QUANTIFICATION OF QUALITY ATTRIBUTES, FUNCTIONAL COMPOUNDS, AND ANTIOXIDANT CAPACITY OF BLACKBERRY AND BLACKBERRY WINES

By

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Bachelor of Science

New Mexico State University

Las cruces, NM

2007

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May 2010

QUANTIFICATION OF QUALITY ATTRIBUTES, FUNCTIONAL COMPOUNDS, AND ANTIOXIDANT CAPACITY OF BLACKBERRY AND BLACKBERRY WINES

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ACKNOWLEDGMENTS

I would like to say a great thank you to my advisor, Dr. William McGlynn, for his immense support, kind advice, and help. He always provided a great deal of support and encouragement throughout my research. I also say thank you to my committee members Dr. Eric Stafne and Dr. Danielle Bellmer for their help, guidance and support related to my research as well as my studies. Special thanks are dedicated to Dr. Mark Payton for providing me guidance and support in statistical analysis. Furthermore, I would like to say thank you to the Oklahoma State University Cimarron Valley Research Station in Perkins, Oklahoma and the University of Arkansas Fruit Substation in Clarksville, Arkansas for providing blackberry cultivars for the research. In addition, I would like to thank Terry Toomy at the Toomey's Thornless Blackberry Farm in Broken Arrow, and Sue Gray, OSU Tulsa County Cooperative Extension Oklahoma, as well as Kendra Woodburn and John Ridgeway at the Fruit Research Station in Clarksville, Arkansas for collecting blackberries and providing samples for research. I would like to express my appreciation to the members of my lab group, especially Darren Scott, Richelle Stafne, and Yannis Oikonomakos for their help, support and friendship. Especially I would like to say thank you to Richelle Stafne for providing and sharing her sensory evaluation data for 2008-harvest blackberries. I also appreciate the Food and Agricultural Products Centers and Department of Horticulture and Landscape Architecture for providing facilities related to my research project. I would also like to thank the North American Bramble Growers Research Foundation for partially funding my project. Finally, I would like to thank my parents, my brothers, and other family members and friends for all of their support and encouragement.

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CHAPTER I

INTRODUCTION

High intake of fruits and vegetables has been shown to prevent degenerative diseases such as coronary heart disease and cancer (Wang and Lin 2000; Siriwoharn and others 2004; Cho and others 2005; Jiao and others 2005; Reyes-Carmona and others 2005; Dai and others 2007; Wang and Xu 2007; Acosta-Montoya and others 2010). Since the 1990s, experts have come to recommend an increase in the consumption of fruits and vegetables to five or more daily servings in order to provide a desirable intake of antioxidants and improve human health (Garcia-Alonso and others 2003). Consuming different colored fruits and vegetables may serve to enrich the diet with health-enhancing compounds that act synergistically to provide enhanced protection from oxidative stress (Hunter and others 2008).There is a need to identify and quantify these important compounds in fruits and vegetables. Within the same fruit species, the growing season, variety, environmental and climatic conditions, plant disease stress, soil type, geographic location, processing storage condition, and maturity seem to influence the concentration of phenolic compounds (Sellappan and others 2002; Garcia-Alonso and others 2003).

Antioxidant activity is defined as the ability to reduce free radical formation and scavenge reactive oxygen species (Reyes-Carmona and others 2005). Usually, molecules that function as antioxidants can donate single electrons or hydrogen atoms to quench

free radicals. High levels of free radicals in the human body are known to increase electrophilic reactions, which can lead to oxidative changes to lipids, proteins, and nucleic acids in the body (Deighton and others 2000). Plant-derived antioxidants function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, and enzyme inhibitor and synergists (Wang and Lin 2000). Antioxidant capacity methods differ in their way of generating free radicals, the strategy to measure the end point of the inhibition reaction, and the sensitivity towards the different reducing molecules in the sample (Vasco and others 2008). Different cultivars grown in the same region or season consistently show difference in antioxidant capacity. Antioxidant capacity varies with cultivar (genotypes), growing temperature, growing season, maturity at harvest and environmental stress (Reyes-Carmona and others 2005).

Though we seldom think of blackberries as medicinal these days, consumption of berry fruits has attracted interest lately because they are high in antioxidants that help to reduce oxidative stress. Blackberries are a good source of antioxidants due to their high concentration of phenolic compounds. The phenolic compounds found in blackberries have been linked to a reduced risk of degenerative diseases such as cardiovascular disease and cancer (Reyes-Carmona and others 2005).

Phenolic compounds are considered to be non-nutrient, biologically active compounds. The functionality of these compounds is expressed through their action as an inhibitor or an activator for a large variety of mammalian enzyme systems and as metal chelators and scavengers of free oxygen radicals. Among the phenolic substances, flavonoids, particularly anthocyanins, are of interest because of their high occurrence in foods such as fruits and vegetables (Sellappan and others 2002; Garcia-Alonso and others

2003). Anthocyanin and total phenolic level have shown substantial variation among genotypes and among years due to genetic differences among genotypes and environmental and genetic variation for those traits (Clark and Talcott 2002).

There are positive correlation between antioxidant capacity and phenolics. Phytochemicals responsible for the antioxidant capacity in berries are most likely to be phenolic acids, anthocyanins and other flavonoid compounds (Reyes-Carmona and others 2005). Phenolic content variation is due to differences in variety, climate, ripeness, and extraction method. Antioxidant capacity may be underestimated comparing fruit samples and individual compounds because there are interactions with other phenolic compounds or other compounds that can happen in complex mixture such as fruit extracts and do not occur in pure compounds (Vasco and others 2008). Total phenolics have been generally overestimated because the measurement has included non-phenolic compounds such as sugars or ascorbic acid which interfere in the Folin-Ciocalteu reaction (Mertz and others 2007).

There is a good deal of research on the health benefits of blackberries related to phenolics and anthocyanin content but not much research on the health benefits of different cultivars. This research is focused on two different cultivars, Apache and Ouachita, grown in both Arkansas and Oklahoma.

Specific Study Objectives

1. Investigate two cultivars developed and suited for cultivation in the Midwest region in order to determine if one or another cultivar has relatively higher quality and antioxidant capacity both as whole berries and as wine.

2. Determine if two growing locations – central Arkansas and eastern Oklahoma – have an influence on the quality and antioxidant capacity of whole berries and wine.
3. Measure antioxidant capacity of blackberries at different stage of processing.
4. Investigate consumer's preference for one or another cultivar and/or growing location and to assess the impact of winemaking technique on wine quality and antioxidant capacity.

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CHAPTER II

REVIEW OF LITERATURE

History of blackberry

The Blackberry, genus *Rubus*, subgenus *Rubus*, is a member of one of the most diverse genera of plants. The genus *Rubus* is comprised of no fewer than twelve subgenera, of which four produce edible fruit. Asia is a major center of diversity for *Rubus* (Deighton and others 2000), which suggests that Asia may be where the genus evolved. In addition to being prized as a food, blackberries have traditionally been used for medicinal purposes as well. Blackberries were used in 16th-century Europe as a medicinal plant to treat infections of mouth and eyes (Dai and others 2007).

Types of blackberries

Blackberries have three different types based on the cane architecture: erect, semi-erect and trailing. Erect type blackberries produce primocanes from buds at the base of floricanes at the crown or from buds on roots. The semi-erect type or trailing type of blackberries produces new primocanes from buds on the crown. Generally primocanes are vegetative in the first year and fruit the second year on the entire length of the floricane.

In the world, 50% of the cultivars are semi-erect type, and 25% erect and trailing types (Strik and others 2007).

The fruiting season for blackberries is usually: mid May to August for erect type, July to October for semi-erect type and late June to August for trailing type (Strik and others 2007). Erect types of blackberries are large clustered and produce large, sweet fruit, and are the most winter hardy. Semi-erect types of blackberries are vigorous, have large fruit clusters, show high productivity and are late ripening. The trailing type of blackberries are large roundish to elongated, wine-colored to black fruit of distinctive flavor, and early ripening. Thornless cultivars have originated as mutation of thorny types (Moore and Skirvin 1990) but also as a result of breeding.

Blackberries are used for freezing, canning, jams, jellies, syrups, bakery filling, ice cream topping and other foods. Erect and semi-erect types of blackberries are suitable for fresh market because they have firmer texture and relatively longer shelf life than trailing blackberries. Trailing type blackberries are often used for processing since these have a distinctive flavor, aroma, smaller seeds, and softer texture than that of two other types (Moore and Skirvin 1990).

Blackberry harvesting

Blackberries can be picked as frequently as every second or third day. When blackberries are harvested by hand, the ideal time is in the early morning after the dew has evaporated since the temperature is cool and the berries are easy to remove. However, berries should never be picked when wet with dew or rain since moisture will facilitate

the growth of spoilage microorganisms. On the other hands, when berries are harvested by machine, night time is the best as the fruits are more easily removed and harvesting at night maximizes uniformity in fruit maturity as well as fruit aroma, flavor and percent soluble solids. Harvested berries should be stored in the shade. Field heat should be removed by rapid cooling in order to extend the shelf life of the fruit (Moore and Skirvin 1990; Strik and others 2007).

The hand picking method relies on fruit color which can be a poor ripeness indicator. Black color develops in blackberries before they are fully mature, possibly resulting in the harvest of sour, unripe fruit. Mechanical harvesters have been developed to rapidly harvest erect-type blackberries for processing. Machines selectively harvest higher quality fruit than hand pickers because machines utilize a shaking principle that removes only fully ripe fruit. Machine harvested berries tended to be larger and had a higher percentage of total soluble solids, lower acidity, and superior color than hand-harvested berries in a study by Halat and others (1997). In this study, machine harvested fruit were rated superior to hand harvested fruit for berry wholeness, flavor and color.

One of the major problems associated with the production of blackberries is cold injury, which hinders the expansion of blackberry production in northern part of the United States. To prevent this, ‘tunnels’ are sometimes used to protect against adverse weather; these tunnels also help to protect the crop from insects and some diseases; this may lead to higher yields and better shelf life. Another common production problem is sunburn injury to the fruit. This injury causes some white drupelets to form on the fruit, which render the fruit worthless for most market (Strik and others 2007).

Worldwide production of blackberries

In 2005, 20,035 ha^2 of blackberries were cultivated worldwide, which yielded 14,292 Mg^2 of blackberry production. Europe had the largest area planted (7,692 ha^2; 38.4%) followed by North America (7159 ha^2; 35.7%), Central America (1,640 ha^2; 8.2%), South America (1,597 ha^2; 8.0%), and Asia (1,550 ha^2; 7.7%). Serbia (5,300 ha^2, 69% of European production) had the largest area of given over to blackberry cultivation not only in Europe but also in the world. The worldwide production of blackberries showed that the North America produced the highest amount of 59,123 Mg^2 (42.1%), followed by Europe 43,000 Mg^2 (30.7%), Asia 26,350 Mg^2 (18.8%), and South America 6,380 Mg^2 (4.5%). Compared to the cultivation area, Europe showed the second highest number. North America, primarily the United States of America, possessed the highest production of blackberries in the world. Oregon and California produces large amount of blackberries in the United States. Oregon produced 65% of the production of trailing types of blackberries i.e. 'Marion' blackberries (61%). Asia ranked third in the worldwide production of blackberries. China produced most of the berries and semi-erect types are grown widely there (Strik and others 2007).

Blackberry cultivars: Ouachita and Apache

Ouachita and Apache are the two major cultivars for this research. Both cultivars are erect thornless types of blackberries. These cultivars were developed at the University of Arkansas Agricultural Experiment Station Fruit Substation at Clarksville, Arkansas by

John R Clark and James N Moore in 1990 for Ouachita and 1988 for Apache. Both cultivars have Navaho as a parent. The common characteristics of these cultivars are early ripening and high yield of fruit i.e. five to seven pounds per plant. There are some characteristics in which they differ.

The origin of ‘Ouachita’ cultivar was from a hand pollinated cross of Navaho and Arkansas selection 1506 (A-1506). The advantage of the new cultivar is that had more erect-canes than that of their parents. In addition, the new cultivar was earlier ripening and produced a larger fruit size than the parent of ‘Navaho’. Also, it produced more fruit than the parent A-1506 cultivar. The ripening time (mid-season starts from mid June) is one week earlier than that of Navaho. The fruit is large (6-7g) and slightly larger (1-1.5g) than that of Navaho. This fruit is as firm as the parents of Navaho so it allows for a relatively long storage period after harvest. Ouachita berries are relatively higher in soluble solids (10-12%) than other cultivars (8-10%). The high soluble solid contents are likely inherited from ‘Navaho’. However, being high in soluble solids may at times be a drawback as berries with very low acidity can have a flat flavor (Clark and Finn 2008). The cold hardiness is up to -17^{o}C. The usage of this berry is mainly for fresh consumption (Clark and others 2006).

The origin of the Apache cultivar was from a hand pollinated cross of ‘Navaho’ and Arkansas Selection 1007 (A-1007). The advantage of the new cultivar was earlier ripening, better fruit firmness, greater vigor, and better fruit flavor than that of A-1007. The ripening time for Apache starts five days later than that of ‘Navaho’; it also has a 10 day shorter ripening period which means more concentrated fruit ripening. The ‘Apache’ berries are also well adapted in different climate region as well as soil types. ‘Apache’

had relatively bigger seeds than ‘Ouachita’ cultivar. Furthermore, this cultivar has large-sized fruit (8- 10g) – about double the size of Navaho. Larger is thought to be better but it may present difficulties in certain instances, such as fully filling clamshell containers to make the desired net product weight (Clark and Finn 2008). The fruit is slightly less firm than that of ‘Navaho’ so it has relatively less storage time. The soluble solid content tends to be slightly less than ‘Ouachita’ as well as ‘Navaho’. The cold hardiness is -20°C. The usage of ‘Apache’ is for both fresh fruit and processed products (Clark and others 2006).

Blackberry quality attributes

Blackberry quality attributes have been shown to vary by region as well as species and cultivar. In a study by Reyes-Carmona and others (2005), relatively low soluble solids contents were recorded for all the Mexican genotypes compared with North pacific region. Oregon grown blackberries had relatively high soluble solids levels, presumably because these genotypes are adapted to photoperiods in the summer that are much longer due to high latitudes than those from in Mexico in any season. Longer photoperiod leads to higher soluble solids (Reyes-Carmona and others 2005).

Titratable acidity decreases significantly during ripening while total soluble solids increase significantly during ripening. Tropical highland blackberries exhibit higher acidity and much lower soluble solid than do blackberry cultivars grown in temperate climates (Acosta-Montoya 2010).

Blackberry sensory attributes

The perceived taste of blackberries is heavily influenced by sugar and organic acid content; organic acid content is reflected in the pH value of the berry tissue (Thomas and others 2005). Higher total soluble solid contents are normally associated with preferred fruit flavor/taste. Fruit flavor and taste is also dependent on a balance between sugars and acids present in the ripe fruit. Thus, high total soluble solids and a correspondingly high titratable acidity typically corresponds to a sweet, better tasting fruit (Thomas and others 2005).

A high concentration of anthocyanins and acidity is desirable in fruit juices to achieve and maintain a desirably red-purple color. Thus, a high level of acidity is considered desirable in blackberries for juice production (Halat and others 1997).

