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Blackberries have been enjoyed for their delicious flavor and high nutritional value for hundreds of years. In North America, blackberries were cultivated since 1850-1860. Blackberries have their characteristic and unique aromas depending on the cultivar and growing conditions. The objective of this study was to evaluate the impact of seasonal variation and growing environment on blackberry aroma.

Volatile compositions of 'Marion' and 'Thornless Evergreen' blackberries from three growing seasons were analyzed using gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS). Seasonal variations were observed for some volatile compounds in these two blackberry cultivars. In 'Marion', the compounds were mainly acids, while in 'Thornless Evergreen', they were mainly acids, alcohols, and a few terpenoids. Although

seasonal variations were present, it was generally observed that the most abundant volatiles in 'Marion' blackberry were acetic, 2/3-methylbutanoic, hexanoic and decanoic acids and linalool, whereas the most abundant volatiles in 'Thornless Evergreen' were 2-heptanol, hexanol, octanol, α-pinene, nopol and *p*-cymen-8-ol. Compared with 'Marion', 'Thornless Evergreen' contained significantly more total volatiles, especially in alcohols, terpenoids and phenols, whereas 'Marion' contained more organic acids. Odor Activity Values (OAVs) were used to determine the most potent odorants in each cultivar. The compounds with high odor activity values (OAV > 10) in 'Marion' were ethyl hexanoate, β-ionone, linalool, 2-heptanone, 2-undecanone, α-ionone and hexanal. The compounds with high odor activity values (OAV >10) in 'Thornless Evergreen' were ethyl hexanoate, 2-heptanone, ethyl 2-methylbutanoate, 2-heptanol, 3-methylbutanal, α-pinene, limonene, *p*-cymene, linalool, *t*-2-hexenal, myrtenol, hexanal, 2-methylbutanal and sabinene.

To investigate the influence of growing environment on blackberry aroma, aroma extraction dilution analysis (AEDA) was employed to characterize the aroma profile of 'Chickasaw' blackberries from two growing regions in United States: Oregon and Arkansas. Eighty-seven odorants were detected in the two berries, in which seventy-seven could be identified. Comparative AEDA analysis showed that the berries grown in these two regions had similar aroma compositions, however, those odorants had various aroma impacts in each region. The compounds with high FD factors in Oregon's 'Chickasaw' were ethyl butanoate, linalool, methional,

trans,cis-2,6-nonadienal, *cis*-1,5-octadien-3-one, and 2,5-dimethyl-4-hydroxy-3(2H)-furanone, whereas in the 'Chickasaw' grown in Arkansas, they were ethyl butanoate, linalool, methional, ethyl 2-methylbutanoate, β-damascenone and geraniol. The flavor formation in 'Chickasaw' blackberries was largely influenced by their growing environment.

IMPACT OF SEASONAL VARIATION AND GROWING ENVIRONMENT ON BLACKBERRY AROMA

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APPROVED:

Major Professor, representing Food Science and Technology

Head of the Department of Food Science and Technology

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Yuanyuan Wang, Author

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CHAPTER I

GENERAL INTRODUCTION

INTRODUCTION

Blackberries (*Rubus* L) are native to North and South America, Europe, and Asia. Today this popular fruit is widely distributed over all land areas except for polar ice caps (Pritts and Handley, 1994). In North America, blackberry has long been a favorite food and people enjoy its delicious flavor and high nutritional value. Recent studies show that blackberries contain high levels of phenolic compounds that may reduce the risk of chronic diseases such as coronary heart disease, cancer, and diabetes (Heinonen, et al., 1998, Meskin, 2004, Scalbert and Williamson, 2000). Blackberries are also a good source of ellagic acid, which is reported as a potential anticarcinogen (Wada and Ou, 2002).

Cultivation of blackberries in North America started around 1850-1860. Many new blackberry cultivars have been developed since that time. Among these new cultivars, there are remarkable improvements in production, fruit quality, and disease resistance. 'Marion' and 'Thornless Evergreen' are the most important trailing blackberry cultivars planted in the Pacific Northwest (Strik, 1992). First released in 1956, 'Marion' is now the predominant blackberry cultivar in this region, accounting for 50% of the total hectarage, and is continually to be widely planted. 'Marion' has developed an outstanding reputation primarily because of its high fruit quality, including its unique and delicious flavor, small

seed size, and good texture for processing (Finn and Strik, 1997). 'Thornless Evergreen' is the second most widely planted trailing cultivar in the Pacific Northwest (Waldo, 1977). Compared to 'Marion', 'Thornless Evergreen' offers the advantage of higher yields and a greater tolerance to cold temperatures. 'Chickasaw' is a high quality, productive blackberry that was first developed in Arkansas and released in 1998 (Clark and Moore, 1999). 'Chickasaw' produces attractive, large, and firm fruit with a good flavor typical for erect blackberries. While 'Marion' and 'Thornless Evergreen' are primarily grown for processed markets, 'Chickasaw' is a fresh market cultivar.

Although sensorial qualities, in particular aroma, can influence consumers' acceptance and purchase preferences, the aroma composition of blackberry has hardly been studied. Most blackberry studies have investigated the volatile composition of the 'Evergreen' cultivar, the predominant cultivar planted world-wide before the 1980's (Georgilopoulos and Gallois, 1987a, Georgilopoulos and Gallois, 1987b, Georgilopoulos and Gallois, 1988, Gulan, et al., 1973, Houchen, et al., 1972, Humpf and Schreier, 1991, Li, et al., 1998, Scanlan, et al., 1970). Very few publications report aroma-active compounds in blackberries. Recently, Klesk and Qian (2003a, 2003b) studied aroma compounds in 'Marion' and 'Thornless Evergreen' blackberry using dynamic headspace GC/Olfactometry (GCO) and aroma extract dilution analysis (AEDA) technique. Results showed that the most important aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries are 2,3-butanedione, 2-heptanol, linalool, dimethyltrisulfide,

1-penten-3-one, methional, ethyl 2-methylbutanoate, benzaldehyde, hexanal, 2,5-dimethyl-4-hydroxy-3-(2H)- furanone, 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone, 4-hydroxy-5-methyl-3(2H)-furanone, and 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone. Because there is no single compound having a "typical" blackberry odor, the authors concluded that the aromas of 'Marion' and 'Thornless Evergreen' blackberries were probably mixtures of these compounds in certain proportions.

Blackberry aroma is mainly formed during a brief ripening period and is affected by genetic and environmental factors. Genetic factors such as genotype makeup and metabolism are the primary determinants of fruits flavor, while environmental factors related to fruit growth and cultivation, such as climate, pre- and post-harvest treatment, soil and fertilization, also affect flavor formation. The cultivar and environmental influences on flavor formation have been examined in several small fruits, including strawberry, raspberry, blueberry and grape (Belancic, et al., 1997, Bureau, et al., 2000, Forney, 2001, Forney, et al., 2000, Hanson, et al., 1994, Klesk, et al., 2004, Marais, et al., 1999, Moore, et al., 2002, Reynolds, et al., 1996), however, no work has been done on blackberry.

