), A-2419, A-2473, APF-185, and APF-190 were straight to slightly convex.

Selections A-2108, A-2252, A-2434, APF-227, 'Natchez', 'Navaho', and 'Tupy' were convex. Wada and colleagues (2010) found that blackberry pyrene shape and structure can be used to identify the genotype and that the pyrene shape, with respect to the lower edge, for 'Navaho' and 'Tupy' was convex. 'Natchez' was classified as straight to slightly convex and slightly convex to convex, 'Ouachita' as straight, and 'PrimeArk® 45' as straight to slightly convex (Bruce & Perkins-Veazie, 2012).

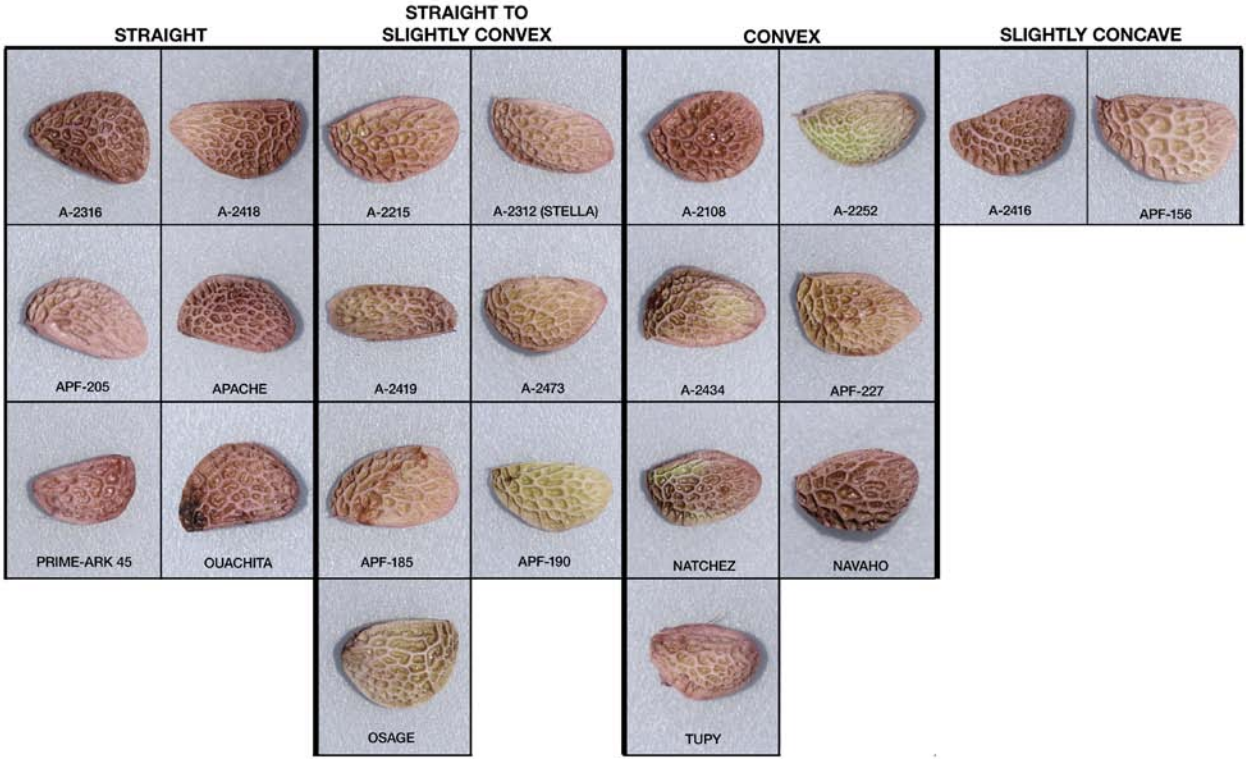

Figure 1. Images of individual pyrenes from blackberry genotypes, Clarksville, AR, 2012.
[z] Based on the shape of the raphe, the lower edge, pyrenes were visually classified into three groups: straight, slightly concave, and convex as categorized by Wada and Reed (2010). Pyrene sizes in the images are not to scale relative to each other.