Phenolics in balckberries

Berry fruits contain high levels of phenolic compounds such as hydroxybenzoic acid and hydrozycinnamic acid derivatives, anthocyanins, flavonols, catechins and tannins (Garcia-Alonso and others 2003). The major phenolics found in blackberries are ellagitannins and anthocyanins. Ellagitannin derived from ellagic acid, belong to hydrolysable tannin class of phenolics contain one or more hexa-hydroxydiphenic acid moieties, esterified to a polxol most often to β-D glucose. Ellagitannins such as lambertianin C and sanguiin H-6 are the main types of phenolics found in blackberries, more than 92 % in red fruit to almost 61% in fully ripe fruit. Ellagitannin contents in

tropic highland blackberry are high when compare with other blackberry cultivars such as Marion and Evergreen grown in template climates. Although Elllagitannins decrease steadily during ripening, tropical highland blackberry (*R. adenotrichus*) contains more ellagitannin than pomegranate even though pomegranate is commercially known for this particular property. The tropical highland blackberry fruit presents the highest levels of ellagitannin found in any edible fruit.

Blackberry total phenolic contents significantly decreases as the fruit matures from the green to ripe stage. That trend is reversed as blackberries begin to senesce; blackberry total phenolics increase from ripe to over-ripe stages. Evergreen berries were slightly higher than those of Marion during under-ripe and ripe stage (Siriwoharn and others 2004). During storage, the phenolic composition varied significantly depending on the temperature and time of storage. Total ellagic acid increased during storage but increase was lower in refrigerated sample at 8°C (Garcia-Alons and others 2003).

The polyphenolic compounds of blackberry seeds have not been characterized; they may represent an untapped potential source of nutraceuticals and natural antioxidants. For example, in a study by Siriwoharn and Wrolstad (2004) total phenolics contents and antioxidant activity as measured by ORAC for seeds were about twice higher than for whole berries. For seeds total anthocyanins and total phenolics were 14 times higher in Marion than Evergreen.

Anthocyanins and ripeness stages

As noted above, the principle types of phenolics found in blackberries are flavonoids, especially anthocyanins. Anthocyanins are water soluble pigments that exist in blackberries and produce red, blue or purple colors (Hassimotto and others 2008). Blackberries contain relatively higher amounts of anthocyanins than other berry fruits (Dai and others 2009). Because the drupelet skin is quite thin and the flesh is very dark in blackberries, larger berry sizes appear to have little effect on anthocyanin contents (Moyer and others 2002). While total phenolic contents tend to decrease during ripening, total anthocyanin pigments and antioxidant capacity tend to increase (Acosta-Montoya and others 2010). As anthocyanins increase with ripening, the hue value of the fruit significantly decreases, indicating darker surface color for fruits. Glycosides and acylglycosides of anthocyanin may be important as potential replacements for synthetic food colorants, and in human nutrition as protecting agent against some diseases (Acosta-Montoya 2010).

Total anthocyanin contents for whole berries was about 15 times higher than that of seeds. Marion berries were approximately 1.5 times higher than Evergreen in total anthocyanins (Siriwoharn and Wrolstad 2004).The greatest losses in total anthocyanins were observed during the first three months of storage. It showed that there is a very sharp decrease stored at high temperature of 30 ℃ so maintain low temperature from 8℃ to 21℃ is important (Garcia-Alonso and others 2003).

Health benefits of anthocyanins

Flavonoids such as anthocyanin, proanthocyanidin, flavonones, and flavonols have a protective effect in the human body (Moyer and others 2002). Proanthocyanidins have occasionally been found in whole berries whereas it has never been detected in berries without seeds (Siriwoharn and Wrolstad 2004). Flavonoids are believed to protect against free radical damage and low density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilatation of arteries. Some other health benefits of consuming fruits and vegetables possibly linked to flavonoids are the following: reduced blood pressure, improved immune system functions, detoxification of contaminants or pollutants, reduced inflammation (Wang and Lin 2000). Aside from general antioxicant activity, antiviral and other antimicrobial effects have also been observed (Sellappan and others 2002;Reyes-Carmona and others 2005).

In a study by Patras and others (2009) blackberry purees' greater retention of anthocyanins was noted as compared to thermally treated purees and total anti-radical power of high pressure treated samples (400-600 MPa) were significantly higher than in fresh and thermally processed samples. High pressure processing at moderate temperatures can maintain nutritional quality of purees and could be used for commercial product.

Anthocyanins are regarded as important antioxidant in berry fruits, but in humans the bioavailability of dietary anthocyanins is low. Bioavailability differs greatly between the various polyphenols, and the most abundant polyphenols are not necessarily those that have the best bioavailability profile, either because they have a lower intrinsic

activity or because they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated (Pantelidis and others 2007).

Factors influencing phenolics and anthocyanins contents in blackberries

Biosynthesis of natural products is influenced by location, weather, and harvest period and etc (Ou and others 2002). Wild blackberries have shown the highest total phenolics and anthocyanins contents, presumably due to greater exposure of the unsheltered wild plant to extremes of temperature, and stress from pests and disease (Acosta-Montoya and others 2010). Phenolic synthesis works as defense mechanism. For example, wild varieties grown extensively in tropical highlands have characteristic of high acidity and a distinctive flavor. Tropical climate's stressful environmental changes such as drought during the dry season, high relative humidity during the rainy season, high solar irradiation levels, extreme temperatures, and attack by insects and pathogens could enhance antioxidant production as plants use them to detoxify free radicals (Acosta-Montoya and others 2010). Different growing regions and genotypes exhibit significant differences in phenolics and anthocyanin contents (Reyes-Carmona and others 2005).

In the study by Dai and others (2009), total phenolic contents and anthocyanin contents were comparable between cultivars of the same harvest year. A decrease in cyanidin-3-glucoside was observed with increased storage temperature. Temperature and time contributed to the loss of anthocyanins, total phenolics and antioxidant activity while the effect of light was insignificant. Elevation of pH, and/or temperature

accelerated anthocyanin degradation. Total anthocyanins decreased much more rapidly than total phenolics in biological buffers.

Role of Antioxidants

Antioxidants play an important role in the body's defense system against reactive oxygen species (ROS), which are harmful by-products generated during normal cell aerobic respiration (Ou and others 2002). Reactive oxygen species such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen($^1O_2$) can damage proteins, lipids, enzymes, DNA and RNA (Wang and Jiao 2000). This can lead to cell or tissue injury associated with degenerative diseases and potentially disrupts cell functions and cause genetic mutations. Antioxidants are important for preventing reactive oxygen or nitrogen species from causing aging and pathogenesis (Ou and others 2001). The hydroxyl radical is the most reactive free radical and can be generated by superoxide anion and hydrogen peroxide reacting in the presence of metal ions. Nitrite occurring in fruits or vegetables can also be transformed to nitrite by reduction reactions with the action of bacteria in human bodies. Nitrites may transform into nitrosamine which is a procarcinogenic substances (Jiao and others 2005).

Antioxidants are substances that delay the oxidation process by inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Thomas and others 2005; Cespedes and others 2008). Antioxidant activity can be divided into two types. Primary antioxidants act to scavenge ROS or to break the chain in the generation of ROS. Secondary antioxidants act by deactivating metal ions, e.g. by

chelation, inhibiting the breakdown of lipid hydroperoxides to volatile products, regenerating 'primary' antioxidants or quenching singlet oxygen (Hunter and others 2008). Dietary phenolics, such as those found in blackberries, are multifunctional antioxidants either acting as reducing reagents, hydrogen donating antioxidants, and/or single oxygen free-radical quenchers.

Flavonoids and antioxidant activity in blackberries

Studies examining the relationship between chemical structure and functional activity in phenolic antioxidants indicate that a loose relationship exists between the numbers of free, aromatic –OH groups and antioxidant activity (Deighton and others 2000). Flavonoid antioxidant capacity is related mainly to structural characteristics such as the presence of an o-diphenyl group in the B ring, the presence of a 3-hydroxyl group connected to a double bond between C_2-C_3 and adjacent to a 4-oxo function in the C ring, and the hydroxylation pattern, mainly in C_5 and C_7 in the A ring (Hassimotto and others 2008). The absence or replacement of some of these structural characteristics reduces or inhibits antioxidant capacity (Hassimotto and others 2008).

As noted above, anthocyanins, the glycoside form of the anthocyanidins are potential antioxidants in blackberries (Deighton and others 2000; Hassimotto and others 2008). An important factor associated with antioxidant capacity is the surface area and surface-to-volume ratio of the berries. A small berry with high ratio would be expected to process a relatively high concentration of anthocyanins and flavonoids on a per weight basis and hence possess high antioxidant potential (Deighton and others 2000).

Blackberries have been observed to have higher hydrogen peroxide scavenging capacity as compared to strawberries after both 7and 14 days of storage (Chanjirakul and others 2007). Blackberry have also been shown to have high antioxidant capacity against superoxide radical scavenging, peroxy radical scavenging and hydroxyl radical scavenging (Wang and Jiao 2000).

Oxygen Radical Absorbance Capacity Assay

The oxygen radical absorbance capacity (ORAC) assay measures the antioxidant capacity, which is influenced by degree of pigmentation and anthocyanin contents (Clark and Talcott 2002). ORAC is a measure of total antioxidant capacity of a food including vitamin C, phenolics, anthocyanins and polyphenols (Perkins-Veazie and Kalt 2002). The ORAC assay has been widely applied to the assessment of free radical scavenging capacity of human plasma, proteins, DNA, pure antioxidant compounds and antioxidant plant or food extracts. ORAC values are influenced by genotypes, cultivars, and maturity stages (Elisia and others 2007).

The Oxygen radical absorbance capacity (ORAC) value reflects the peroxy radicals scavenging activity induced by 2-2`-azobis (2-aminopropane) dihydrochloride (AAPH) at 37°C. The protective effect of an antioxidant is measured by assessing the area under the fluorescence decay curve (AUC) of the sample as compared to that of Trolox – a water-soluble derivative of Vitamin E. The ORAC assay provide a unique and complete assessment in which the inhibition time and inhibition degree are measured as the reaction goes to completion. The $ORAC_{FL}$ assay provides a direct measure of

hydrophilic antioxidant activity against peroxyl radicals. Fluorescein (FL, PKa=6.4) is a synthetic compound with high quantum yield of fluorescence at pH greater than 7.0 and long wavelengths (492/515 nm, exitaton/emission). Since FL is pH sensitive, sample always need to be diluted greatly with phosphate buffer at about pH7.0 or higher before analysis (Ou and others 2001).

Mechanism of the ORAC assay

The basic antioxidant mechanism has two categories: preventive antioxidant, and chain-breaking. Preventive antioxidants inhibit or retard the formation of free radicals from their unstable precursors. Examples of preventive antioxidants include superoxide dismutase, catalase, peroxidase, and tansferrin; all of these inhibit the formation of ROS. Chain breaking antioxidants interrupt the radical chain reaction. Formation of a delocalized stable radical does not continue the chain reaction or else continues it with low efficiency. With chain-breaking antioxidants there are two different kinds of transfers: single electron transfer (SET) and hydrogen atom transfer (HAT). The SET mechanism is strongly solvent dependent due to solvent stabilization of the charged species. The HAT mechanism is based on the ability of antioxidant to donate a hydrogen atom to the oxygen radical, resulting in the formation of a stable antioxidant radical. Chain breaking antioxidants donate a labile hydrogen atom to a peroxyl radical much more rapidly than the peroxyl radical reacts with substrate. The resulting species is stable and is not able to continue the autoxidation of the chain (Ou and others 2002).

To compare with other antioxidant capacity assays, the $ORAC_{FL}$ assay utilizes a radical initiator to create a peroxyl radical and the peroxyl radical abstracts a hydrogen atom from a donor molecule, preferably from an antioxidant molecule. As a result, the reaction between the peroxyl radical and the indicator fluorescent probe may be inhibited (Ou and others 2002). So, ORAC directly measures the antioxidant activity of chain-breaking antioxidants against peroxyl radicals (Hunter and others 2008).

The Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) assays are different in that a single electron is transferred from the antioxidant molecule to the pro-oxidant. Hence, these assays do not measure chain-breaking antioxidant activity or preventive antioxidant activity (Ou and others 2002). The TEAC method involves the activation of metmyoglobin with hydrogen peroxide in the presence of $ABTS^{+\cdot}$ to produce the radical cation. The FRAP method is based on the chemical reaction whereby Fe (II) interacts with hydrogen peroxide to produce hydroxyl radicals, the most harmful reactive oxygen species (Hunter and others 2008). Both the TEAC and the FRAP methods assume that the redox reaction will proceed so fast that all reactions are complete within a short period of time. These two methods measure only the reducing capability of an antioxidant in relation to Fe (III) and $ABTS^{+\cdot}$ which may not be relevant to overall antioxidant activity under a variety of conditions (Ou and others 2001; Mermelstein 2008).

ORAC values related to blackberry maturity and storage

Studies have shown that different maturity stages of blackberries had an effect on the antioxidant activity of the fruit (Siriwoharn and others 2004). Green berries had the highest ORAC values and pink berries had the lowest. In both Marion and Evergreen blackberries, the under-ripe stage had the lowest ORAC values (Siriwoharn and others 2004). In another study, berries harvested later had slightly higher ORAC values than berries harvested early in the season (Perkins-Veazie and Kalt 2002). Total anthocyanin contents increased after storage at temperatures above 5°C. On the other hand, ORAC tended to decrease with storage, while total phenolics increased or stayed the same (Perkins-Veazie and Kalt 2002).

Different laboratory methods of extraction and analysis may contribute to the variance seen in reported levels of anthocyanins, phenolics, and antioxidants (Moyer and others 2002). Genetic and environmental factors, such as cultivars, maturity, UV light exposure, and harvesting method, play an important role in berry composition. The level of phenolics and the antioxidant capacity of blackberries are influenced by maturity and there is pronounced variation among cultivars (Siriwoharn and others 2004).

Correlation between total phenolics, total anthocyanins, and ORAC

ORAC values have been highly correlated with total phenolics and anthocyanins (Stintzing and others 2002; Cespedes and others 2008). ORAC values and anthocyanin contents showed no correlation at the green berry stage but did show correlations at the pink to ripe berry stages (Wang and Lin, 2000). The correlation between total phenolics

and ORAC values has been observed to be slightly higher than between anthocyanins and ORAC values (Moyer and others 2002).

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CHAPTER III

WHOLE BLACKBERRY AND BLACKBERRY JUICE

ABSTRACT

Blackberry (*Rubus* sp.) is a good source of antioxidants and contains high amounts of phenolic compounds, mainly anthocyanins. Anthocyanins, a part of the flavonoid family, are common pigments in many berry fruits and have been studied for their health benefits. Two blackberry cultivars (Apache and Ouachita) that are commonly grown in the Midwest were harvested from two locations, one in Oklahoma and one in Arkansas. Assays were conducted to determine total phenolics and anthocyanin contents. Oxygen Radical Absorbance Capacity (ORAC) assays were also conducted in order to measure antioxidant activity. Qualitative tests such as pH, titratable acidity, soluble solids were conducted to measure the quality of blackberry fruit. Sensory tests were also performed in order to evaluate subjective impressions of quality. Whole blackberries had the highest concentrations of total phenolics and anthocyanins, as well as the highest ORAC values. Overall, Apache berries had higher concentrations of phenolics, anthocyanins and higher ORAC values than Ouachita. In addition, blackberries harvested in 2009 had higher concentrations of phenolics, anthocyanins and ORAC values than those harvested in 2008 blackberries.

INTRODUCTION

Phenolic compounds are considered non-nutrient biologically active compounds. Berry fruits are high in phenolic compounds such as hydroxybenzoic acid and hydrozycinnamic acid derivatives, anthocynins, flavonols, catechins and tannins. Blackberries 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 and others 2008; Hassimotto and others 2008). Blackberry contains high amounts of cyanidin-3-glycosides (Fan-Chiang and Wrolstad 2005; Elisia and others 2007). Various anthocyanin-containing extracts from plants and fruits have been shown to reduce oxidative stress-associated degenerative diseases, inflammatory diseases and cancer (Wang and Lin 2000; Siriwoharn and others 2004; Cho and others 2005; Jiao and others 2005; Reyes-Carmona and others 2005; Dai and others 2007; Wang and Xu 2007; Tiwari and others 2008; Dai and others 2009; Acosta-Montoya and others 2010).