The objectives of this study were to evaluate the seasonal variation of 'Marion' and 'Thronless Evergreen' blackberry volatiles and the impact of growing environment on 'Chickasaw' blackberry aroma. The results of this study could help to better understand the important aroma contributors to blackberries and facilitate future blackberry breeding programs.

This study was carried out in two parts: "Seasonal variation of volatile composition and odor activity value of 'Marion' and 'Thornless Evergreen' blackberries", and "Impact of growing environment on 'Chickasaw' blackberry aroma evaluated by gas chromatography olfactometry dilution analysis". The objective of the first part was to provide comparative quantitative results on the volatile compositions of the two cultivars through three harvest seasons (years 1999, 2001, and 2002) using gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS) and calculate odor activity values of some important aroma compounds. The objectives of the second part were to identify the aroma compounds in 'Chickasaw' blackberry using gas chromatography/olfactometry (GCO), to compare the aroma profile in 'Chickasaw' blackberries grown in Oregon and Arkansas by aroma extraction dilution analysis (AEDA) technique, and to elucidate the influence of growing environment on aroma quality.

LITERATURE REVIEW

Blackberries, often termed "caneberries" or "brambles", belong to the genus *Rubus,* subgenus *Eubatus*, and include a diverse group of plants. They are native to North and South America, Europe, and Asia. Today this popular fruit is widely distributed over all land areas except for polar ice caps. In North America, blackberry has long been a favorite fruit, not only due to the good flavor but also to its high nutritive value. Blackberries are good sources of carbohydrates, dietary

fiber, vitamins and minerals, whereas virtually no fat (Ravai, 1996). In addition, blackberries have high content of phenolic compounds that may reduce the risk of chronic diseases such as coronary heart disease, cancer, and diabetes (Heinonen, et al., 1998, Meskin, 2004, Scalbert and Williamson, 2000).

The cultivation of blackberries in the North America started around 1850-1860. The 'Evergreen' and 'Himalaya' types from Europe were among the first to be grown. In 1867, 18 blackberry cultivars were listed and most of them were native seedlings and selections. By the late 19th century, some commercial blackberry cultivars had been developed, including Lawton, Logan, Young, Eldorado, and Snyder. In the United States, blackberry production reached 40, 000 acres in 1948, mainly in Texas, Oklahoma, Michigan, Arkansas, Missouri, Ohio, New Jersey, Tennessee, Kentucky, West Virginia, Pennsylvania, New York, North Carolina, Illinois, Washington, Oregon, and California. Today, all the Pacific Coasts states are active blackberry producers. The South Central region is also an important production region. In the North and much of the East there is active interest in thornless types. There has been a remarkable increase in production, fruit size, disease resistance, and longer shelf life (Pritts and Handley, 1994).

The Blackberry Production in the Pacific Northwest

Today, the Pacific Northwest is the leading blackberry production region in the world as its climatic and soil conditions are very ideal for growing blackberries.

Based on the growth habit, the blackberries planted in the Pacific Northwest could be classified into three types: trailing, erect, and semi-erect. Trailing and semi-erect types require support, whereas erect types do not. Currently, trailing type are the predominant blackberries planted in the Pacific Northwest, accounting for 98% of the total blackberry hectarage. The hectarage of erect and semi-erect types in this region is relatively small, but will likely increase in future (Strik, 1992).

Trailing Blackberry Cultivars

'Marion', 'Thornless Evergreen', and 'Boysen' are the most important trailing blackberry cultivars planted in the Pacific Northwest. 'Marion' is a raspberry-blackberry hybrid cultivar and was first released in 1956. Now it is the predominant blackberry cultivar in this region, accounting for 50% of the total hectarage, and is continually to be widely planted (Strik, 1992). 'Marion' is highly prized for its unique and good flavor, small seed size, and good texture for processing (Finn and Strik, 1997). Despite its relatively sensitivity to cold injury and thorny canes, 'Marion' is still the top choice for new plantings. 'Thornless Evergreen' is the second most widely planted trailing cultivar in the Pacific Northwest. A thornless clone of this cultivar was selected from wild harvested stands. Compared to 'Marion', 'Thornless Evergreen' offers the advantage of higher yields and a greater tolerance to cold temperatures. However, the fruit quality is inferior to 'Marion'.

'Boysen' is also a raspberry-blackberry hybrid cultivar. Due to its very unique flavor and appearance, 'Boysen' is sold with cultivar identification (Hall, et al., 2002). The seed sizes of 'Boysen' are bigger than those of 'Marion', but the flavor, color and texture of 'Boysen' are very desirable for processing. Other trailing blackberry cultivars planted in the Pacific Northwest include 'Kotata', 'Silvan', 'Olallie', 'Waldo', and 'Logan' (Strik, 1996).

Erect Blackberry Cultivars

'Navaho', 'Cherokee', and 'Shawnee' are the most commonly planted erect cultivars in the Pacific Northwest. 'Cherokee' and 'Shawnee' are the two mostly planted cultivars in Oregon, while 'Cherokee' is preferred by growers. It is generally accepted that 'Cherokee' has superior growth, yield, and fruit quality to 'Shawnee'. In California, 'Cherokee', 'Shawnee', and 'Comanche' are planted in similar proportions. 'Cheyenne' and 'Choctaw' were also planted in some areas of the Pacific Northwest.

Semi-erect Blackberry Cultivars

'Chester Thornless' and 'Hull Thornless' dominated the hectarage of semi-erect blackberry crop in the Pacific Northwest. Growers in Oregon prefer 'Chester Thornless' because it has very high yields (averaging over 12 tons/acre),

firm and medium size of fruit, and good flavor. However, in California both cultivars are choices for new plantings. In Washington, 'Chester' is the predominant semi-erect cultivar planted.

The Flavor Formation of Fruits

To fully understand the aroma of fruits, it is not only necessary to know their volatile compositions, but also the metabolisms of these flavor compounds. Fruit aroma composition is specific to species and often to genotype, however, it does not imply that the metabolic pathways are totally different. Some metabolic pathways are shared by most fruits. Generally, volatile biogeneration in fruit uses three main chemical compound classes: fatty acids, amino acids, and carbohydrates.

Fatty Acid Metabolism

Fatty acids are the major precursors of many volatile compounds responsible for fruit flavor. Fatty acids in general are catabolized though two main oxidative pathways: β-oxidation and lipoxygenase (LOX) pathway (Sanz, et al., 1997). During the fruit ripening period, β-oxidation pathway produces the "primary aroma", the aroma generated in intact fruits (Schreier 1984). Fatty acids, more precisely acyl-CoA derivatives, are metabolized to shorter chain

acyl-CoAs by sequentially losing two carbons during each round of the β-oxidation cycle. The enzymes involved in the oxidation cycle for saturated fatty are, in order, acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, acetyl-CoA acetyltransferase, and thiolase. In most cases, the acyl-CoAs resulting from β-oxidation are converted into esters via alcohol acyltransferase (Paillard, 1979).