Nutraceutical analysis. 'Natchez' (81.8 mg ellagic acid eqv/100 g) had the highest total ellagitannins and 'Tupy' (26.1 mg ellagic acid eqv/100 g) the lowest (Table 4). Total flavonols ranged from 9.5 mg rutin eqv/100 g ('Ouachita') to 36.8 mg rutin eqv/100 g (APF-227). Total anthocyanins ranged from 145 mg acy/100 g (A-2473) to 373 mg acy/100 g ('Apache'). Lewers

et al. (2010) reported total anthocyanins that ranged from 141 to 432 mg of cyanidin-3-glucodside eqv/100 g. Selections A-2316 (1200 mg gallic acid eqv/100 g), APF-156 (1178 mg gallic acid eqv/100 g), and 'Natchez' (1086 mg gallic acid eqv/100 g) had the highest levels of total phenolics and 'Ouachita' (515 mg gallic acid eqv/100 g) the lowest compared with values ranging from 114 to 1056 mg gallic acid eqv/100 g reported by Howard and Hager (2007). The ORAC ranged from 63.9 µmol Trolox eqv/g (APF-205) to 213.2 µmol Trolox eqv/g ('Prime-Ark® 45'). ORAC means reported by Moyer, Hummer, Finn, Frei, and Wrolstad (2002) and Lewers et al. (2010) for blackberries ranged from 13 to 146 µmol Trolox eqv/g and from 27 to 88 µmol Trolox eqv/g, respectively. 'Prime-Ark® 45' and 'Natchez' had strikingly higher ORAC values that were significantly higher than all other genotypes in this study. Among all genotypes, levels for total anthocyanins, total phenolics, total flavonols, total ellagitannins, and ORAC were among the highest for 'Natchez' compared to other tested genotypes.

Table 4
Nutraceutical attributes (based on fresh weight) for blackberry genotypes, Clarksville, AR, 2012.

Genotype	Total ellagitannins (mg ellagic acid eqv/100 g)	Total flavonols (mg rutin eqv/100 g)	Total anthocyanins (mg acy/100 g)	Total phenolics (mg gallic acid eqv/100 g)	ORAC[z] (µmol Trolox eqv/ g)
A-2108	48.4 bcdefgh[y]	15.7 efgh	343 abc	642 cdef	88.5 b
A-2215	32.9 fgh	11.9 gh	339 abc	635 cdef	83.4 b
A-2252	45.9 cdefgh	17.9 cdefgh	286 abcdef	894 abcdef	84.9 b
A-2312 (Stella)	34.1 defgh	26.3 abcde	181 fgh	662 cdef	71.0 b
A-2316	60.3 abcd	21.9 cdefg	267 abcdefgh	1200 a	90.3 b
A-2416	55.1 bcdef	20.8 cdefg	229 cdefgh	1029 abc	92.5 b
A-2418	59.6 abcde	28.2 abc	263 abcdefgh	982 abcd	102.1 b
A-2419	40.3 cdefgh	16.2 defgh	207 efgh	665 cdef	83.1 b
A-2434	52.9 bcdefg	33.5 ab	329 abcde	814 abcdef	78.4 b
A-2473	39.0 cdefgh	15.1 fgh	145 h	659 cdef	77.6 b
APF-156	38.7 cdefgh	13.5 fgh	177 fgh	1178 a	80.8 b
APF-185	38.3 cdefgh	13.5 fgh	279 abcdefg	933 abcde	92.3 b
APF-190	33.7 efgh	17.2 cdefgh	157 gh	525 ef	70.2 b
APF-205	30.0 fgh	12.6 gh	180 fgh	602 def	63.9 b
APF-227	74.7 ab	36.8 a	155 gh	839 abcdef	74.2 b
Apache	40.9 cdefgh	27.1 abcd	373 a	891 abcdef	90.6 b
Natchez	81.8 a	36.6 a	359 ab	1086 ab	202.1 a
Navaho	41.3 cdefgh	18.7 cdefgh	301 abcdef	955 abcd	82.3 b
Osage	36.4 defgh	27.8 abc	333 abcd	662 cdef	86.3 b
Ouachita	28.6 gh	9.5 h	208 defgh	515 f	70.7 b
Prime-Ark® 45	64.4 abc	24.0 bcdef	210 defgh	909 abcdef	213.2 a
Tupy	26.1 h	22.8 bcdefg	239 bcdefgh	714 bcdef	68.3 b