Anthocyanins are water-soluble glycosides of polyhydroxyl and polymethoxyl derivatives of 2-phenylbenzopyrylium. Anthocyanins exist at low pH as a flavylium cation which is their naturally occurring form. The flavylium cation is highly electron deficient, which leads to its potent reactivity with free radicals and oxygen-reactive species. Anthocyanins have been observed to have higher antioxidant activity than vitamin E, ascorbic acid, and β-carotene (Dai and others 2007). The strong antioxidant activity of anthocyanins is attributed to free radical scavenging properties associated with the hydroxyl groups attached to the molecules phenolic ring structure. The antioxidant efficiency of anthocyanins in preventing oxidation of human low density lipoprotein has

been shown to be influenced by the number of hydroxyls on the B ring of the anthocyanin molecule (Wang and Lin 2000; Elisia and others 2007).

Blackberry extract and concentrate has been widely used as a natural colorant in beverages, baked products, chewing gums, jellies, purees, and fruit wine making (Jiao and others 2005; Hager and others 2008). Blackberry juice concentrate is used in beverage and syrup formations for natural colorant applications and in nutraceutical preparations (Fan-Chiang and Wrolstad 2005). Anthocyanins are very stable under acidic condition: but under normal processing and storage conditions readily convert to colorless derivatives and subsequently to insoluble brown pigment (Dai and others 2009). The loss of color and increased browning during the production and/or storage of processed foods may be influenced by maillard browning, enzymatic browning, ascorbic acid degradation, and the polymerization of anthocyanins with other phenolics (Garcia-Alonso and others 2003). Some other factors that cause color changes are pH, light, oxygen, heat, sulfur dioxide or sulfate salt, metal ions, and co-pigments (Tiwari and others 2008). To prevent color changes, heat treatment in used to help increase the shelf life of the foods (Fan-Chiang and Wrolstad 2005). Blackberry juice by-product is a potential source of nutraceutical ingredient (Hager and others 2008).

There is a need to identify and quantify some of important antioxidant compounds in fruits and vegetables. Within the same fruit type, the growing season, cultivar, environmental and climatic conditions, plant disease, soil type, geographic locations, processing storage condition, and maturity seems to influence the concentration of phenolic compounds (Garcia-Alonso and others 2003). This research is focused on two different cultivars, Apache and Ouachita, grown in both Arkansas and Oklahoma.

MATERIALS AND METHODS

Berry collection

The two blackberry cultivars, Apache and Ouachita, were collected from two different locations in Arkansas and Oklahoma. The Arkansas blackberries were collected from the University of Arkansas Fruit Research Substation in Clarksville, Arkansas. The elevation at this location is 273 m (895 ft) above sea level and the average annual precipitation is 125.5 cm (49.4 in). Plants were spaced 61 cm (2 ft) apart in rows, row middles were spaced at 3.7 m (12 ft), and the plots were trellised. Harvest of blackberries began in May and berries were collected every Monday and Thursday. Berries were collected after they turned fully black. Berries were placed into polyethylene bags and frozen within one hour of harvest for subsequent storage.

Oklahoma-grown blackberries were collected from Toomey's Thornless Blackberry Farm in Broken Arrow, Oklahoma. The elevation at this location is 238m (780 ft) above sea level and the average annual precipitation is 107.7 cm (42.4 in). Plants were spaced 1.2 m (4 ft) apart in row. Harvest started in the third week of June and ended in third week of July. Berries were collected after they turned fully purplish black. Berries were placed into polyethylene bags and frozen within one hour of harvest for subsequent storage.

Berry Sample Storage, Handling, and Preparation

Sample storage

All blackberry samples were frozen and stored at -16°C (3°F) after collection and until further processing or analysis. For each blackberry cultivar and growing location, one 100g subsample (2008) and one 200g subsample (2009) of whole frozen berries were removed from the large storage bag and sealed into quartz-sized zip-lock polyethylene bags for subsequent preparation of whole-berry extracts.

General sample preparation.

Frozen blackberry samples were placed into a cooler at 4°C (39°F) and allowed to thaw for 24-48 hours prior to subsequent processing and analysis.

Preparation of whole berries for sensory analyses.

Blackberry samples were thawed as described above. A total of 120 blackberries was removed from frozen storage for each cultivar-growing location combination, sealed into separate polyethylene bags, and allowed to thaw in refrigerated storage at 4°C (39°F) for approximately 48 hours.

Preparation of whole berry extracts.

The whole berry extracts were shown in Figure 1. Berries were thawed as described above, then removed from the cooler and allowed to come to room

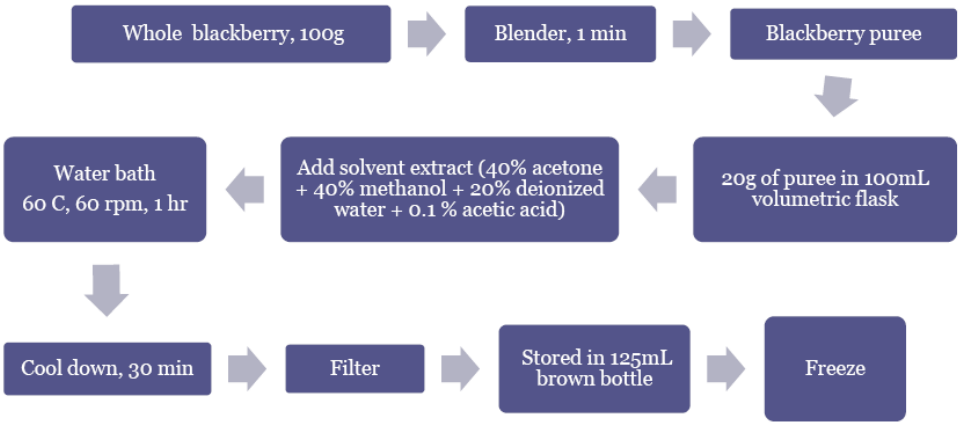
temperature (~2 hours). The contents of each blackberry sample bag were blended for a total time of about 1 minute with a Hamilton Beach Custom Grind Coffee Grinder (Hamilton Beach, Washington, NC, USA) using the "percolator" setting. Blackberry puree samples were then sealed into polyethylene bags for extraction. Samples not extracted the same day were placed in frozen storage at -15℃ (5℉) for subsequent extraction.

Prepared blackberry purees were extracted as follows 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, and 0.1% Acetic acid (Spectrum Quality Products, Gardena, CA, USA). All percentages were volume percentage.

Twenty grams of blackberry puree were weighed into a 100mL volumetric flask. The flask was filled to volume with the extraction solvent described above and the flask was mixed using a vortex agitator for about 10 to 20 seconds. The flask was then placed in a reciprocal shaking water bath model 50 (Precision Scientific, Winchester, VA, USA) and held at 60℃ for 1 hour with an agitation rate of 60 rpm. Each flask was mixed 3 to 4 times during the first 10 minutes of extraction to remove gas bubbles. After 1 hour in the water bath, the flask was removed and allowed to cool approximately 45 minutes. Cooled extracts were then filtered through a funnel lined with miracloth 1R filter paper (Calbiochem, La Jolla, CA, USA). Samples were filtered into 125 ml brown glass bottles. The bottles were capped, the caps were wrapped with plastic film, and the sealed bottles were placed in frozen storage at -15℃ (5℉) for subsequent analyses.

Two replicate extract samples were prepared from each puree sample.

Figure 1. Blackberry extract procedures



Preparation of blackberry juice samples from 2009 harvest.

Blackberry lots from each of the four (2x2) combinations of growing location and cultivar were removed from frozen storage and thawed in a cooler at 4°C (39°F) as described above. Berry samples were mixed every two hours for the first six hours of thawing to facilitate the process. When the berries were totally thawed, the weight of each lot was measured. Each cultivar-location combination lot of berries was then pressed using a 25 liter bladder press (Zambelli Enotech,Camisano Vicentino, Italy) to express the juice. Four samples of about 100 ml were removed from each lot, sealed in 125 ml brown glass bottles, and frozen for subsequent analyses.

Whole Berry Sensory Analysis

The blackberries were evaluated using affective (preference) testing. Evaluations were based on a 9-point hedonic scale with responses ranging from dislike extremely to

like extremely. The 9 points correlate to the panelists' degree of liking. The descriptors (see appendix) used for each point were: dislike extremely, dislike very much, dislike moderately, dislike slightly, neither like nor dislike, like slightly, like moderately, like very much and like extremely. A random three-digit number was assigned to each blackberry cultivar and growing location combination.

Samples were allowed to come to room temperature before serving. Four intact blackberries were presented to each panelist for each cultivar-growing location combination. The approximate amount of sample each panelist received was 30g for the Apache cultivar and 20g for the Ouachita cultivar. Each panelist received each combination of cultivar and growing location in random order. A total of 39 panelists participated in 2008 and 36 in 2009. The panelists were untrained. The panelists were instructed to clean their palettes between samples by drinking water.

Juice and Berry Puree Quality Analyses

Soluble solids

Percent soluble solids were measured using a Leica Auto ABBE refractometer (Buffalo, NY, USA) with sample temperature compensation.

pH

The pH of the blackberry juice was measured using an Accumet AB 15 pH meter (Buffalo, NY, USA).

Titratable Acidity

A 5mL sample of blackberry juice was diluted to 105 ml using deionized water. The titratable acidity was then measured as % citric acid using a 809 Titrando automatic titrator (Metrohm ion analysis, Herisau, Switzerland). The sample was titrated with 0.1N NaOH to an endpoint of pH 8.2. The sample was agitated throughout measurement using a magnetic stir-bar. Two duplicate readings were taken from each berry puree and juice sample.

Juice and Whole-berry Extract Chemical Analyses

Total Phenolic Content

Total phenolics were measured using the method of Singleton and Rossi (1965). Briefly, 0.5 ml of prepared, filtered blackberry extract or blackberry juice was added to 1 ml of Folin-Ciocalteu reagent (Fluka Biochemika, Steinheim, Switzerland) and 5 ml of deionized water to in a 25 ml volumetric flask. The contents were mixed and allowed to stand for 5-10 minute at room temperature (~25℃). 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 hours. 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 per 100 g starting puree (GAE/100g). 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.

Total Monomeric Anthocyanin Contents

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 was added to a 25 ml volumetric flask. The flask was then brought to volume with pH 1 potassium phosphate buffer. One 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 minutes. Then the absorbance of each solution was measured at 520 nm and 700nm using a Beckman DU 520 (Brea, CA, USA) spectrophotometer. Deionized water was used as the blank. An overall absorbance value was calculated for each sample as follows:

$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5}$

Using this value, the monomeric anthocyanin pigment concentration in the original sample was calculated using the following formula:

Monomeric anthocyanin pigment (mg cyanidin-3-glucoside/100 ml juice or mg cyanidin-3-glucoside/100g whole berries) = (A * MW * [1/ε]*DF * 100)

Where:

- A is the absorbance.
- MW is the molecular weight of cyanidin-3-glucoside, 457.16 g.
- ε is molar extinction coefficient of cyanidin-3-glucoside, 29,600.

- DF is the dilution factor for the sample.
- 100 is a correction factor to convert to concentration of monomeric anthocyanin per 100 g of whole berries or 100 ml of juice.

All samples were measured in duplicate (berry extract) or triplicate (juice and unracked wine).

Juice and Whole-berry Extract Antioxidant Activity Analyses

Oxygen Radical Absorbance Capacity (ORAC) Assay

ORAC assays were conducted using the method first developed by Ou and others (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 485nm and an emission wavelength of 520nm. The microplate reader was controlled using HTSoft software 2.0. We used fluorescein disodium (Sigma-Aldrich, St-Louis, MO, USA) at a concentration of about 0.3 μM as a fluorescent probe and target of free radical attack, Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, (Fluka Chemika, Steinheim, Switzerland) at a concentration of 10μM as a standard antioxidant, and AAPH (2,2'-azobis[2-amidino-propane] dihydrochloride, Waco Chemicals Inc., Richmond, VA, USA) at a concentration of about 122 mM as a free peroxyl radical generator. All reagents were prepared in 75mM phosphate buffer, which was adjusted to final pH of 7.0 with HCl or NaOH as needed.

A 48-well clear polystyrene microplate (Falcon Plate 48-well, BD Falcon, San Jose, CA, USA) was used for ORAC readings. Briefly, each well was filled with 160

μL of fluorescein. For "blank" wells, 20 μL of phosphate buffer was added, followed by 20 μL of AAPH. For "standard" wells, 20 μL of Trolox was added, followed by 20 μL of AAPH. And for "sample" wells, 20 μL of either whole-blackberry extract or blackberry juice was added, followed by 20 μL of AAPH. 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 decay curve.

Prepared plates were placed into the microplate reader immediately after the AAPH was added and plate reading was initiated. The total run time for each assay was 70 minutes at 37^{o}C and the microplate reader was programmed to record fluorescence every two minutes. This gave a total of 35 reading cycles, which was adequate time to allow at least a 90% degradation of the fluorescein. 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 fi = 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) per gram of fresh blackberry tissue or blackberry juice.

Statistical Analysis

Statistical analyses were performed using SAS version 9.2 (SAS institution, Cary, NC, USA). For all analyses except for sensory evaluations, an analysis of variance for each set of data was conducted using a completely randomized design. The sensory evaluations were analyzed using a randomized block design wherein the blocking variable was panelist. Means were separated using least significant differences (LSD) with a 95% confidence interval ($p<0.05$).

RESULTS AND DISCUSSION

Sensory Analysis

Sensory analysis results are presented in Figures 2-5. Figure 2 compares the Ouachita berries from the two locations; Figure 3 compares the Apache berries from the two locations; Figure 4 compares the two cultivars grown in Arkansas; Figure 5 compares the two cultivars grown in Oklahoma. The blackberry sensory data from 2008 were previously reported by Stafne (2009). All data shown represent a two-year average. The most striking statistically significant differences noted are those between Ouachita berries grown in Arkansas and Oklahoma (Figure 2). Every attribute evaluated was more intense in the Apache berries. Differences were not seen in the Apache berries (Figure 3), in which Oklahoma-grown berries were rated as significantly more acidic than Arkansas-grown berries. Similarly, few significant differences were seen between

Apache and Ouachita berries grown in Arkansas (Figure 4); Ouachita berries were rated firmer and seedier than Apache berries. More differences were seen in the Oklahoma-grown berries (Figure 5); Apache was rated as being sweeter, more flavorful, and more acidic.