In the case of unsaturated fatty acids, an auxiliary enzyme is required to complete the oxidation. After three cycles of β-oxidation on oleoyl-CoA, (*Z*)-3-enoyl-CoA is produced. Enoly-CoA isomerase isomerizes (*Z*)-3-enoyl-CoA to (*E*)-2-enoyl-CoA, so the latter could be catalyzed by enoyl-CoA hydratase in the next step. For polyunsaturated fatty acids, the process is similar to that for oleoyl-CoA but one more auxiliary enzyme is required. The product of enoyl-CoA hydratase is *R*-(–)-3-hydroxyacyl-CoA from polyunsaturated fatty acids, instead of the (*S*)-(+)-enantiomer produced from saturated fatty acids. The (*S*)-(+) isomer is formed via 3-hydroxyacyl-CoA epimerase, thereby it could be used by 3-hydroxyacyl-CoA dehydrogenase of the normal β-oxidation cycle (Sanz, et al., 1997).

Through plant cell disruption, such as crushing and slicing, lipoxygenase (LOX) pathway produces a variety of C_6 and C_9 aroma compounds, which are so-called "secondary flavors". Many fruit acids, alcohols, aldehydes, and esters are derived from LOX degradation of linoleic and linolenic acids (Olias, et al., 1993, Pérez, et al., 1999, Stone, et al., 1975). This enzymatic oxidative

degradation starts with acylhydrolases, which free polyunsaturated fatty acids from glycolipids, phospholipids, or triacylglycerols. Through the action of LOX and LOX isozymes, linoleic and linolenic acids degrade and produce fatty acid hydroperoxides. The site of oxygen incorporation could be preferentially at C_9, C_{13}, or non-specifically at either carbon, depending on the plant source and isozymes present. Hydroperoxide lyase converts these fatty acid hydroperoxides to aldehydes and oxoacids (Sanz, et al., 1997).

Based on the substrate specificity, there are three classes of hydroperoxide lyases: C_9-, C_{13}-, and non-specific hydroperoxide lyases. The substrate specificity of hydroperoxide lyases can determine the aroma composition in many fruits. Hydroperoxide lyase cleaves 13-hydroperoxylinoleic acid to 12-oxo-(9*Z*)-dodecenoic acid and hexanal. For 13-hydroperoxylinolenic acid, the same acid is obtained, but the aldehyde is (3*Z*)-hexenal. When the substrates are 9-hydroperoxides of linoleic and linolenic acids, the products are 9-oxononanoic acid and (3*Z*)-nonenal or (3*Z*,6*Z*)-nonadienal respectively (Sanz, et al., 1997). Compounds with a (3*Z*)-enal structure are isomerized to the (2*E*)-enal form by (3*Z*,2*E*)-enal isomerase in most plants. Before or after the isomerization, alcohol dehydrogenase acts on these unsaturated carbonyls to produce the corresponding alcohols. These alcohols are the natural substrates for alcohol acyltransferase and could be converted to esters (Sanz et al, 1997).

Amino Acid Metabolism

Amino acids also represent an important source of volatile compounds contributing to the aroma of fruits, either as direct or indirect precursors. As direct precursors, the metabolism of amino acids could generate alcohols, carbonyls, acids, and esters, either aliphatic, branched or aromatic (Sanz, et al., 1997). These volatile compounds contribute to and in some cases determine the primary aroma of many fruits. Radioactive labeling studies showed that the transformation of these amino acids was similar to that of Ehrlich pathway in yeast (Sanz, et al., 1997). Three enzymatic classes, aminotransferase, decarboxylase, and alcohol dehydrogenase were involved in this transformation.

In the first step, the amino acid degrades and a 2-oxoacid is produced by an amino acid-specific 2-oxoglutarate aminotransferase. The 2-oxoacid is decarboxylated in the next step by an enzymatic complex similar to pyruvate dehydrogenase (decarboxylating) or 2-oxoglutarate dehydrogenase in the Krebs cycle (Sanz, et al., 1997). After this, the resulting aldehydes are converted to alcohols or acids via alcohol dehydrogenase or aldehyde oxidase, respectively. Acyl-CoA products generated by the 2-oxoglutarate dehydrogenase are transformed into esters via alcohol acyltransferase.

Aromatic amino acids (tyrosine, phenylalanine) are important precursors for a group of compounds with phenolic and spicy odor notes. A different volatile metabolic pathway has been suggested for aromatic amino acids, in which cinnamic acid and its *p*-hydroxy derivative (*p*-coumaric acid) are suggested as the

starting intermediates. Cinnamic acid is derived from phenylalanine and transformed into to *p*-coumaric acid and benzoic acid derivatives. Para-coumaric acid is also derived from tyrosine and gives rise to phenolic derivatives (Sanz, et al., 1997).

As indirect precursors, amino acids are transformed into new amino acids through different metabolic pathways to generate volatile compounds. Sulfur-containing volatile compounds are produced from these amino acid derived compounds after enzymatic degradation upon cell disruption (Sanz, et al., 1997). This pathway is mainly found in vegetables, but also exists in fruits.

Carbohydrate Metabolism

A few classes of aroma compounds in fruits are directly generated from carbohydrates. In them, terpenes are produced from carbohydrates metabolism through the isoprenoid pathway (Sanz, et al., 1997). Mevalonic acid (MVA) is thought to be the first specific precursor in terpene biogenesis, which is then converted to isopentenyl diphoshate (IPP), the hypothetical 'active isoprene' unit from which all isoprenoid compounds are built.

Firstly, MVA is transformed into mevalonic acid diphosphate (MVAPP) by two steps of phosphorylation via mevalonate kinase and 5-phosphomevalonate kinase sequentially. Then IPP is formed by decarboxylation and dehydration of MVAPP via MVAPP decarboxylase. A molecule of IPP is isomerized to the dimethylallyl diphosphate form (DMAPP) by isopentenyl diphosphate isomerase.

Finally, prenyltransferases catalyze the condensation of DMAPP and IPP to produce geranyl diphosphate (GPP), the direct precursor of monoterpenes. From GPP, volatile monoterpenes are produced through the enzymatic reactions of hydrolysis, cyclations, and oxidoreductions (Sanz, et al., 1997).

Furanones are also generated from carbohydrate metabolism. In them, 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (furaneol) and its derivative 2,5-dimethyl-4-methoxy-3-(2H)-furanone (mesifurane) were identified as important aroma compounds in many fruits, such as pineapple, red raspberry, strawberry, mango, arctic raspberry, grapefruit, and tomato (Sanz, et al., 1997, Schwab, 1998). Despite the importance of furanones for fruit aroma, little is known about their biosynthesis and metabolism. Based on the study of strawberries, it was demonstrated that furaneol and its derivatives mesifurane and 2,5-dimethyl-4-acetoxy-3-(2H)-furanone (furaneol acetate) were formed from D-fructose in a biological Maillard reaction (Schwab, 1998).

Esters are one of the largest groups of volatile compounds in fruit aroma. They are formed through the enzymatic actions on alcohols and acyl CoA's that are derived from both fatty acid and amino acid metabolism (Wyllie and Fellman, 2000). However, little is known about the final step of esterification reaction. Studies in micro-organisms indicated that two enzymes were implicated in ester formation: alcohol acyltransferase (AAT) and esterase. AAT could transfer an acyl moiety of an acyl-CoA onto the corresponding alcohol, while esterase hydrolyzes esters. These enzymatic activities have been described in fruits. The

major factors affect the ester biogeneration are fruit ripeness, availability of substrates, the substrate specificity of alcohol acyltransferases for both acyl-CoAs and the corresponding alcohols (Sanz, et al., 1997).