[z] Oxygen radical absorbance capacity=ORAC.
[y] Genotypes were evaluated in triplicate (n=3). Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD.

Correlations within and between descriptive sensory analysis and composition attributes. Since there were many attributes in this study that were significantly correlated, only selected correlations are reported. Correlations are shown within each category of data (descriptive, basic composition, berry and pyrene attributes, and nutraceutical) and between data from the different categories. As expected, there are significant correlations within each

category. For all genotypes, positive and negative correlations were significant at r=0.98–0.70 (p<0.001), r= 0.69-0.56 (p<0.01), and r= 0.55-0.45 (p<0.05).

Flavor intensity was positively correlated to sweetness (r=0.47). Sweetness was negatively correlated to sourness (r=-0.74). Soluble solids content was positively correlated to pH (r=0.50) and soluble solids/titratable acidity ratio (r=0.60), but negatively correlated to titratable acidity (r=-0.46). Berry pH was positively correlated to soluble solids/titratable acidity ratio (r=0.96) and negatively correlated to titratable acidity (r=-0.91). Titratable acidity was negatively correlated to soluble solids/titratable acidity ratio (r=-0.90).

Berry weight was positively correlated to berry volume (r=0.93), pyrenes/berry (r=0.70), and pyrene weight/berry (r=0.78). Berry volume was positively correlated to pyrenes/berry (r=0.58), and pyrene weight/berry (r=0.67). Pyrenes/berry was positively correlated to pyrene weight/berry (r=0.84) and pyrene weight/berry weight ratio (r=0.63); it was negatively correlated to soluble solids (r=-0.64). Pyrene weight/berry was positively correlated to pyrene weight/berry weight ratio (r= 0.80).

Total ellagitannins was positively correlated to total flavonols (r=0.72), total phenolics (r=0.62), and ORAC (r=0.66).

For relations between descriptive and composition attributes, pyrene weight/berry was positively correlated to descriptive-evaluated overall seediness (r=0.51). Pyrene weight/berry weight ratio was also positively correlated to total ellagitannins (r=0.58), ORAC (r=0.60), and descriptive-evaluated overall seediness (r=0.70); this correlation describes the importance of pyrenes for health-promoting factors along with sensory perception of “seediness”. Berry pH was negatively correlated to total phenolics (r=-0.57) and sourness (r=-0.75). Titratable acidity was positively correlated to total phenolics (r=0.57), and sourness (r=0.82). Soluble solids

content/titratable acidity ratio was negatively correlated to total phenolics (r=-0.51) and sourness (r=-0.68), which is expected because high levels of phenolics provide a bitter taste and enhanced flavor is dependent upon low titratable acidity and perceived sourness. Total ellagitannins (r=0.57) and ORAC (r=0.54) were positively correlated to overall seediness.

Conclusion

The findings determined by this study on fresh blackberries may have the potential to provide much needed data for fruit breeders. Characteristics of fresh blackberry fruits used in this investigation varied between genotypes, some of which are likely to be improved. A finding from this study revealed that even though the sensory scores for overall seediness varied, there was a positive correlation between overall seediness and pyrene weight/berry weight ratio. The correlations within and between sensory analysis, composition attributes, and nutraceutical attributes generated by this study provide a source of information on fresh blackberries and can contribute to genotype improvement efforts at the UASDOA.