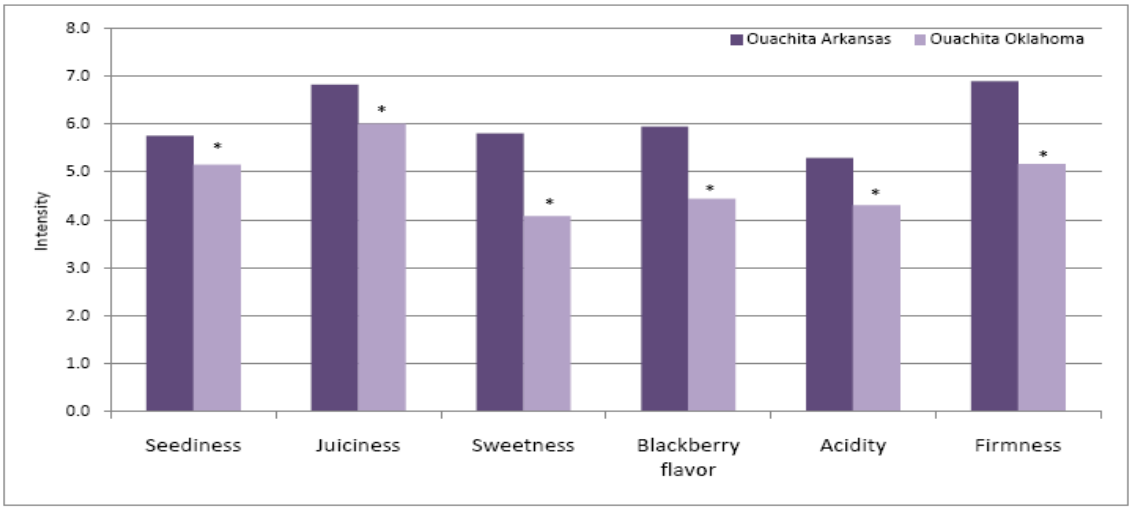


Figure 2. Sensory evaluation scores for 2008 and 2009 Ouachita blackberries. All six attributes are based on the mean value of n=39 (2008) and n=36 (2009).
* Arkansas-Ouachita was significantly different than Oklahoma-Ouachita for all attributes evaluated. ($p<0.05$).

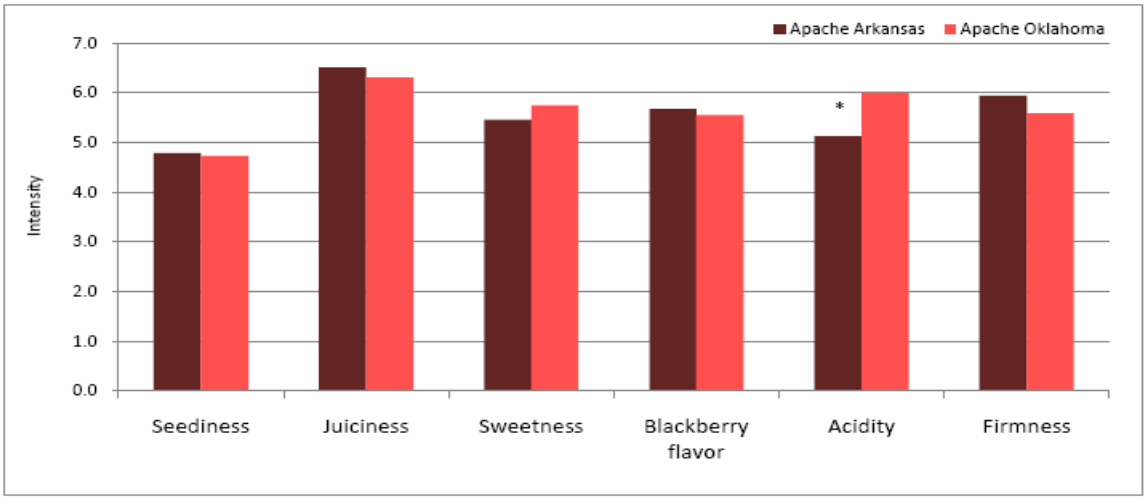


Figure 3. Sensory evaluation scores for 2008 and 2009 Apache blackberries. All six attributes are based on the mean value of n=39 (2008) and n=36 (2009).
* Arkansas-Apache was significantly different than Oklahoma-Apache in perceived acidity ($p<0.05$).

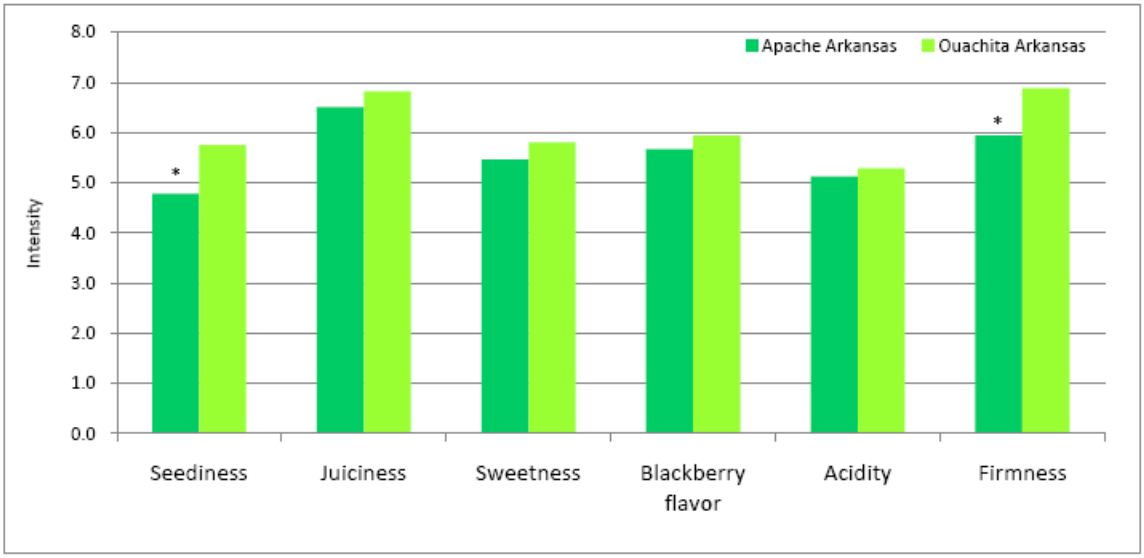


Figure 4. Sensory evaluation scores for 2008 and 2009 Arkansas blackberries. All six attributes are based on the mean value of n=39 (2008) and n=36 (2009).
* Arkansas-Apache was significantly different than Arkansas-Ouachita for seediness and juiciness ($p<0.05$).

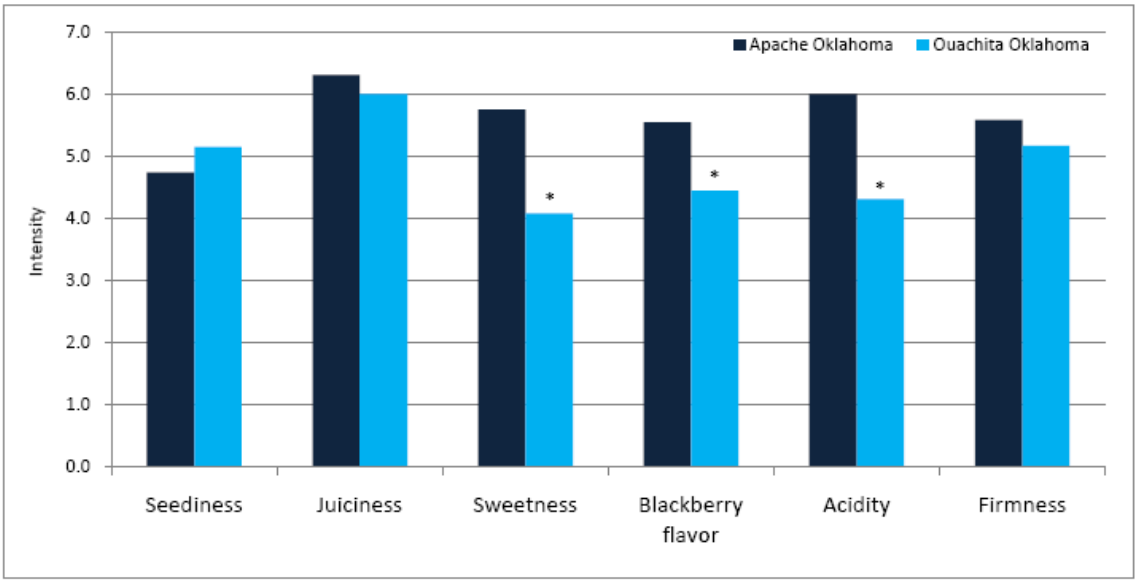


Figure 5. Sensory evaluation scores for 2008 and 2009 Oklahoma blackberries. All six attributes are based on mean values of n=39 (2008) and n=36 (2009).
*Oklahoma-Apache was significantly different than Oklahoma-Ouachita for sweetness, intensity of blackberry flavor, and acidity ($p<0.05$).

Quality Attributes

Soluble solids

Soluble solids values are presented in Table 1. Results showed higher soluble solids values than Wang and others (2008) but showed slightly lower value than Siriwoharn and others (2004). Soluble solid contents measures the sugar contents of the fruit. Usually soluble solids values are influenced by both environmental factors and farm management. Sugar content typically increases with milder weather conditions, especially dry and sunny conditions near the time of harvest. Sugar contents may also vary according to soil types. Farm management may influence sugar content by affecting degree of ripeness, disease control and others factors. As fruit ripens, the sugar content of the fruit increases. This produces a sweeter taste.

Overall, blackberries from 2008 showed slightly lower soluble solid values than blackberries from 2009. However, Oklahoma-Ouachita was an exception to that pattern at the sample from the year 2009 had much lower soluble solids content than that the sample from 2008. Between the two cultivars, Apache had slightly higher values than Ouachita for both years. Of the two locations, berries grown in Arkansas showed slightly higher values than those grown in Oklahoma for both years. However, no statistically significant differences were seen among soluble solid values when averaged over two years.

Table 1. Soluble solids content (°brix) of 2008 and 2009 blackberries.

Samples	2008 Juice	2009 Juice
Arkansas-Apache	10.15	11.20
Oklahoma-Apache	10.45	10.60
Arkansas-Ouachita	9.45	11.05
Oklahoma-Ouachita	9.50	7.35

1. n=2
Note: No significant differences ($p < 0.05$) were observed among the samples.

pH

Blackberry pH values are presented in Table 2. The pH values of blackberries harvested in 2008 were slightly lower than those harvested in 2009. Between the two cultivars, Ouachita had slightly higher pH values than Apache for both years. Comparing the two growing locations, blackberries grown in Oklahoma showed higher pH values than those grown in Arkansas for both years. However, no statistically significant differences were seen among pH values.

When fruits ripen, there is a relative increase in sugar decrease in acid. Blackberries from Oklahoma were harvested at a purplish black color, not a fully black color as the Arkansas berries were. This may explain the some of the differences in pH values that we observed. Also, the different soil types may also have had some influence.

Table 2. Mean[1] pH values of 2008 and 2009 blackberries.

Samples	2008 Juice	2009 Juice
Arkansas-Apache	3.09	3.26
Oklahoma-Apache	3.19	3.33
Arkansas-Ouachita	3.17	3.58
Oklahoma-Ouachita	3.37	3.70

1. n=2.
Note: No significant differences ($p < 0.05$) were observed among any of the samples.

Titratable acidity

Titratable acidity values are shown in Table 3. Results showed somewhat higher titratable acidities than reported by Wang and others (2008) and Siriwoharn and others (2004). Overall, blackberries from 2008 had higher acidity than blackberries from 2009. Between the two cultivars for both years, Ouachita showed higher titratable acidity values than Apache. Between the two growing locations, in 2008 the berries grown in Oklahoma showed higher titratable acidity values than the berries grown in Arkansas. The opposite was true, however, in 2009. Overall, no statistically significant differences were seen among titratable acidity values.

The titratable acidity of Oklahoma berries showed a substantially different pattern from year to year. Even though the ripening stages were the same between the two years, weather condition may have influenced the acidity of the fruit. The weather in Oklahoma was cooler, with more rainfall and less sunshine in 2009 than in 2008. Lack of sunshine or low temperature may reduce the sugar contents of fruit and lead to an increase in acidity.

Table 3. Mean[1] titratable acidity (g/L citric acid) of 2008 and 2009 blackberries.

Samples	2008 Juice	2009 Juice
Arkansas-Apache	13.5	11.3
Oklahoma-Apache	14.0	7.9
Arkansas-Ouachita	15.7	12.4
Oklahoma-Ouachita	17.3	10.8

1. n=2.
Note: No significant differences ($p < 0.05$) were observed among any of the samples.

Total Phenolic Contents

Whole blackberries

Whole blackberry total phenolic contents are shown in Table 4. Overall, our results yielded higher values for total phenolic content than some other studies (Deighton and others 2000; Moyer and others 2002; Sellappan and others 2002; Pantelidis and others 2007; Koca and others 2008; Wang and others 2008). However, we had slightly lower values for total phenolic contents than those reported for Evergreen and Marion blackberries (Siriwoharn and others 2004). One possible explanation for the differences we saw is that the management system in Oregon area may different than in the Midwest regions; this may have lead to relatively higher value of total phenolic contents for the Northwest fruit. Also, the different types of blackberries and the differing weather condition likely influenced the relative total phenolic contents.

Overall, whole blackberry extract from 2009 showed higher value for total phenolic contents than those from 2008. Between the two cultivars, both years showed that Apache had higher values for total phenolic contents than Ouachita. Comparing the two locations, blackberries grown in Oklahoma showed higher values for total phenolic

contents than blackberries grown in Arkansas for both years. However, no statistically significant differences were seen among total phenolics values when averaged over two years (Figure 6).

Part of the variability seen in the data shown in the graph may be due to sampling error. Since only a relatively small sample of berries was used to prepare our extracts, we may not have collected a sufficiently representative sample.

Table 4. Mean total phenolics[1], total anthocyanins[1] and oxygen radical absorbance capacity (ORAC) assay[2] values for 2008 and 2009 blackberries, unracked blackberry wine (2008) and blackberry juice (2009).

Samples	Mean[1] Total Phenolics mg gallic acid equivalent/L	Mean[1] Total Anthocyanins mg cyanidin-3-glucoside/ 100g puree	Mean[2] ORAC Values µmol Trolox equivalent/ g of fresh tissue
Whole berries 2008			
Arkansas-Apache	7290.8	450.00	184.69
Oklahoma-Apache	7219.8	515.25	185.91
Arkansas-Ouachita	5182.4	328.29	142.77
Oklahoma-Ouachita	6360.3	646.26	172.27
Whole berries 2009			
Arkansas-Apache	7496.5	615.14	189.68
Oklahoma-Apache	8069.5	485.13	208.91
Arkansas-Ouachita	6067.7	361.91	165.66
Oklahoma-Ouachita	8751.4	239.93	210.55
Unracked wine 2008			
Arkansas-Apache	2799.4	9.25	15.94
Oklahoma-Apache	1997.2	6.47	17.10
Arkansas-Ouachita	1909.7	5.66	12.02
Oklahoma-Ouachita	1772.3	3.76	12.70
Juice 2009			
Arkansas-Apache	2648.9	45.56	20.08
Oklahoma-Apache	2233.9	39.33	18.92
Arkansas-Ouachita	2651.2	35.74	17.47
Oklahoma-Ouachita	2652.8	36.85	21.10

1. n=2 (whole berries), n=3 (unracked wine and juice).
2. n=4 (all samples).