Factors Affecting the Development of Volatile Compounds in Fruits

Genetic Factors

The genetic composition determines the enzyme systems and their activities during flavor formation. Each fruit species has its own metabolic pathway and produces its characteristic flavor profile that could be distinguished from other species. In the same fruit species, it is also not uncommon to detect aroma differences among cultivars. However, in most cases these differences are due to quantitative difference in flavor composition rather than qualitative differences.

Paillard (1967) investigated the volatile compounds in nine apple cultivars and found that all the cultivars contained the same volatiles but the proportions were different. Later, "Charm" analysis was applied to determinate the aroma-active compounds in 40 apple cultivars (Cunningham, et al., 1985). Some common volatile compounds were found to be important in all of these apples. However, the aroma was not the result of same compounds in every cultivar and no two cultivars showed close resemblance. Similar results were also observed in strawberries (Forney, et al., 2000), raspberries (Honkanen and Hirvi, 1990b), and

tomatoes (Baldwin, et al., 1991a, Baldwin, et al., 1991b). Forney and Jordan detected a 35-fold difference in the quantity of volatiles in five strawberry cultivars, including 'Annapolis', 'Cavendish', 'Honeoye', 'Kent', and 'Micmac'. Butanoates and hexanoates are the main esters in strawberries, but their relative proportions vary among cultivars (Forney, et al., 2000). In raspberries, the concentrations of several important aroma compounds including α- and β-ionones were found to be higher in fruits from wild species than fruits from commercially cultivated. Additionally, wild raspberry genetypes contained numerous aroma compounds that were not present in cultivated raspberries (Honkanen and Hirvi, 1990c). Sensory test results showed that the flavor of fresh wild raspberries is much stronger and more pleasant than the cultivated raspberries (Honkanen and Hirvi, 1990b). In tomato, the differences in volatile composition among cultivars were detected by qualitative and quantitative techniques (Baldwin, et al., 1991a, Baldwin, et al., 1991b).

Molecular biological techniques have been applied to investigate the flavor formation mechanisms. The fate of amino acids in relation to aroma biogenesis was studied in strawberries using the in vitro growth approach (Pérez, et al., 2002). Incubations of strawberries with L-isoleucine resulted in an increase in 14 aroma compounds, a 7-fold increase in the sum of 2-methylbutanoate esters, and a double production of 2-methylbutyl esters compared to those of the control berries. Tomato flavor appears to be related to the ethylene production, which is synthesized by the aminocyclopropanecarboxylic acid (ACC) synthase. When the

expression of ACC synthase was inhibited, tomato fruit produced low levels of many important flavor volatiles and the flavor quality was deteriorated (Baldwin and Scott, 1992, Baldwin, et al., 2000). Transgenic fruit with antisense pectinmethylesterase that demethylates pectin in cell walls, contained lower levels of methanol, while fruit with downregulated phytoene synthase, an enzyme involved in carotenoids pathway, had lower levels of carotenoid-derived volatiles.

Environmental Factors

Pre-harvest factors

Pre-harvest factors, such as temperature, water availability, soil, climate, and chemical application, significantly affect flavor formation in plants. The influence of climate on carrot flavor was demonstrated. The same carrot cultivar was grown in controlled environments which simulated the climatic conditions in California, Florida, and Wisconsin (Simon, et al., 1980). Flavor and compositional differences were observed among the three carrots. The amounts of carotenoids and terpenes were higher in carrots planted under the simulated Wisconsin condition than those in California and Florida conditions.

Crop nutrition and fertilizer treatment also influence the production of volatile compounds. The flavor of onion, garlic, *A. vineale*, cabbage, mustard and watercress largely depends on the sulphate content of the growth medium (Freeman and Mossadeghi, 1970, Freeman and Mossadeghi, 1971, Freeman and Mossadeghi, 1972a, Freeman and Mossadeghi, 1972b). Onions grown under sulphate

deficiency lacked their typical flavor. Somogyi et al (1964) demonstrated that the quantities of volatiles increased in 'McIntosh' apples with nitrogen fertilizers. Tomato fruit treated with increased levels of nitrogen (N) and potassium (K) fertilizers showed increased levels of titratable acidity, soluble solids, and several volatiles, but lower flavor scores (Wright and Harris, 1985).

Water avaiablity is another factor that influences flavor formation during fruit growth. Plentiful rainfall often causes large lush fruits with reduced flavor, while inadequate rainfall results in smaller sized fruits with more intense flavor (Freeman, 1979).

The quantity and quality of sunlight during fruit growth can also affect the fruit flavor and volatile composition. In the early ripening stage of 'Syrah' grapes, direct sunlight on fruits promoted the formation of carotenoid precursors (Razungles, et al., 1998). In another study, effects of the modification of whole vine or individual cluster light environment by shade cloth from berry set to maturity were studied on the volatiles and glycoconjugated in 'Muscat' (Bureau, et al., 2000). Results showed that the artificially shaded bunches contained lower levels of monoterpenols and C13 norisoprenoids than sun-exposed berries and berries from naturally shaded bunches. Treatments affecting ethylene metabolism can also affect flavor formation in fruits. In pears, pre-harvest treatment with aminoethoxyvinylglycine (AVG, an ethylene production inhibitor) suppressed the volatile production by 50%, while fruit treated with ethylene exposure, the volatile production increased (Khan, et al., 2001, Romani, et al., 1983).

Harvest maturity

The formation of volatile compounds during the ripening is dynamic. Volatiles change qualitatively and quantitatively. Furanones and esters are the main aroma compounds in strawberry, but they are not detected until fruit are over 50% red. C_6 compounds such as hexanal, *cis*-3 hexenal, and hexanol were the main compounds in immature fruits. During maturation the levels of C_6 compounds decreased drastically, whereas furanones and esters increase significantly (Ménager, et al., 2004). In cantaloupe esters increased progressively after the pollination and the following ripening stage, while most aldehydes increased in early growth stages and then tapered off with increasing harvest maturity (Beaulieu and Grimm, 2001).

Fruits should be harvested at optimal eating quality. However, today the trend is to pick fruits that are not fully ripe. These fruits are firm and suffer less physical damage during shipping and handing, and have extended shelf life. Literatures generally indicate that once the fruit are picked and artificially ripened, its flavor develops differently from the unpicked fruit. The characteristic flavor of peach is from lactones and their concentrations increase as the stage of maturity advances. Artificial ripening of peaches does not result in similar levels of lactones in the naturally ripened fruit. These peaches only contained about 20% of the total lactones compared with the tree-ripen peaches. The levels of benzaldehydes and total esters were also lower in the artificially ripened peaches (Do, et al., 1969). Studies on tomato fruit indicated that the flavor of artificially

ripen fruits was not only reduced in comparison to the vine-ripen product, but also was abnormal in character. It was suggested that immature tomatoes lack equivalent enzyme systems as the vine-ripen tomatoes (Yu, et al., 1967, Yu, et al., 1968a, Yu, et al., 1968b, Yu, et al., 1968c, Yu and Spencer, 1969, Yu and Spencer, 1970). During the ripening stage, more enzyme systems develop that are important for flavor formation. Therefore, it is possible that the artificially ripen fruit is short of the characteristic tomato flavor due to the lack of corresponding enzyme systems.