References

Bruce, M., & Perkins-Veazie, P. (2012, July). *Physical characteristics of pyrenes as a means to identify blackberry and raspberry cultivars.* Paper presented at the Annual Conference of the American Society for Horticultural Science, Miami, FL.

Cho, M. J., Howard, L. R., Prior, R. L., & Clark, J. R. (2004). Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry, and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture, 84*(13), 1771-1782. doi:10.1002/jsfa.1885

Clark, J. R. (1999). The blackberry breeding program at the University of Arkansas: Thirty-plus years of progress and developments for the future. *Acta Horticulturae, 505*, 73-77.

Clark, J. R. (2005). Changing times for eastern United States blackberries. *HortTechnology, 15*(3), 491-494.

Clark, J. R., & Finn, C. E. (2008). New trends in blackberry breeding. *Acta Horticulture, 777*, 41-47.

Clark, J. R., Howard, L. R., & Talcott, S. (2002). Antioxidant activity of blackberry genotypes. *Acta Horticulturae, 585*, 475-480.

Clark, J. R., Stafne, E. T., Hall, H. K., & Finn, C. E. (2007). Blackberry breeding and genetics. *Plant Breeding Reviews, 29*, 19-144.

Connor, A. M., Finn, C. E., & Alspach, P. A. (2005). Genotypic and environmental variation in antioxidant activity and total phenolic content among blackberry and hybridberry cultivars. *Journal of the American Society for Horticultural Science, 130*(4), 527-533.

Darrow, G. M., & Sherwood, H. (1931). Seed and berry size of cane fruits. *Proceedings of the American Society for Horticultural Science, 28*, 194-199.

Finn, C. E., & Clark, J. R. (2012). Blackberry. In: Badenes, M. L. & Byrne, D. H., (Eds.), *Handbook of Plant Breeding: Volume 8: Fruit breeding.* (pp. 151-190). New York, NY: Springer.

Hager, T. J., Howard, L. R., Liyange, R., Lay, J. O., & Prior, L. R. (2008). Ellagitannin composition of blackberry as determined by HPLCESI-MS and MALDI-TOF-MS. *Journal of Agricultural and Food Chemistry, 56*(3), 661-669. doi:10.1021/jf071990b

Hager T., Prior, R., & Howard, L. (2008). Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blackberry products. *Journal of Agricultural and Food Chemistry, 56*(3), 689-695. doi:10.1021/jf071994g

Hager T., Prior, R., & Howard, L. (2010). Processing and storage effects on the ellagitannin composition of processed blackberry products. *Journal of Agricultural and Food Chemistry, 58*(22), 11749-11754. doi:10.1021/jf102964b

Hall, H. K., Stephens, M. J., Stanley, C. J., Finn, C. E., & Yorgey, B. (2002). Breeding new 'Boysen' and 'Marion' cultivars. *Acta Horticulture, 585*, 91-95.

Howard, L. R., & Hager, T. J. (2007). Berry fruit phytochemicals. In Y. Zhao (ed.), *Berry fruit: Value-added products for health promotion* (pp. 73-104). Boca Raton, FL: CRC Press.

Lewers, K. S., Wang, S. Y., & Vinyard, B. T. (2010). Evaluation of blackberry cultivars and breeding selections for fruit quality traits and flowering and fruiting dates. *Crop Science, 50*(6), 2475-2491. doi:10.2135/cropsci2010.02.0097

Liu, R. H. (2003). Health benefits of fruits and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition, 78*(3), 517S-520S.

Moore, J. N., Brown, G. R., & Brown, E. D. (1974). Relationships between fruit size and seed number and size in blackberries. *Fruit Varieties Journal, 28*, 40-50.

Moore, J. N., Lundergan, C., & Brown, E. D. (1975). Inheritance of seed size in blackberry. *Journal of the American Society for Horticultural Science, 100*(4), 377-379.

Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., & Wrolstad, R. E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium, rubus* and *ribes. Journal of Agricultural and Food Chemistry, 50*(3), 519-525.