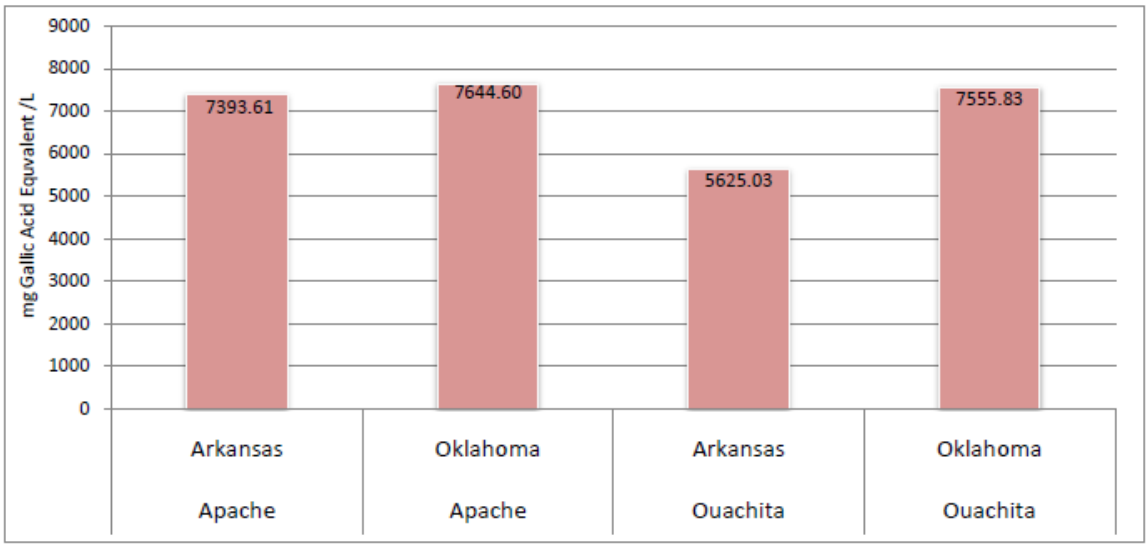


Figure 6. Total mean (n=4) phenolic contents for blackberries averaged over two years. Note: No significant differences ($p<0.05$) were observed among berry samples.

Unracked wine and blackberry juice

Total phenolic contents for unracked wine (2008) and blackberry juice (2009) are shown in Table 4 above. Because these samples were not replicated in both years, our comparisons here must remain tentative because we cannot separate the possible influences of harvest year and fermentation technique on the results. However, some interesting results were observed. The total phenolic contents for both the wine and the juice were observed to decrease by at least one third compared to those of the whole extracts. Between the two cultivars, Apache-derived juice and unracked wine had higher values for total phenolic contents than Ouachita. Juice and unracked wine made from blackberries grown in Arkansas showed higher values for total phenolics than those grown in Oklahoma. Interestingly, there was not a dramatic decrease in total phenolic contents between juice and unracked wines in products made from Apache berries

(Figure 7). In any case no statistically significant differences were seen among total phenolics values for juices and unracked wines.

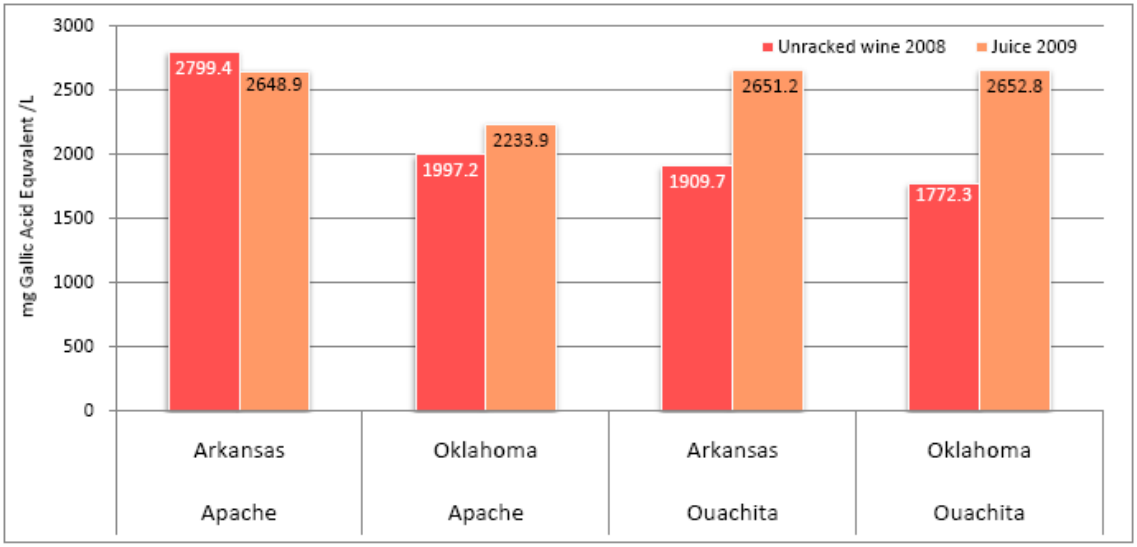


Figure 7. Total phenolic contents of unracked wine (2008) and blackberry juice (2009). Note: No significant differences ($p<0.05$) were observed among unracked wine and juice samples.

Total Monomeric Anthocyanin Contents

Whole blackberries

Whole blackberry anthocyanin contents are shown in Table 4 above. The anthocyanin contents we measured are higher than those reported for some other cultivars (Moyer and others 2002; Sellappan and others 2002; Siriwoharn and others 2004; Pantelidis and others 2007; Wang and others 2008). However, our results were within the range of values (7-495 mg cy-3-glu/100g) shown in studies by Koca and others (2008) as well as the range (1-1186 mg cy-3-glu/100g) reported by Deighton and others (2000). Between the two cultivars, Apache had higher total anthocyanin contents than that of Ouachita for both years. No consistent pattern in anthocyanin contents was seen

with respect to cultivar. Overall, no statistically significant differences were seen among total anthocyanin contents when averaged over two years (Figure 8)

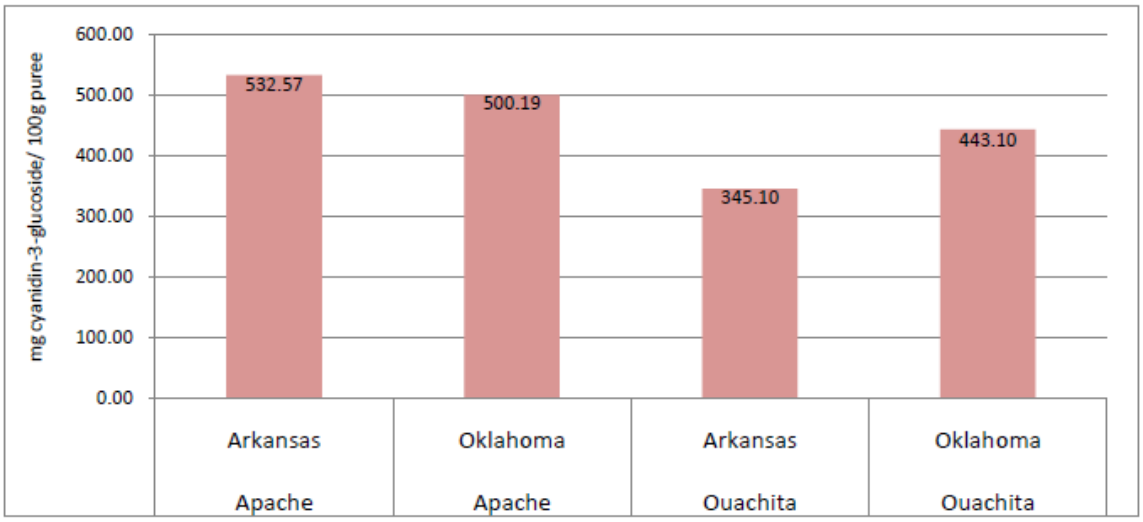


Figure 8. Total monomeric anthocyanin contents for blackberries averaged over two years.
Note: No significant differences ($p<0.05$) were observed among berry samples.

Unracked wine and blackberry juice

Total monomeric anthocyanin contents for unracked wine (2008) and blackberry juice (2009) are shown in Table 4 above. As noted above, because these samples were not replicated in both years our conclusion here must remain tentative. Arkansas-Apache juice was found to have significantly higher anthocyanin contents than Arkansas-Ouachita juice; this was the only statistically significant difference seen among the samples ($p<0.05$). More interestingly, total anthocyanin contents were seen to drop dramatically between juice and fermented but unracked wine samples (Figure 9). The drop was proportionally much greater than the drop seen in total phenolics (Figure 7). Between the two cultivars, Apache consistently had slightly higher total anthocyanin contents than Ouachita. No clear pattern was seen with respect to growing location.

Because anthocyanin is a water soluble pigment, it passes readily into the juice during blackberry processing. Once removed from its tissue matrix, however, it tends to become unstable and the color may fade over time. It is important to note that processing technique was different for our juice samples as compared to our unracked wine samples. In case of the juice samples, we pressed the berries as the first step in processing. Thus, we would expect the juice samples to have experienced relatively little loss of anthocyanins. For the unracked wine, hoever, we fermented the whole berries first and then pressed out the liquid. It is possible that some degradation of anthocyanins occurred during the fermentation process. This would likely explain the differences seen in total anthocyanin contents.

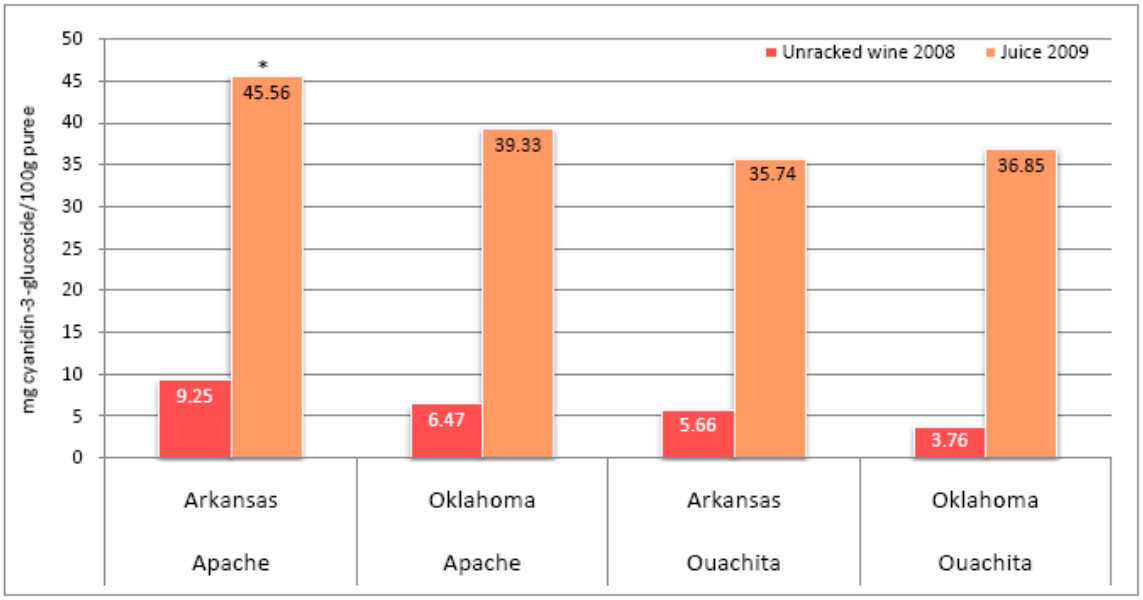


Figure 9. Total monomeric anthocyanin contents of unracked wine (2008) and blackberry juice (2009).
*Apache-Arkansas juice was significantly different than Ouachita-Arkansas juice ($p < 0.05$).
Note: No significant differences ($p<0.05$) were observed among unracked wine samples.

Oxygen radical absorbance capacity (ORAC) assay

Blackberry Extracts

Whole blackberry ORAC values are shown in Table 4 above. Results showed ORAC values two to three-fold higher than ORAC values observed in some other studies of blackberries (Moyer and others 2002; Siriwoharn and others 2004; Wang and others 2008). This might be due to the fact that the different extraction method and different weather condition can increase the antioxidant capacity. Usually, phenolic contents increase in harsh weather condition. Overall, our blackberry samples from the 2008 harvest had lower ORAC values than those harvested in 2009. Between the two years, the weather in 2009 was harsher than 2008 and this may have led to the observed increase the ORAC values. Among the four blackberry cultivar-location samples harvested in 2008, Oklahoma-Apache had the highest ORAC value, followed by Arkansas-Apache, Oklahoma-Ouachita, and Arkansas-Ouachita. For the 2009 harvest, Oklahoma-Ouachita had the highest ORAC value, followed by Oklahoma -Apache, Arkansas-Apache, and Arkansas-Ouachita. For both cultivars and both years, Apache had higher ORAC values than Ouachita. Comparing the two growing locations, blackberries grown in Oklahoma showed higher ORAC values than berries grown in Arkansas for both cultivars. Averaged over two years, we observed a statistically significant difference ($p<0.05$) between Arkansas-Ouachita and Oklahoma-Ouachita (Figure 10) with Oklahoma Ouachita being higher. This may be due to the fact that the weather conditions in Oklahoma tend to be more variable and somewhat harsher than those in Arkansas. Harsher weather (e.g. more extreme temperature swings, longer dry periods, etc.) may stimulate higher antioxidant content.

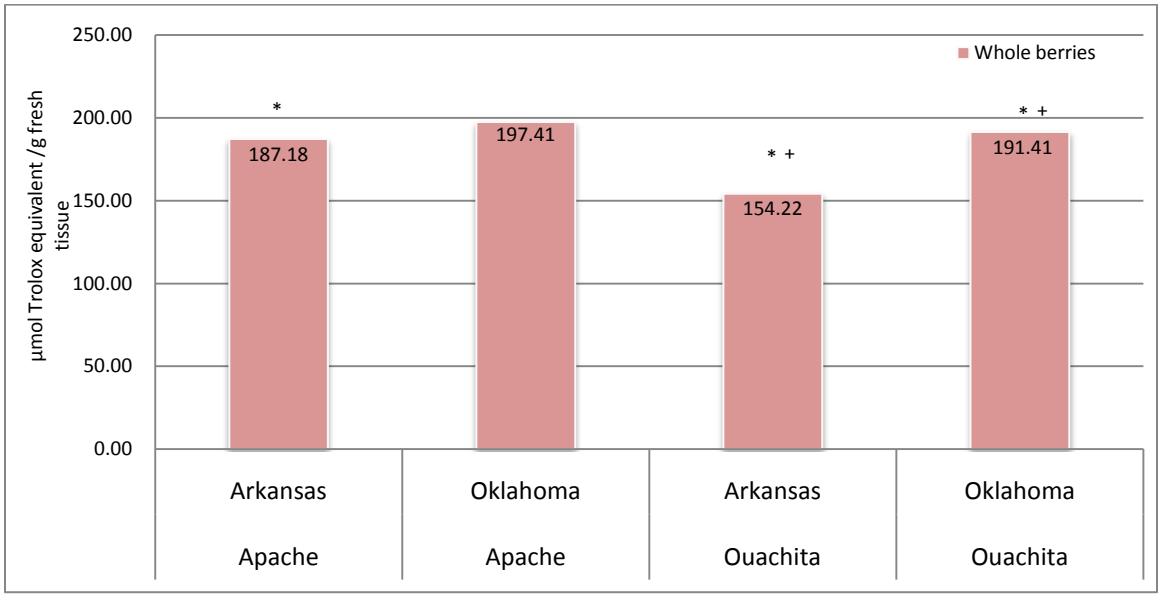


Figure 10. Oxygen radical absorbance capacity (ORAC) assay values for blackberries averaged over two years.
* Arkansas-Apache was significantly different ($p<0.05$) than Arkansas-Ouachita.
$^{+}$ Arkansas-Ouachita was significantly different ($p<0.05$) than Oklahoma-Ouachita.

Unracked blackberry wine (2008) and blackberry juice (2009)

ORAC values for unracked wine (2008) and blackberry juice (2009) are shown in Table 4 above and Figure 11. The ORAC value decreased dramatically after processing. Overall we see a dramatic reduction – about 90% – in ORAC values between whole berries on the one hand and juice and unracked wine on the other. No clear pattern was seen in the ORAC results relative to cultivar or growing location for the juice and unracked wine. It is interesting to note that we did not see as dramatic a drop in ORAC values going from juice to unracked wine as we did in anthocyanin contents. The drop more closely resembles that seen for total phenolic contents. This observed drop may be

due to the fact that antioxidant capacity is typically more closely related to total phenolic contents than to total anthocyanin contents.