Post-harvest factors

Various storage techniques are used in order to maximize the shelf-life, control post-harvest decay, and eliminate pests. These techniques include temperature control, irradiation, chemical application, and modified atmosphere.

One extreme example of the flavor quality affected by storage conditions comes from the studies on banana. Banana volatile production is an exponential function of storage temperature from 5 to 25°C. Temperatures lower than 5°C resulted in a lack of characteristic banana flavor, while temperatures higher than 27°C resulted in abnormal flavor development (Paillard, 1981).

Tomatoes stored at 2, 5, 10, and 13°C contain lower levels of important volatiles, less ripe aroma, and more off-flavors compared to fruit stored at 20°C (Maul, et al., 2000). Winter pears, unlike other fruits, required a period of time at

-1 to 0°C to ripen with good quality (Knee, 1987). Pears need to be stored at a low temperature for a certain number of days and then the temperature was increased to 20°C to develop good pear flavor. The length of chilling period is cultivar dependant with a range from 15 to 60 days (Gerasopoulos, 1988, Hansen, 1966, Richardson and Gerasopoulos, 1994).

Heat treatments used for pre-conditioning and decay control can also resulte in altered volatile profiles (McDonald, et al., 1996). The effects of hot-water treatments on the flavor development of three olive cultivars were investigated. The thermal treatments affected the aroma quality differently among cultivars, but in general the amounts of C5 compounds and C6 aldehydes were decreased in all three cultivars after the heat treatment (Pérez, et al., 2003). Freezing is another widely used method for maintaining fruit quality during long-term storage (Skrede, 1996). However, the freezing and thawing process can change the flavor quality (Larsen and Poll, 1995, Ueda and Iwata, 1982).

Controlled atmosphere (CA) storage altered flavor of apples and reduced volatile emission compared to that of air-stored fruit (Mattheis, et al., 1995). When apples were stored at 5% CO_2 + 2% O_2 atmosphere after 5 1/2 months, the flavor production was nearly completely inhibited (Paillard, 1981).

Modified atmosphere (MA) techniques (e.g., package and edible coatings) are widely used to extend storage life. Levels of O_2 and CO_2 can be reduced as CA does. However, when the O_2 levels are below the tolerance limit of fresh fruits, anaerobic respiration increases and off-flavors are detected. The effect of

edible coating on flavor quality and the levels of volatile compounds have been investigated in citrus (Cohen, et al., 1995), apple (Saftner and Conway, 1999), and mango (Baldwin, et al., 1999). Results indicated that the coating barrier entrapped volatiles and induced anaerobic respiration and the synthesis of ethanol and acetaldehyde(Baldwin, et al., 1999).

Volatile compounds were investigated from strawberries stored at 1°C under modified atmosphere packaging (MAP) conditions in high barrier pouches flushed with either carbon dioxide, mixed gases, or air (Shamaila, et al., 1992). After 10 days storage, the total amount of volatiles of MAP strawberries was less than those of unpackaged strawberries. The sensory ratings for strawberry odor and overall quality were also declined for MAP strawberries compared to unpackaged strawberries. This was confirmed by another study in which strawberry fruit stored in a CO_2 saturated atmosphere showed significant changes in volatile levels (Dourtoglou, et al., 1995).

In addition to CA and MA techniques, the effects of other gaseous treatments on flavor development in fruits have been reported. Ethylene plays an important role as a modulator of ripening in climacteric fruits and the application of ethylene to synchronize ripening has been practiced for years on banana and tomato (Defilippi, et al., 2004). 1-Methylcyclopropene (MCP), an ethylene action inhibitor, can totally inhibit ethylene production in fruits, thus influences the flavor development (Abdi, et al., 1998, Fan, et al., 1999, Golding, et al., 1998). Methyl jasmonates (MJ) is another growth regulator modulating climacteric fruit ripening.

Effects of MCP and MJ treatments on the volatile compound production in apples were investigated by Fan and Mattheis (1999). The production of ethylene and many volatile alcohols and esters were inhibited by MCP treatment alone or by a combination treatment. Precursor atmosphere (PA) technology is a short-time storage biotechnology that uses intact and mature fruit cells as a biocatalyst to produce fruit flavor (Berger, et al., 1992). The fruits are exposed to a controlled atmosphere containing vapors of precursor substrate, e.g., volatile alcohols. Studies showed that the application of acetaldehyde vapors to blueberries, tomatoes, and pears led to the enhancement of the fruit sensory quality including an increase in the sugar content, sugar-acid ratio, and hedonic sensory rating, while the application of ethanol led to similar but limited enhancement of sensory quality (Paz, et al., 1981).

Blackberry Aroma

Compared to other small fruits such as raspberry or strawberry, the analysis of blackberry aroma is limited (Ancos, et al., 2000, Azodanlou, et al., 2003, Hakala, et al., 2002, Honkanen and Hirvi, 1990a, Shamaila, et al., 1993, Zabetakis and Holden, 1997). The early stage studies of volatile analysis for blackberries mainly focused on 'Evergreen' cultivar, the predominant blackberry planted world-wide before the 1980's (Georgilopoulos and Gallois, 1987a, Georgilopoulos and Gallois, 1987b, Georgilopoulos and Gallois, 1988, Gulan, et al., 1973,

Houchen, et al., 1972, Humpf and Schreier, 1991, Li, et al., 1998, Scanlan, et al., 1970).

Georgilopouls and Gallois did a thorough study on the volatile compounds in fresh and heated blackberries (Georgilopoulos and Gallois, 1987a, Georgilopoulos and Gallois, 1987b). A total of 245 compounds were identified in fresh 'Thornless Evergreen' blackberries. Based on chemical classes, the volatiles in fresh blackberries could be divided into alcohols (65.28%), ketones (8.25%), aldehydes (5.12%), esters (3.64%), ethers (1.40%), phenols (1.31%), lactones (0.95%), furans (0.03%), acetals (0.02%), hydrocarbons (trace amount), and acids (trace amount). The aroma of fresh blackberries was mainly due to 2-heptanol, *p*-cymen-8-ol, 2-heptanone, 1-hexanol, α-terpineol, pulegone, 1-octanol, isoborneol, myternol, 4-terpineol, carvone, elemicine, and nonanal. These volatile compounds represented 67% of the total odorous profile. In heated blackberries juices of 'Evergreen' and two wild blackberry genotypes, alcohols, furans and aldehydes were the predominant chemical classes (Georgilopoulos and Gallois, 1988). 2-Hepatnol was the most abundant compound in 'Evergreen', whereas furfural was the most abundant compound in the two wild genotypes. Compared with fresh berries, alcohols were still the predominant chemical class, while the levels of esters decreased. Aldehydes, γ-butyrolactone, γ-hexalactone, and ethyl acetate were present in higher levels in heated juices.