Narayana, K. R., Reddy, M. S., Chaluvadi, M. R., & Krishna, D. R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacology, 33*, 2-16.

Ness, A. R., & Poulens, J. W. (1997). Fruit and vegetables, and cardiovascular disease: A review. *International Journal of Epidemiology, 26*, 1-13.

Prior, R., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., … Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry, 46*(7), 2686-2693.

Reyes-Carmona, J., Yousef, R. A., Martínez-Peniche, G. G., & Lila, M. A. (2005). Antioxidant capacity of fruit extracts of blackberry (*Rubus* sp.) produced in different climatic regions. *Journal of Food Science, 70*(7), 497-503. doi:10.1111/j.1365-2621.2005.tb11498.x

Sariburun E., Şahin, S., Demir, C., Türkben, C., & Uylaşer, V. (2010). Phenolic content and antioxidant activity of raspberry and blackberry cultivars. *Journal of Food Science, 75*(4), 328-335. doi:10.1111/j.1750-3841.2010.01571.x

Seeram, N. P. (2008). Berry fruits: Compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *Journal of Agricultural and Food Chemistry, 56*(3), 627-629.

Seeram, N. P., Adams, L. S., Zhang, Y., Lee, R., Sand, D., Scheuller, H. S., & Heber, D. (2006). Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro. Journal of Agricultural and Food Chemistry, 54*(25), 9329-9339.

Sellappan S., Akoh, C. C., & Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry, 50*(8), 2432-2438.

Siriwoharn, T., Wrolstad, R. E., Finn, C. E., & Pereira, C. B. (2004). Influence of cultivar, maturity, and sampling on blackberry [Rubus L. hybrids] anthocyanins, polyphenolics, and antioxidant properites. *Journal of Agricultural and Food Chemistry, 52*(26), 8021-8030.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*(1), 49-55.

Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruit, and cancer prevention: A review. *Journal of the American Dietetic Association, 96*(10), 1027-2039.

Strik, B. C., Clark, J. R., Finn, C. E., & Bañados, M. P. (2007). Worldwide blackberry production. *HortTechnology, 17*(2), 205-213.

Takeda, F. (1993). Characterization of blackberry pyrenes [Abstract]. *HortScience, 28*, 488.

Tomlik-Wyremblewska, A., Zieliński, J., & Guzicka, M. (2010). Morphology and anatomy of blackberry pyrenes (*Rubus* L., Rosaceae) elementary studies of the European representatives of the genus *Rubus* L. *Flora, 205*(6), 370-375.

Van der Sluis, A. A., Dekker, M., De Jager, A., & Jongen, W. M. F. (2001). Activity and concentration of polyphenolic actioxidants in apple: Effects of cultivar, harvest year, and storage conditions. *Journal of Agricultural and Food Chemistry, 49*(8), 3606-3613.

Wada, S., Nonogaki, H., & Reed, B. M. (2010). Identifying blackberry cultivars by seed structure (#EM9002). Retrieved from Oregon State University Extension Publications Online Catalog: http://hdl.handle.net/1957/16304.

Wada, S., & Reed, B. M. (2010). Seed coat morphology differentiates blackberry cultivars. *Journal of American Pomological Society, 64*(3), 152-161.

Wang, S. Y., Galletta, G. J., & Maas, J. L. (1997). Chemical characterization of 24 strawberry cultivars and selections. *Journal of Small Fruit and Viticulture, 5*(1), 21-36. doi:10.1300/J065v05n01_02

Wang, W. D., Xu, S. Y., & Jin, M. K. (2009). Effects of different maceration enzymes on yield, clarity and anthocyanin and other polyphenol contents in blackberry juice. *International Journal of Food Science and Technology, 44*, 2342-2349. doi:10.1111/j.1365-2621.2007.01637.x

Zheng, W., & Wang, S.Y. (2003). Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and Food Chemistry, 51*(2), 502-509.