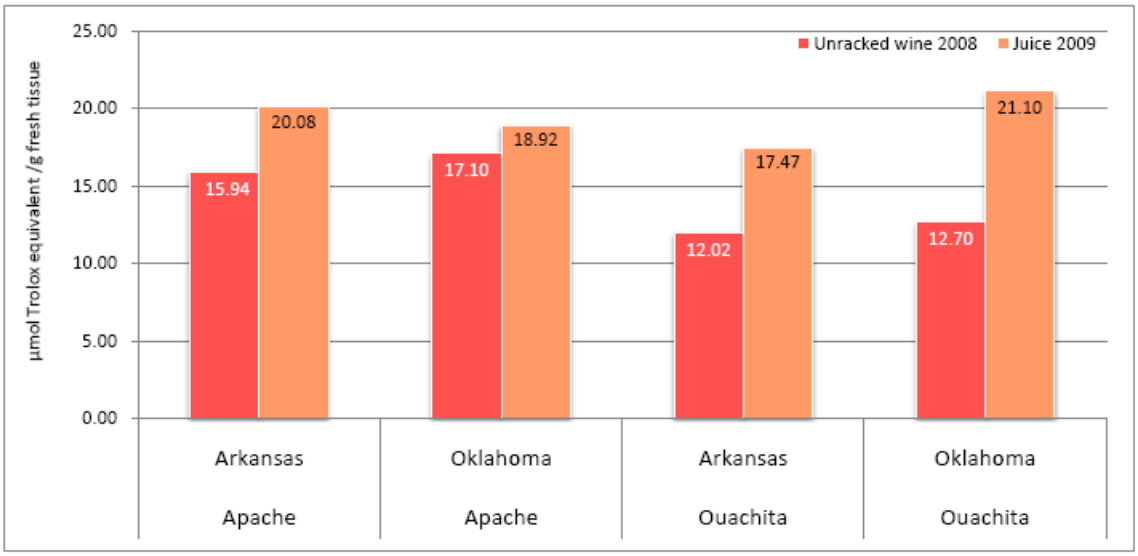


Figure 11. ORAC assay value of unracked wine (2008) and blackberry juice (2009). Note: No significant differences ($p<0.05$) were observed among unracked wine and juice samples.

Correlations

Not surprisingly, total phenolic contents was found to have a strong positive correlation [correlation coefficient = 0.758 ($p<0.0001$)] with ORAC value in whole berries. However, this correlation did not hold true for the juice or unracked wine samples.

CONCLUSIONS

The acidity, sweetness and flavor affected the sensory ratings for the blackberry fruit. The blackberries that were higher in titratable acidity and pH showed were more

preferred in terms of blackberry flavor. Also, the blackberries with higher soluble solids content were more preferred as well. Both cultivars were rated high in blackberry flavor. Other researchers have noted that a relatively high ratio between titratable acidity and soluble solids content has been linked to greater preference (Thomas and others 2005). In this experiment, the Ouachita berries grown in Arkansas had the highest ratings for sweetness and blackberry flavor and were preferred to Ouachita berries grown in Oklahoma. They also tended to be rated higher than Apache berries grown in Arkansas, though the differences were not statistically significant. However, for berries grown in Oklahoma, the Apache berries tended to be more preferred than the Ouachita berries with significant differences seen in sweetness, flavor, and acidity. Thus, the Apache cultivar may tend to produce more preferred berries in Oklahoma whereas the Ouachita cultivar may tend to produce more preferred berries in Arkansas.

This study showed that whole berry extracts had higher concentration of total phenolics and monomeric anthocyanins as well as higher ORAC values as compared to both juice and unracked wine. No statistically significant differences were seen in total phenolic contents among whole berry samples or among juice and unracked wine samples. Similarly, no statistically significant differences were seen in monomeric anthocyanin contents among whole berry samples. Apache tended to have higher concentrations of total phenolics and anthocyanins compared to Ouachita.
More differences were seen among the samples with respect to ORAC values. Oklahoma berries tended to have higher ORAC values than Arkansas berries; some significant differences were observed. This mirrored the results seen for total phenolic contents. Apache berries tended to have higher ORAC values than Ouachita berries, but this trend

was not universal and was not statistically significant except in the case of Arkansas-Apache juice versus Arkansas-Ouachita juice. This may suggest that processing introduced a good deal of variability into antioxidant content and activity in processed blackberry products regardless of differences seen in the starting intact blackberries.

Overall, the quantification of antioxidant activities of blackberries was limited to total phenolics, total anthocyanins and peroxyl radicals scavenging as measured by the ORAC assay. Thus, it may be beneficial for future research to employ additional techniques to identify and quantify other antioxidant compounds in blackberries. Our results indicate that a good deal of antioxidant activity is lost in processing. Therefore, blackberry by-products such as pomace may well be a good source of antioxidants and have possible applications in the manufacture of functional foods and/or nutritional supplements.

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CHAPTER IV

BLACKBERRY WINE

ABSTRACT

Blackberry (*Rubus sp*.) is a good source of antioxidants and contains a high amount of phenolic compounds, mainly anthocyanins. Anthocyanins are very stable at acidic conditions but under processing become unstable and change color from red to brown pigments. Two blackberry cultivars (Apache and Ouachita) that are commonly grown in the Midwest were harvested from two locations, one in Oklahoma and one in Arkansas. Assays were conducted to determine total phenolics and total anthocyanin contents. The Folin-Ciocalteu method was used to measure total phenolic contents and the pH differential method was used to evaluate anthocyanin contents. Oxygen Radical Absorbance Capacity (ORAC) assays were also conducted in order to measure antioxidant activity. Titratable acidity, pH and alcohol content were measured in order to see the difference between blackberry wines made from two cultivars grown in two locations. All of the blackberries were collected during summer 2008 and summer 2009. Wines made from Oklahoma-grown berries tended to have higher total phenolic contents than wines made from Arkansas-grown berries. No consistent cultivar effect was

observed. Wines made from Apache berries tended to have slightly higher total anthocyanins than wines made from Ouachita berries. No consistent location effect was observed. ORAC values in the wines were significantly correlated with anthocyanin content and not total phenolics content. Wines made from Oklahoma-grown berries tended to have slightly higher ORAC values than wines made from Arkansas-grown berries. No consistent cultivar effect on ORAC value was observed in this study.

INTRODUCTION

Grape and wine contain high amount of phenolics, mostly flavonoid (1000-1800 mg/L). Moderate consumption of alcohol reduces the risk of coronary heart disease. Wine has more beneficial effect than beer and spirits. Drinking both wine and beer reduces the risk of cardiovascular death. Phenolic compounds present in red wine cause an increase in serum total antioxidant capacity when ingested and thereby inhibit low density lipoprotein (LDL) oxidation which is an early step in atherogenesis. Other beneficial effects of red wines are anticancer, antioxidant, and anti-amyloidogenic (Yildirim and others 2005).

Blackberries are high in phenolic compounds especially anthocyanins. Anthocyanin is a color pigment of blackberries which gives red, blue, and purple. Anthocyanins are one of the stronger antioxidants found in berry fruit and have been linked to prevention of disease caused by oxidative stress. Blackberry juice extract has been widely used as a natural colorant in beverages, baked products, jellies and wines

(Jiao and others 2005; Hager and others 2008). Anthocyanin is very sensitive to pH and easily changes color while processing. Anthocyanins are very stable at acidic condition but under processing may change color from purplish red to colorless derivatives and subsequently to insoluble brown pigments (Dai and others 2009).

Two major anthocyanins found in blackberry were cyanidin-3-glucoside and cyanidin-3-rutinoside. When juice is fermented to wine, anthocyanin, especially cyanidin-3-glucoside is dramatically lost (Fan-Chiang and Wrolstad 2005). Depectinizing and pasteurizing increase color extraction and total juice yields, inactivate enzymes, and improve the flavor and aroma of wine. On the other hand, heating darkens blackberry juice (Rommel and others 1992). Storage time had a greater influence on color than storage temperature. Percentage of polymeric color tends to increase during fermentation and storage. Wines with low polymeric color contents were less bitter and astringent. Red raspberry wines contained more stable anthocyanin pigments and formed considerably less haze and sediments than strawberry and blackberry wines. Blackberry wine has been shown to have relatively more sediment than raspberry wine (Rommel and others 1990). According to Rommel and others (1992), blackberry wine from the Evergreen cultivar had not been commercially successful because of excessive haze and sediment formation along with color loss and browning in the wines during storage.

Early addition of SO_2 to the musts protects anthocyanins, avoiding both their direct oxidation and the oxidation of phenolic acids to o-chinones, molecules that are highly reactive toward anthocyanins. Addition of SO_2 during the pre-fermentative phases has been shown to yield wines richer in catechin – this is of interest for the production of

healthier red wines since this molecule has been shown to inhibit the growth of human cell lines originating from cancers of the prostate (Gumbuti and others 2007).

There is a good deal of research on grape wines and the health benefits of anthocyanins and berry processing. However, there are fewer research reports on berry fruit wines, perhaps because such wines are not as commonly consumed as grape wines in general. This research is focused on making blackberry wine with two cultivars – Apache and Ouachita – that are grown in both Oklahoma and Arkansas in order to determine if the two growing locations and/or the two cultivars have an influence on the quality and antioxidant capacity of the wines produced. We also sought to assess the impact of winemaking technique on wine quality and antioxidant capacity.

MATERIALS AND METHODS

Berry collection

The two blackberry cultivars, Apache and Ouachita, were collected from two different locations in Arkansas and Oklahoma. The Arkansas blackberries were collected from the University of Arkansas Fruit Research Substation in Clarksville, Arkansas. The elevation at this location is 273 m (895 ft) above sea level and the average annual precipitation is 125.5 cm (49.4 in). Plants were spaced 61 cm (2 ft) apart in rows, row middles were spaced at 3.7 m (12 ft), and the plots were trellised. Harvest of blackberries began in May and berries were collected every Monday and Thursday. Berries were

collected after they turned fully black. Berries were placed into polyethylene bags and frozen within one hour of harvest for subsequent storage.

Oklahoma-grown blackberries were collected from Toomey's Thornless Blackberry Farm in Broken Arrow, Oklahoma. The elevation at this location is 238m (780 ft) above sea level and the average annual precipitation is 107.7 cm (42.4 in). Plants were spaced 1.2 m (4 ft) apart in row. Harvest started in the third week of June and ended in third week of July. Berries were collected after they turned fully purple. Berries were placed into polyethylene bags and frozen within one hour of harvest for subsequent storage.

Berry Sample Storage, Handling, and Preparation

Sample Storage

All blackberry samples were frozen and stored at -17°C (1°F) after collection and until further processing.

General sample preparation.

Frozen blackberry samples were placed into a cooler at 4°C (39°F) and allowed to thaw for 24-48 hours prior to subsequent processing.

Blackberry Wine Processing -- 2008 harvest

Blackberry wine processing for the 2008 harvest is shown in Figure 12. Blackberry lots from each of the four (2x2) combinations of growing location and

cultivar were removed from frozen storage and thawed in a refrigerator at 4°C (39°F) as described above. When the berries were totally thawed, each of the four samples (Apache, AR; Apache, OK; Ouachita, AR and Ouachita, OK) was weighed and the weights were recorded. The starting weight of each batch was approximately 14kg (30 lbs). A small sample the free juice associated with the thawed berries was collected from each sample batch of blackberries and the soluble solids content of each sample batch was measured using a Leica Auto ABBE refractometer (Buffalo, NY, USA) with temperature compensation. The soluble solids contents of each batch was adjusted to approximately 20 °Brix by adding granulated white sugar as needed. We assumed that 4 kg of berries equaled approximately 3.8 liters (1 gallon) in volume. Each sample of whole berries was then treated as follows:

- Inoculated at a rate of 2g/kg must with Fermirouge (DSM, Delft, Netherlands) yeast, which was first rehydrated 15:1 with warm water.
- Added Lallzyme C (Scott laboratories, Petaluma, CA, USA) and Lafase® (Scott laboratories, Petaluma, CA, USA) fruit pectinases at a rate of 1g/10kg must.
- Added Fermaid K (Presque Isle Wine Cellars, North East, PA, USA) at a rate of 1.3g/kg 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 about 30 ppm.

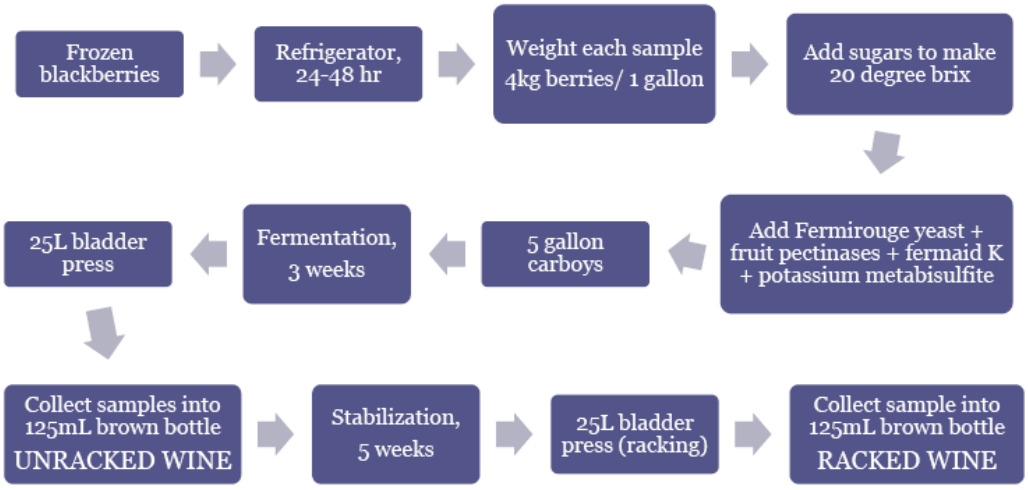
The inoculated berries were then transferred to either 11.4 liter (3-gallon) or 19.0 liter (5-gallon) glass carboys that had been cleaned and sanitized with 300 ppm potassium

metabisulfite solution fermentation. 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 about three weeks, as judged by no further evolution of CO_2 gas and negligible amounts of residual sugars. Once the fermentation was complete, each sample of fermented juice was transferred to a 25 liter bladder press (Zambelli Enotech,Camisano Vicentino, Italy) in order to separate skins and seeds from the fermented juice. After pressing, the fermented juice was transferred to either 11.4 liter (3-gallon) or 19.0 liter (5-gallon) cleaned and sanitized glass carboys for stabilization. Four samples of about 100 ml were removed from each lot, sealed in 125 ml brown glass bottles, and frozen for subsequent analyses.

The blackberry wine samples were racked off of the lees after about five weeks of stabilization. Racking involves decanting the liquid portion of the wine off of the sediment that collects at the bottom of the vessel. Racking helped to remove skins and seeds and acted to clarify the fermented blackberry juice. Free SO_2 levels were monitored periodically. About 0.8g of additional potassium metabisulfite was added to maintain the SO_2 desired concentration, about 50 ppm. Four samples of the final racked wine were removed from each of the four cultivar-location combinations and held in frozen storage at -15℃ (5℉) for subsequent analyses.