The effects of heating treatment on the aroma profile of blackberry juice were also examined by Georgilopoulos and Gallois (Georgilopoulos and Gallois,

1988). Furans (42%) were found to be the most abundant chemical class followed by aldehydes (21%) and alcohols (10%). The aroma of this juice was mainly due to furfural, 3-methyl-1-butanol, phenylacetaldehyde, and *trans*-furan linalool oxide. Although numerous compounds were identified in blackberries, none of them was described as "characteristically blackberry" aroma. Therefore, blackberry aroma was considered as a complex and delicate mixture.

Very few publications report aroma-active compounds in blackberries. Recently, Klesk and Qian investigated the aroma-active compounds in 'Marion' and 'Thornless Evergreen' blackberries using dynamic headspace GC/Olfactometry (GCO) and aroma extract dilution analysis (AEDA) technique (Klesk and Qian, 2003a, Klesk and Qian, 2003b). Fifty-eight compounds were identified in both berries via dynamic headspace sampling (Klesk and Qian, 2003a). Comparisons between cultivars shows that except for esters ('Marion' = 1.8 × 'Thornless Evergreen') and alcohols ('Thornless Evergreen' = 2.25 × 'Marion'), both cultivars containe equal numbers of aroma compounds in each chemical class. Judged by odor intensities, 2,3-butanedione, 2-heptanol, linalool, dimethyltrisulfide, 1-penten-3-one were possibly the most important aromas in 'Marion', while 2,3-butanedione, ethyl 2-methylbutanoate, methyl hexanoate, α-terpineol, *allo*-ocimene, *l*-carvone, and 1-penten-3-one, β-pinene, 3-methylbutanal, and methional were possibly the most important aromas in 'Thornless Evergreen'. By the application of AEDA, 63 aromas were identified in 'Marion' and 'Thornless Evergreen' blackberries (Klesk and Qian, 2003b). Again, the comparison of

cultivars indicated that both berries have comparable compound types and numbers. However, those compounds showed widely different aroma impact in two blackberries. Based on the flavor dilution (FD) factors, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, hexanal, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone, 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone, 4-hydroxy-5-methyl-3-(2*H*)-furanone, 4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone, 5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone, thiophene, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, 2-methylthiophene, and methional were possibly the most potent odorants in 'Thornless Evergreen' (FD 512-2048). Except for ethyl 2-methylpropanoate, same compounds were detected as major contributors to 'Marion' aroma, but the FD factors (8-256) varied significantly from 'Thornelss Evergreen'.

CHAPTER II

SEASONAL VARIATION OF VOLATILE COMPOSITION AND ODOR ACTIVITY VALUE OF 'MARION' (*Rubus* L) AND 'THORNLESS EVERGREEN' (*R. laciniatus* L.) BLACKBERRIES

MICHAEL QIAN AND YUANYUAN WANG

Journal of Food Science

525 W. Van Buren St., Suite 1000, Chicago, ILL 60607

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ABSTRACT

Volatile compositions of 'Marion' and 'Thornless Evergreen' blackberries from 3 growing seasons were analyzed using gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS). Although seasonal variations were present for both cultivars, it was generally observed that the most abundant volatiles in 'Marion' blackberry were acetic, 2/3-methylbutanoic, hexanoic and decanoic acids, ethanol, and linalool, whereas the most abundant volatiles in 'Thornless Evergreen' were 2-heptanol, hexanol, octanol, α-pinene, nopol, and *p*-cymen-8-ol. Compared with 'Marion', 'Thornless Evergreen' contained significantly more total volatiles, especially in alcohols, terpenes and terpenoids, and phenols, whereas 'Marion' contained more organic acids. Odor Activity Values (OAVs) were determined to identify each cultivar's most potent odorants. The compounds with the high odor activity values (OAV > 10) in 'Marion' were ethyl hexanoate, β-ionone, linalool, 2-heptanone, 2-undecanone, α-ionone, and hexanal. The compounds with the high odor activity values (OAV >10) in 'Thornless Evergreen' were ethyl hexanoate, 2-heptanone, ethyl 2-methylbutanoate, 2-heptanol, 3-methylbutanal, α-pinene, limonene, *p*-cymene, linalool, *t*-2-hexenal, myrtenol, hexanal, 2-methylbutanal, and sabinene.

KEY WORDS:

'Marion'; 'Thornless Evergreen'; blackberry; seasonal variation; aroma; OAV

INTRODUCTION

Blackberry flavor is mainly formed during a brief ripening period and is influenced by internal and external factors. Internal factors are based on plant characteristics such as metabolism and genetic makeup, while external factors depend on fruit growth and cultivation concerns such as climate, soil, fertilization, pre- and post-harvest treatment, and harvest date (Boschetti, et al., 1999, Forney, 2001, Forney, et al., 2000, Naumann and Wittenburg, 1980, Paillard, 1981). Major factors affecting blackberry taste, such as sugars, acids, titratable acidity, and Brix, have been studied by many researchers (Fan-Chiang, 1999, Fitelson, 1970, Plowman, 1991, Sapers, et al., 1985, Widdowson and McCance, 1935, Wrolstad, et al., 1980). However, compared to other small fruit such as raspberry or strawberry, the analysis of blackberry aroma is limited (Ancos, et al., 2000, Azodanlou, et al., 2003, Hakala, et al., 2002, Honkanen and Hirvi, 1990a, Shamaila, et al., 1993, Zabetakis and Holden, 1997). Most blackberry studies investigated the volatile compounds in 'Evergreen' cultivar, the predominant cultivar planted world-wide before the 1980's (Georgilopoulos and Gallois, 1987a, Georgilopoulos and Gallois, 1987b, Georgilopoulos and Gallois, 1988, Gulan, et al., 1973, Houchen, et al., 1972, Humpf and Schreier, 1991, Li, et al., 1998, Scanlan, et al., 1970). Since the early 1980's, 'Marion' replaced the 'Thornless Evergreen' as the leading blackberry cultivar planted in the Pacific Northwest (Finn, et al., 1997, Strik, 1992). Compared with 'Thornless Evergreen', 'Marion' is highly preferred by consumers for its aromatic bouquet and intense flavor.

Very few publications report aroma-active compounds in blackberries. Turemis and others (2003) examined the aroma compositions of 5 blackberry cultivars using immersion solid phase micro extraction technique and found furfural and its derivative were to be the most abundant aromatic compounds in those blackberries, while 5-hydroxymethyl furfural to be the "the main specific blackberry-like aromatic compound". Klesk and Qian (2003a, 2003b) studied aroma compounds in 'Marion' and 'Thornless Evergreen' blackberry using dynamic headspace GC/Olfactometry (GC/O) and aroma extract dilution analysis (AEDA) technique and found that the most important aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries are 2,3-butanedione, 2-heptanol, linalool, dimethyltrisulfide, 1-penten-3-one, methional, ethyl 2-methylbutanoate, benzaldehyde, hexanal, 2,5-dimethyl-4-hydroxy-3-(2*H*)- furanone, 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone, 4-hydroxy-5-methyl-3(2*H*)-furanone, and 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone. Because there is no single compound having a "typical" blackberry odor, the authors conclude that the aroma of 'Marion' and 'Thornless' blackberries is probably a mixture of these compounds in certain proportions. Blackberry aroma, particularly 'Marion' blackberry aroma, is still poorly understood.

The goal of this work was to provide comparative quantitative results on the volatile compositions of the 2 cultivars through 3 harvest seasons (years 1999, 2001, and 2002), using gas chromatography-flame ionization detection (GC-FID)

and GC-mass spectrometry (GC-MS) and calculate odor activity values of some important aroma compounds.

MATERIALS AND MATHODS

Chemicals

Ethyl undecanoate, used as an internal standard, and ethyl decanonate were purchased from Eastman (Rochester, N.Y., U.S.A.). Ethyl hexadecanoate and β-caryophyllene were purchased from Pfaltz & Bauer (Waterbury, Conn., U.S.A.). Limonene, butyl acetate, octyl acetate, 2-heptanone, α- and β-pinene, α-terpineol, 2-nonanone, and 2-undecanone were obtained from K&K Laboratories (Jamaica, N.Y., U.S.A.). Ethyl hexanoate, ethanol, 2-methylpropanol, hexyl acetate, acetic acid, α- and β-ionone, butanoic acid, ethyl acetate, ethyl 2-methylbutanoate, eugenol, 2-heptanol, hexanal, hexanoic acid, *t*-2-hexenal, linalool, linalool oxide, borneol, 3-methylbutanal, 2-methylbutanoic acid, *t*-2-hexenoic acid, octanoic acid, decanoic acid, octanol, phenylethyl alcohol, *t*-3-hexenol, *c*is-3-hexenol, *t*-2-hexenol, *cis*-2-hexenol, camphene, α-phellandrene, α-terpinolene, theaspirane, myrtenal, *p*-cymene, γ-terpinene, eugenol, 2-methyl-3-buten-2-ol, 1-penten-3-ol, 3-methylbutanol, *t*-2-hexenyl acetate, butanol, 2-butanol, benzyl alcohol, 2-pentanone, acetoin, hexanol, heptanol, nonanol, 2-nonanol, decanol, 2-butanone, sabinene, ethyl octanoate, citronellol, 2-pentanol, 1-terpineol, and myrtenol were obtained from Aldrich Chemical Co. Inc. (Milwaukee, Wis., U.S.A.). Sodium chloride was obtained from Fisher

Scientific (Fair Lawn, N.J., U.S.A.). Diethyl ether was obtained from Honeywell Internal Inc. (Muskegon, Mich., U.S.A.). Pentane was obtained from Malinckrodt Baker Inc. (Philipsburg, N.J., U.S.A.).

Blackberry samples

'Marion' and 'Thornless Evergreen' blackberries were grown in Woodburn, Oregon, from 5- and 10-year-old plants. The fruits were machine- and hand-harvested, washed, graded, individually quick-frozen (IQF), and stored at –18 °C. One box of each cultivar (13.6 kg, frozen 5 months) from the 1999, 2001, and 2002 growing seasons, were transported on ice to the laboratory, and stored at –23 °C.

Extraction of volatile compounds

Three hundred grams of IQF berries were thawed at room temperature for 3 h. The berries were blended in a glass blender jar (Waring Products Division, Dynamics Corp. of America, New Hartford, Conn., U.S.A.) for a total of 40 s. Ethyl undecanoate as the internal standard was added before blending. The puréed fruit was transferred to a 1-L Erlenmeyer flask covered with alumina foil and extracted with 100 mL of distilled pentane:diethyl ether (1:1 v/v) on a platform shaker (Innova 2300; New Brunswick Scientific, Edison, N.J., U.S.A.) at 125 rpm, for 3 h. The solvent and juice were poured into a separatory funnel. The juice was drawn off and returned to the fruit; the organic phase was retained. The extraction procedure was repeated twice, yielding a total volume of 280 mL

solvent. Volatile compounds were recovered from the organic extract by using solvent-assisted flavor evaporation (SAFE) at 50 °C under vacuum (Engel, et al., 1999). The organic SAFE extract was dried with anhydrous Na_2SO_4, concentrated to 2 mL by solvent evaporation, and reduced to its final volume of 0.2 mL with a flow of nitrogen. This extraction was done in triplicate for each cultivar and growing season.

GC-FID analysis

The analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID). Samples were analyzed on a DB-Wax column (60 m × 0.32 mm i.d. cross-linked polyethylene glycol 20M, 0.5 μm film thickness; J&W Scientific, Folsom, Calif., U.S.A.). Injector and detector temperatures were 250 °C, the helium column flow rate was 2.0 mL/min at 25 °C, and the 2-μL sample injections were splitless. The oven temperature was programmed for a 4 min hold at 35 °C, then 35 °C to 235 °C at 2 °C/min (30 min hold). Retention indices were estimated in accordance with a modified Kovats method (Van and Kratz, 1963).

GC-MS analysis

The same samples as used for GC-FID analysis (2- μL splitless injections) were analyzed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 Mass Selective Detector (MSD). System software control and data

management/analysis was performed through Enhanced ChemStation Software, G1701CA v. C.00.01.08 (Agilent Technologies, Inc., Wilmington, Del., U.S.A.). Volatile separation was achieved with the same DB-Wax capillary column used in the GC-FID analyses. A constant helium column flow rate was set at 2 mL/min and the same GC oven temperature programming was set as for the GC-FID analysis. Injector, detector transfer line, and ion source temperatures were 250, 280, and 230 °C, respectively. Electron impact mass spectrometric data from m/z 35-300 was collected using a scan rate of 5.27 /s, with an ionization voltage of 70 eV. Retention indices were estimated in accordance with a modified Kovats method (Van and Kratz, 1963). Compound identifications were made by comparing mass spectral data from the Wiley 275.L (G1035) Database (Agilent), and confirmed by comparing Kovats retention indices (RI) to the standards or RI reported in the literature.

Quantitative analysis

Volatile compound concentrations in parts per million (ppm) were calculated based on individual volatile peak area from GC-FID response comparison to the peak of the internal standard. Each tabulated experimental value corresponds to the average of the 3 extraction replicates. Odor activity values (OAV) were calculated by dividing the concentrations of aroma compounds in blackberries with their sensory threshold (Patton and Josephson, 1957).

Statistical analysis

An analysis of variance (ANOVA) was used to test the variances of volatile concentrations from growing season and cultivar. The statistical analysis was performed using the S-PLUS Version 6.1 software (Insightful Corporation, Seattle, Wash., U.S.A.).

RESULTS AND DISCUSSION

The volatile compositions (ppm) for 'Marion' and 'Thornless Evergreen' through 3 growing seasons are given in Table 2.1. Qualitative and quantitative volatile differences can be inferred between the 2 cultivars by using two-way ANOVA (growing season and cultivar). Based on chemical classes or biological origins, these compounds can be divided into acids, alcohols, esters, aldehydes, ketones, terpenes and terpenoids, and phenols. Based on the total concentration for each chemical class, the most abundant volatiles in 'Marion' were acids, followed by alcohols, terpenes and terpenoids, ketones, esters, aldehydes, and phenols. Comparatively, in 'Thornless Evergreen' the most abundant volatiles were alcohols, followed by terpenes and terpenoids, acids, phenols, ketones, esters, and aldehydes.