Figure 12. Blackberry wine processing 2008 flowchart

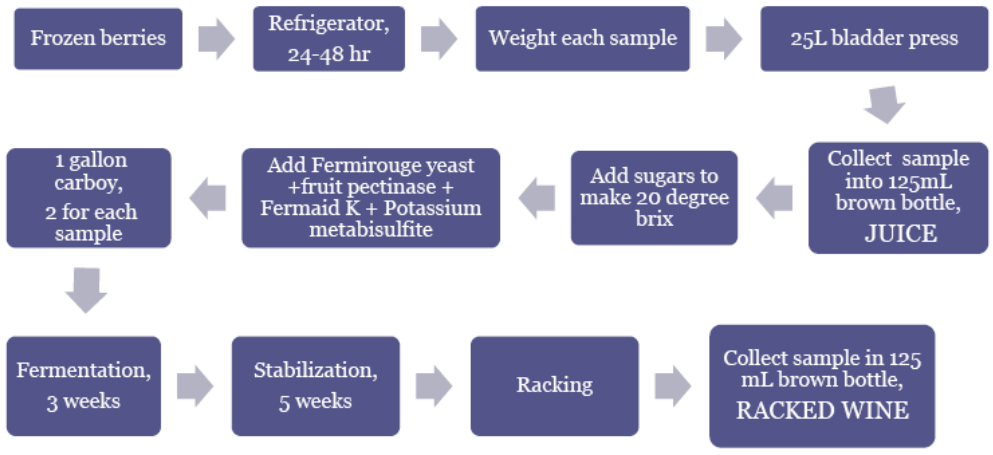


Blackberry Wine Processing -- 2009 harvest

Blackberry wine processing for the 2009 harvest is shown in Figure 13. Blackberry samples from the 2009 harvest were thawed and prepared for processing just as those from the 2008 harvest had been. Soluble solids content was adjusted to about 20 °Brix with added white, granulated sugar. Whole berries were then pressed using a 25 liter bladder press (Zambelli Enotech, Camisano Vicentino, Italy). Each sample of pressed juice was then inoculated with yeast, treated with pectinases, fortified with yeast nutrient, and dosed with SO_2 just as the whole berries from the 2008 harvest had been. The same brands and the same addition rates were used. The inoculated juice was then transferred to either 11.4 liter (3-gallon) or 3.8 liter (1-gallon) glass carboys that had been cleaned and sanitized with 300 ppm potassium metabisulfite solution for fermentation. The carboys were sealed with rubber stoppers and S-type airlocks filled with a 300 ppm potassium metabisulfite solution.

The fermentation was completed in about three weeks, as judged by no further evolution of CO_2 gas and negligible amounts of residual sugars. The blackberry wine samples were racked off of the lees after about five weeks of stabilization. Free SO_2 levels were monitored periodically. Once the fermentation was complete, the wines were transferred into 3.8liter (1 gallon) cleaned and sanitized glass carboys for stabilization. Four samples of the wine were removed from each of the four cultivar-location combinations and held in frozen storage at -15℃ (5℉) for subsequent analyses.

Figure 13. Blackberry wine processing 2009 flowchart



Wine Quality Analyses

pH

The pH of the blackberry wine was measured using an Accumet AB 15 pH meter (Fisher Scientific, Fair Lawn, NJ, USA).

Titratable Acidity

A 5mL sample of blackberry wine was diluted to 105 ml using deionized water. The titratable acidity was then measured as % citric acid using a 809 Titrando automatic titrator (Metrohm ion analysis, Herisau, Switzerland). The sample was titrated with 0.1N NaOH to an endpoint of pH 8.2. The sample was agitated throughout measurement using a magnetic stir-bar. Two duplicate readings were taken from the racked wine samples.

Alcohol content

Percent (w/w) alcohol content was measured using an ebulliometer (Dujardin-Salleron, LDS, Accueil Cedex, France). Calculation of the alcohol percentage was based on the boiling point of water compared to the boiling point of the wine. Briefly, a sample cup attached to the ebulliometer was filled with deionized water. The water was heated to boiling by an electric element and the sample chamber was vented through a condenser unit to prevent the escape of vapors. The temperature of the boiling water was measured using a calibrated mercury-in-glass thermometer inserted into the sample chamber. The above process is repeated with a wine sample and the difference between the boiling point of water and the boiling point of wine is used to look up the alcohol concentration using an adjustable wheel that correlates boiling points for water with boiling points for a water alcohol mixture.

Wine Chemical Analyses

Total Phenolic Contents

Total phenolics were measured using the method of Singleton and Rossi (1965) as described in Chapter 3. Triplicate readings were taken from unracked and racked wine samples.

Total Monomeric Anthocyanin Contents

Total monomeric anthocyanins were measured using the pH differential method first described by Giusti and Wrolstad (2000) as described in Chapter 3. Two duplicate assays were performed on unracked and racked wine samples.

Wine Antioxidant Activity Analyses

Oxygen Radical Absorbance Capacity (ORAC) Assay

We conducted ORAC assays on wine using the method first developed by Ou and others (2001), as described in Chapter 3. Quadruplicate assays were performed on unracked and racked wine samples.

Statistical Analysis

Statistical analyses were performed using SAS version 9.2 (SAS institution, Cary, NC, USA). For all analyses an analysis of variance for each set of data was conducted using a completely randomized design. Means were separated where appropriate using least significant differences (LSD) with a 95% confidence interval ($p<0.05$).

RESULTS AND DISCUSSION

Quality attributes

pH

The pH values measured for blackberry wines are shown in Table 5 below. When looking at overall patterns, several trends emerged. First, the pH values of blackberry wine from 2008 were slightly lower than those from 2009. This may be a function of the different wine-making techniques employed, as the 2008 wine was fermented on the skins and seeds. Second, blackberries grown in Oklahoma consistently had higher pH values than blackberries grown in Arkansas. The higher pH values and relatively lower acidity values seen in the Oklahoma berries may be due to the berries grown in Arkansas being harvested at a later stage or ripeness. Ouachita tended to have higher pH values than Apache. However, the only statistically significant difference seen among samples averaged over two years was between Arkansas-Ouachita and Oklahoma-Ouachita (Table 5).

Table 5. Mean[1] pH values of 2008 and 2009 blackberry wines.

Samples	2008 Wine	2009 Wine
Arkansas-Apache	3.22	3.36
Oklahoma-Apache	3.27	3.57
Arkansas-Ouachita	3.22	3.40
Oklahoma-Ouachita	3.40	3.73

1. n=2

Table 6. Significant differences observed in pH values of blackberry wine averaged over two years.

Samples	Mean[1] pH value
Oklahoma Ouachita	3.57 a[2]
Arkansas-Ouachita	3.31 b

1. n=2.
2. Means followed by the same letter are not significantly different (p>0.05).

Titratable acidity

The titratable acidity results are shown in Table 7. Overall, blackberry wine made in 2008 had higher titratable acidity values than wine made in 2009. Once again, this may be a function of how the wines were made. The 2008 wine had more opportunity to extract acids from the skins and especially the seeds than did the wine made in 2009. It is interesting to note that for both years wine made from Ouachita berries showed higher titratable acidity values than wine made from Apache berries. This could be due to the normal variation in acid content that is seen among different cultivars. The one statistically significant difference that was seen over two years was that wine made from Oklahoma-grown Ouachita blackberries was more acidic than wine made from Oklahoma-grown Apache berries (Table 8). No clear trend was seen with respect to growing location.

Table 7. Mean[1] titratable acidity (g/L citric acid) of 2008 and 2009 blackberry wines.

Samples	2008 Wine	2009 Wine
Arkansas-Apache	13.4	11.7
Oklahoma-Apache	13.6	11.6
Arkansas-Ouachita	15.2	12.6
Oklahoma-Ouachita	16.8	13.6

1. n=2.

Table 8. Significant differences observed in titratable acidity values of blackberry wine averaged over two years.

Samples	Mean[1] Titratable Acidity (g/L citric)
Oklahoma-Ouachita	15.2 a[2]
Oklahoma-Apache	12.6 b

1. n=2.
2. Means followed by the same letter are not significantly different (p>0.05).

Alcohol content

The alcohol contents of blackberry wine are shown in Table 9. Alcohol measurements were made using bottled wine from 2008 and racked, but unbottled wine from 2009. Final alcohol content is a function of starting sugar content, and starting sugar contents were adjusted each year to about 20 °Brix. Therefore, as the wines were fermented to dryness, we might expect to see the same alcohol content for all wines tested. However, while there were no statistically significant differences among the samples tested for alcohol content, we measured lower and more variable values among the 2008 wine samples. This may be due to the fact that accurately measuring the

beginning °Brix is more difficult when starting the winemaking process with whole berries rather than pressed juice.

Table 9. Alcohol Contents (% alcohol) of blackberry wines.

Samples	2008 Wine	2009 Wine
Arkansas-Apache	11.9	12.9
Oklahoma-Apache	11.3	12.9
Arkansas-Ouachita	11.3	13.9
Oklahoma-Ouachita	9.2	14.3
Note: No significant differences ($p < 0.05$) were observed among the samples.		

Total Phenolic Contents

Total phenolic contents for blackberry wines are shown in Table 10. Overall, we observed that blackberry wines retained about 26% of the total phenolic contents present in whole berries, about 73% of the total phenolics present in the pressed juice, and about 87% of the total phenolics present in the unracked wine (Tables 4 and 10). Looking at the results by year, we observed that 2008 wines had relatively higher amounts of total phenolics than 2009 wines. Those results fit our expectation given that the 2008 wines had more time to extract phenolic compounds from the skins and seeds than the 2009 wines did. Interestingly, however, the difference was only about 8% -- relatively minor.

Comparing the two cultivars, Apache had slightly higher total phenolics than Ouachita in both growing locations when averaged over two years (Figure 14), although the values were not significantly different. Comparing the two locations, wines made from berries grown in Oklahoma showed a slightly higher amount of total phenolics than

wine made from berries grown in Arkansas when averaged over two years (Figure 14).

However, no statistically significant differences were observed.

Table 10. Mean total phenolics[1], total anthocyanins[1] and oxygen radical absorbance capacity (ORAC) assay[2] values for 2008 and 2009 blackberry wines.

Samples	Mean[1] Total Phenolics mg gallic acid equivalent/L	Mean[1] Total Anthocyanins mg cyanidin-3-glucoside/ 100g puree	Mean[2] ORAC Values µmol Trolox equivalent/ g of fresh tissue
Racked Wine 2008			
Apache AR	1830.4	2.34	11.03
Apache OK	2154.9	4.19	14.97
Ouachita AR	1821.2	2.70	13.87
Ouachita OK	1896.0	1.89	14.19
Racked Wine 2009			
Apache AR	1856.1	5.52	21.20
Apache OK	1676.5	4.92	17.52
Ouachita AR	1721.0	3.67	18.05
Ouachita OK	1856.9	3.38	19.20

1. n=3.
2. n=4.

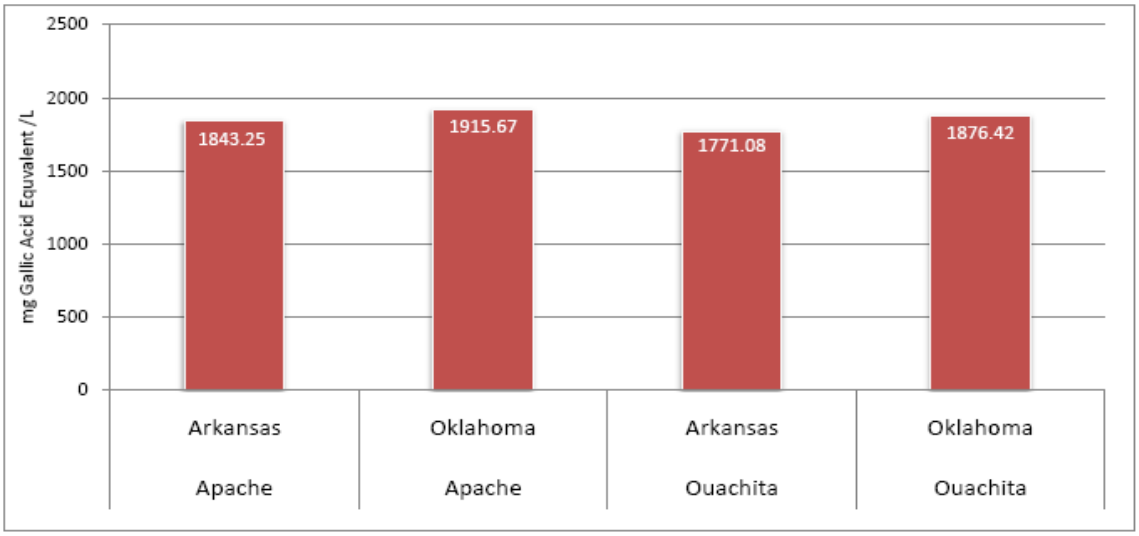


Figure 14. Total phenolic contents for blackberry wines averaged over 2008 and 2009. Note: No significant differences ($p<0.05$) were observed among wine samples.

Total Anthocyanin Contents

Total monomeric anthocyanin contents for blackberry wines are shown in Table 10. Overall, we observed that blackberry wines retained only about 1% of the total anthocyanins present in whole berries, about 9% of the total phenolics present in the pressed juice, and about 57% of the total phenolics present in the unracked wine (Tables 4 and 10). Unlike the results observed for total phenolics, the average anthocyanin contents of the 2008 wines was lower than that seen in the 2009 wines. Thus, the on-the-skin fermentation did not reliably boost the final anthocyanin contents of the wines. A pattern for Apache wines to have slightly higher total anthocyanins than Ouachita wine was observed over both years (Figure 15). No clear pattern was observed with respect to growing location. No statistically significant differences were observed in any case.

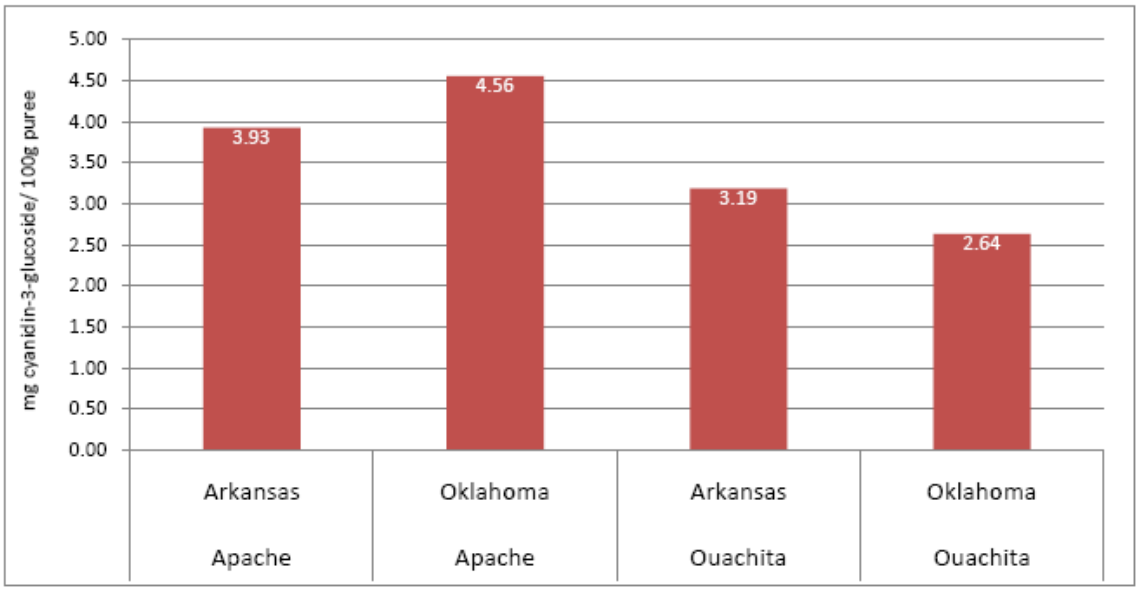


Figure 15. Total anthocyanin contents for blackberry wines averaged over 2008 and 2009.
Note: No significant differences ($p<0.05$) were observed among wine samples.

Oxygen radical absorbance capacity (ORAC) assay

ORAC values for blackberry wines are shown in Table 10. Overall, we observed that blackberry wines retained about 9% of the ORAC value present in whole berries, about 84% of the ORAC value present in the pressed juice, and essentially 100% of the ORAC value present in the unracked wine (Tables 4 and 10).

Looking at the ORAC data, the blackberry wine samples from 2008 consistently had lower ORAC values than blackberry wine samples from 2009. Thus, fermenting blackberry wine on the skin did not lead to increased antioxidant capacity in the wines tested in this experiment. The weather condition may have affected the antioxidant capacity of the wines: harsher weather can increase the antioxidant capacity of the fruit from which the wine is made. Comparing the two harvest years, the weather conditions in 2009 were harsher (drier and hotter) than in 2008 and as a result these may have caused higher ORAC value in the wines.

Assessing the two cultivars, no real pattern was seen in the relative ORAC values of Apache versus Ouachita over the two years studied (Figure 16). Comparing the two growing locations, wine made from blackberries of the same cultivar grown in Oklahoma showed slightly higher ORAC values than wine made from blackberries of the same cultivar grown in Arkansas over the two-year period of the study (Figure 16). No statistically significant differences were observed, however.

Correlation

A significant positive correlation coefficient of 0.846 ($p<0.0001$) between total anthocyanin contents and ORAC value was observed in the blackberry wines. No

significant correlation was observed between total phenolic contents and ORAC value. This is in contradiction to the relationship seen between ORAC value, total phenolic contents, and total anthocyanin contents observed in whole berries. One explanation for this may simply be the degree of variability seen in the total phenolics values measured. Or possibly, as noted previously, the ORAC values may be influenced by fermentation byproducts such that the correlation seen between total phenolics and ORAC values in whole fruit and juice may not hold in wines. Further research would be necessary to explore these possibilities.

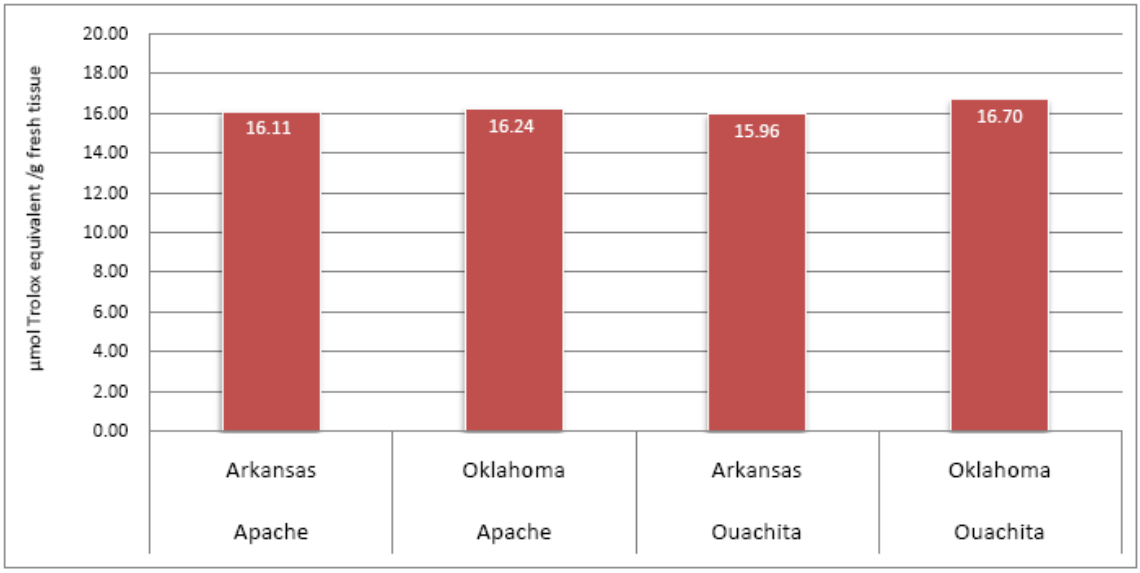


Figure 16. Oxygen Radical Absorbance Capacity (ORAC) assay values for blackberry wines averaged over 2008 and 2009.
Note: No significant differences ($p<0.05$) were observed among wine samples.

CONCLUSIONS

Wines made from Ouachita blackberries tended to have higher titratable acidity values than wines made from Apache blackberries regardless of growing location. Wines made from Oklahoma-grown berries tended to have higher pH values than wines made from Arkansas-grown berries regardless of cultivar. Overall, it appears that wine-making technique may have had a larger influence on basic quality attributes than cultivar or growing location. Fermenting the blackberries before pressing resulted in higher titratable acidity and lower pH values for the final wine. It also appears that fermenting whole blackberries may make it more difficult to adjust starting sugar content and thus to control final wine alcohol content.

Winemaking technique also appeared to have an influence on total phenolic contents, with wines made by pressing berries after fermentation tending to have higher total phenolic contents than wines made from pressed juice. This difference was not dramatic, however. Wines made from Oklahoma-grown berries tended to have higher total phenolic contents than wines made from Arkansas-grown berries. Since Oklahoma has relatively harsher (more variable temperatures and rainfall) weather condition than Arkansas, this may tend to stimulate the plant's defense system to produce higher levels of phenolic compounds. No consistent cultivar effect was observed.

Winemaking technique did not appear to have any effect on total monomeric anthocyanin contents. Wines made from Apache berries tended to have slightly higher total anthocyanins than wines made from Ouachita berries. No consistent location effect was observed.

Winemaking technique did not appear to have any intuitive effect on ORAC values in the wines. Overall ORAC values were slightly higher in the wines fermented from pressed juice rather than whole berries. ORAC values in the wines were significantly correlated with total anthocyanin contents and not total phenolic contents; further research would be necessary in order to determine whether this result would hold true generally. Wines made from Oklahoma-grown berries tended to have slightly higher ORAC values than wines made from Arkansas-grown berries. Although no statistically significant correlation between total phenolic contents and ORAC value was seen, this mirrors the results seen for total phenolic contents. It may be that the somewhat harsher Oklahoma climate puts more stress on the vines, thus favoring the production of phyto-protective phenolic compounds and thereby boosting the ORAC value. Farm management techniques may have influenced the antioxidant content of the blackberries as well. More research would be needed to test this hypothesis. No consistent cultivar effect on ORAC value was observed in this study.

Based on our results, blackberry vinification decreases the total phenolic contents, the total anthocyanin contents, and the antioxidant capacity of whole blackberries significantly. Interestingly, the decrease does not appear to be uniform among constituent compounds nor does it appear to be linear throughout the vinification process. For example, the degree of reduction in total phenolics and especially in anthocyanins between juice, unracked wine sampled just at the end of fermentation, and racked wine was greater than the degree of reduction in ORAC values at the same stages in processing. This suggests that other, unknown factors may be contributing to observed ORAC values in the juices and wines. Perhaps fermentation byproducts are contributing

to the ORAC values observed in the wines. More study is needed to elucidate these possible influences.

Overall, this research showed that blackberries and blackberry products have a distinct advantage in terms of possessing high antioxidant capacity as well as providing a distinct and appealing flavor; this may lead to increasing consumption of blackberry wine in the future. To help insure better quality wines, there is still a need for further research to evaluate cultivars that are able to withstand the unfriendly environmental conditions in Oklahoma while still producing appealing wines. There is also a need for ongoing research designed to further develop winemaking techniques to maximize quality and health-beneficial properties. This study represents a first step in that direction.

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CHAPTER V

CONCLUSIONS AND RECOMMENDATION

Whole Berries:

The acidity, sweetness and flavor affected the sensory ratings for the blackberry fruit. Blackberries rated higher in titratable acidity, pH, and soluble solids were more preferred in terms of blackberry flavor. Overall, both cultivars were rated high in blackberry flavor. Ouachita berries grown in Arkansas had the highest ratings for sweetness and blackberry flavor and were preferred to Ouachita berries grown in Oklahoma. They also tended to be rated higher than Apache berries grown in Arkansas. For berries grown in Oklahoma, the Apache berries tended to be more preferred than the Ouachita berries with significant differences seen in sweetness, flavor, and acidity. Thus, the Apache cultivar may tend to produce more preferred berries in Oklahoma whereas the Ouachita cultivar may tend to produce more preferred berries in Arkansas.

Whole berry extracts had higher concentrations of total phenolics and monomeric anthocyanins as well as higher ORAC values as compared to both juice and wine. No statistically significant differences were seen in total phenolic or anthocyanin contents among whole berry samples. However, Oklahoma berries tended to have higher ORAC values than Arkansas berries; some significant differences were observed. Total phenolic

content was found to have a strong positive correlation with ORAC value in whole berries.

Wine:

Wines made from Ouachita blackberries tended to have higher titratable acidity values than wines made from Apache blackberries regardless of growing location. Wines made from Oklahoma-grown berries tended to have higher pH values than wines made from Arkansas-grown berries regardless of cultivar. Overall, it appears that wine-making technique may have had a larger influence of basic quality attributes than cultivar or growing location. Fermenting the blackberries before pressing resulted in higher titratable acidity and lower pH values for the final wine.

Winemaking technique also appeared to have an influence on total phenolic contents, with wines made by pressing berries after fermentation tending to have higher total phenolic contents than wines made from pressed juice. Wines made from Oklahoma-grown berries tended to have higher total phenolic contents than wines made from Arkansas-grown berries. No consistent cultivar effect was observed.

Winemaking technique did not appear to have any effect on total monomeric anthocyanin contents. Wines made from Apache berries tended to have slightly higher total anthocyanins than wines made from Ouachita berries across the board. No consistent location effect was observed.

Winemaking technique did not appear to have a large effect on ORAC values. Overall ORAC values were slightly higher in the wines fermented from pressed juice rather than whole berries. ORAC values in the wines were significantly correlated with

anthocyanin contents and not total phenolic contents; further research would be necessary in order to determine whether this result would hold true generally. Wines made from Oklahoma-grown berries tended to have slightly higher ORAC values than wines made from Arkansas-grown berries. No consistent cultivar effect on ORAC value was observed in this study.

Based on our results, there is a significant decrease in the total phenolic contents, the anthocyanin contents, and the antioxidant capacity of blackberry wine compared to whole blackberries. Overall, we observed that blackberry wines retained about 26% of the total phenolics, only about 1% of the total anthocyanins, and approximately 9% of the ORAC value present in starting whole berries. This suggests that blackberry processing byproducts such as pomace may be a good source of antioxidants and have possible applications in the manufacture of functional foods and/or nutritional supplements. Even so, on average the blackberry wines possessed antioxidant capacity of about 1600 µmol Trolox equivalents / 100 ml of wine.

Future Research:

Overall, this research showed that blackberries and blackberry products have a distinct advantage in terms of possessing high antioxidant capacity as well as providing a distinct and appealing flavor; this may lead to increasing consumption of blackberry products such as juice and wine in the future. To help insure better quality wines, there is still a need for further research to evaluate cultivars and genotypes that are able to withstand the harsh environmental conditions in Oklahoma while still producing appealing wines. There is also a need for ongoing research designed to further develop

winemaking techniques to maximize quality and health-beneficial properties. Finally, given that we did not observe a uniform decrease in antioxidant compounds and antioxidant capacity going from whole berries to wine, research is needed to develop improved techniques to identify and quantify the relative contributions of antioxidant compounds to overall antioxidant capacity.

APPENDICES

Blackberry total weight before processing

Cultivar	Location	2008 berries	20009 berries
Apache	Arkansas	11.18 kg (24.65 lbs)	16.83 kg (37.10 lbs)
Apache	Oklahoma	19.33 kg (42.62 lbs)	9.06 kg (19.98 lbs)
Ouachita	Arkansas	11.48 kg (25.31 lbs)	14.90 kg (32.84 lbs)
Ouachita	Oklahoma	12.29 kg (27.09 lbs)	15.21 kg (33.54 lbs)

Blackberry total yield after processing

Cultivar	Location	2008 berries*	20009 berries[+]
Apache	Arkansas	5.30 kg (11.68 lbs)	10.98 kg (24.20 lbs)
Apache	Oklahoma	4.53 kg (9.99 lbs)	8.64 kg (19.05 lbs)
Ouachita	Arkansas	8.32 kg (18.33 lbs)	5.31 kg (11.70 lbs)
Ouachita	Oklahoma	4.56 kg (10.06 lbs)	7.94 kg (17.50 lbs)

*denoted as weight from blackberry bottle of wine. [+]denoted as weight from blackberry juice.

Sensory Evaluation sample rubric

Sample:_____

Please take a sip of water, taste the sample, then check the box which best describes your feelings about each of the following attributes.

Seediness:

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]

Juiciness:

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]

Sweetness:

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]

Blackberry Flavor:

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]

Acidity(2008)/ Firmness (2009):

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]

VITA

Youri Joh

Candidate for the Degree of

Master of Science

Thesis: QUANTIFICATION OF QUALITY ATTRIBUTES, FUNCTIONAL COMPOUNDS, AND ANTIOXIDANT CAPACITY OF BLACKBERRY AND BLACKBERRY WINES

Major Field: Food Science

Biographical:

Personal data:
Born in Seoul, Republic of Korea on December 27th, 1980

Education:

- Earned Bachelor of Science in Human Nutrition and Food Science at New Mexico State University, Las Cruces, New Mexico in May 2007.
- Completed the requirements for the Master of Science in food science at Oklahoma State University, Stillwater, Oklahoma in May, 2010.

Experience:

- Internship at Samsung Food Product Center, Suwon, Republic of Korea from January to May 2007
- Graduate Research Assistant at Oklahoma State University, 2010-current

Professional Memberships:

Name: Youri Joh Date of Degree: May, 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: QUANTIFICATION OF QUALITY ATTRIBUTES, FUNCTIONAL COMPOUNDS, AND ANTIOXIDANT CAPACITY OF BLACKBERRY AND BLACKBERRY WINES

Pages in Study: 91 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study:

The objective of the study was: (i) investigate two cultivars, Apache and Ouachita, and two growing locations, Central Arkansas and Eastern Oklahoma, in order to determine their possible influences on the quality and antioxidant capacity of whole berries and wine; (ii) measure antioxidant capacity of blackberries at different stage of processing-whole berries, juice, unracked wine, and racked wine; (iii) investigate consumer's preference for cultivar and/or growing location and assess the impact of winemaking technique on wine quality and antioxidant capacity. The Folin-Ciocalteu method was used to measure total phenolic contents and the pH differential method was used to evaluate total anthocyanin contents. Oxygen Radical Absorbance Capacity (ORAC) assays were also conducted in order to measure antioxidant activity. Qualitative tests such as pH, tiratable acidity, soluble solids were conducted to measure the quality of blackberry fruit. Sensory tests were also performed on whole berries in order to evaluate subjective impressions of quality.

Findings and Conclusions:

Whole berries had the highest total phenolics, total anthocyanins, and ORAC values, followed by juice and wines respectively. Blackberries rated higher in titratable acidity, pH and soluble solids were more preferred in terms of blackberry flavor. The Apache cultivar was preferred among Oklahoma-grown berries while the Ouachita cultivar was preferred among Arkansas-grown berries. Wine processing technique did not significantly affect the antioxidant capacity of blackberry wines; however, berries pressed after fermentation trended toward higher total phenolics while wines made by fermenting pressed juice were numerically higher in total anthocyanins and ORAC values. Oklahoma-grown berries showed slightly higher values for total phenolics, total anthocyanins, and ORAC values. Total anthocyanin content was strongly correlated with ORAC values in wine while total phenolics content was strongly correlated with ORAC values in whole berries and juice. Blackberry processing byproducts may be a good source of antioxidants given that substantial amounts of antioxidants appear to remain in the blackberry pomace after pressing.

ADVISER'S APPROVAL: William G. McGlynn