Based on compound class totals (Table 2.1), 'Thornless Evergreen' contains much greater volatile amounts than 'Marion' (27.33 versus 8.62 ppm). The concentrations of 'Thornless Evergreen' alcohols are 6 times greater than those in

'Marion', while the terpens and terpenoids are 10 times greater. 'Thornless Evergreen' aldehydes, esters, and ketones are, respectively, about 3, 1.5, and 2 times more concentrated than those in 'Marion'.

Table 2.1 data indicates that acids (pungent, cheesy, sour) and alcohols (alcoholic, floral, fruity, green) represent 78.08 % (53.83 + 24.25) of the total volatiles identified in 'Marion'. Terpenes and terpenoids(citrus, piney, terpene-like) account about 10% of the total volatiles while aldehydes (green, fruity, vegetal, 1.62 %), ketones (floral, fruity, pleasant, 5.10 %), esters (floral, fruity, sweet, 4.64%) and phenols (0.23%) account the remaining 12%. In the case of 'Thornless Evergreen', Table 2.1 data shows that alcohols represent 46.62 % of the total volatiles identified, while terpenes and terpenoids account 31.94%. The 6 most abundant volatiles in 'Marion', totaling 5.34 ppm, were acetic, hexanoic, decanoic and 2/3-methylbutanoic acids, ethanol, and linalool. In 'Thornless Evergreen', totaling 14.41 ppm, the 6 most abundant volatiles were 2-heptanol, octanol, α-pinene, hexanol, *p*-cymen-8-ol, and nopol.

Table 2.1 'Marion' and 'Thornless Evergreen' blackberry volatiles (ppm)

DB-Wax RI	Compound	Basis of identification	'Marion' Growing season[a] 2002	2001	1999	ppm[b]	'Thornless Evergreen' Growing season[a] 2002	2001	1999	ppm[b]	Main effect of cultivar (p-value)
	Acids[c]		**5.29**	**5.56**	**3.08**	**4.64**	**5.17**	**1.42**	**1.2**	**2.6**	
1471	acetic acid	MS, RI	3.69bA[d]	0.85aA	0.86aA	1.8	0.08bB	0.02aB	0.03aB	0.04	<0.01*[e]
1642	butanoic acid	MS, RI	0.23a	0.20a	0.12b	0.19	0.12b	0.03a	0.04a	0.07	<0.01
1688	2/3-methylbutanoic acid	MS, RI	0.15bA	0.44a	0.43a	0.34	1.82bB	0.51a	0.52a	0.95	<0.01*
1874	hexanoic acid	MS, RI	0.91aA	3.22bA	1.18aA	1.77	2.27bB	0.66aB	0.43aB	1.12	<0.01*
2002	*t*-2-hexenoic acid	MS, RI	0.03aA	0.13b	0.04a	0.07	0.55bB	0.12a	0.10a	0.26	<0.01*
2085	octanoic acid	MS, RI	0.15	0.19	0.19	0.18	0.06b	0.02a	0.03a	0.04	<0.01
2308	decanoic acid	MS, RI	0.13a	0.53b	0.26a	0.3	0.27b	0.05a	0.05a	0.12	<0.01
	Alcohols[c]		**1.72**	**2.89**	**1.61**	**2.09**	**21.94**	**8.9**	**7.37**	**12.74**	
955	ethanol	MS, RI	0.75aA	1.02aA	0.02b	0.6	0.09B	0.03B	0.02	0.05	<0.01*
1045	2-butanol	MS, RI					0.02	0.01	0.01	0.01	
1060	2-methyl-3-buten-2-ol	MS, RI					0.12	0.09	0.08	0.1	
1113	2-methylpropanol	MS, RI					0.24b	0.03a	0.04a	0.1	
1142	2-pentanol	MS, RI					0.05a	0.02ab	0.03b	0.03	
1167	butanol	MS, RI	0.02A	0.03	0.03	0.03	0.39bB	0.07a	0.06a	0.17	<0.01*
1181	1-penten-3-ol	MS, RI					0.06	0.09	0.04	0.06	
1220	2-methyl/3-methylbutanol	MS, RI	0.02	0.05A	0.04A	0.04	0.03b	0.01aB	0.01aB	0.02	0.02*
1272	3-methyl-3-buten-1-ol	MS, RIL[f]					0.10b	0.03a	0.03a	0.05	
1344	2-heptanol	MS, RI	0.18	0.25	0.37	0.27	4.15	3.95	4.06	4.05	<0.01
1378	hexanol	MS, RI	0.09aA	0.27bA	0.21abA	0.19	4.09bB	1.08aB	0.57aB	1.92	<0.01*
1389	*t*-3-hexenol	MS, RI					0.09b	0.02a	0.01a	0.04	
1410	*cis*-3-hexenol	MS, RI	0.10A	0.11	0.16	0.12	0.26bB	0.13a	0.13a	0.17	<0.01*
1432	*t*-2-hexenol	MS, RI	0.03aA	0.09bA	0.07abA	0.06	0.67bB	0.34aB	0.25aB	0.42	<0.01*
1441	*cis*-2-hexenol	MS, RI					0.02b	0.01a	0.01a	0.01	

Table 2.1 (continued) 'Marion' and 'Thornless Evergreen' blackberry volatiles (ppm)

DB-Wax RI	Compound	Basis of identification	'Marion' Growing season[a] 2002	2001	1999	ppm[b]	'Thornless Evergreen' Growing season[a] 2002	2001	1999	ppm[b]	Main effect of cultivar (p-value)
1481	heptanol	MS, RI					0.15b	0.05a	0.05a	0.08	
1488	6-methyl-5-hepten-2-ol	MS, RIL[g]	0.02A	0.03	0.04	0.03	0.08bB	0.03a	0.04a	0.05	<0.01*
1538	2-nonanol	MS, RI	0.03	0.05	0.05	0.04	0.03			0.03	
1573	octanol	MS, RI	0.09A	0.08A	0.09A	0.09	6.64bB	1.13aB	0.66aB	2.81	<0.01*
1676	nonanol	MS, RI	0.02A	0.03A	0.03A	0.03	0.53bB	0.12aB	0.12aB	0.26	<0.01*
1738	2-undecanol	MS, RIL[h]	0.09a	0.24b	0.11a	0.15					
1787	decanol	MS, RI	0.04A	0.06A	0.03A	0.05	1.54aB	0.42bB	0.24cB	0.73	<0.01*
1912	benzyl alcohol	MS, RI	0.11aA	0.27b	0.20c	0.19	0.64bB	0.27a	0.22a	0.38	<0.01*
1950	phenylethyl alcohol	MS, RI	0.04aA	0.09bA	0.06aA	0.06	1.15aB	0.78bB	0.51cB	0.81	<0.01*
2029	4-phenyl-2-butanol[T]	MS	0.03a	0.07bA	0.05aA	0.05	0.05	0.02B	0.02B	0.03	0.11*
2331	cinnamic alcohol	MS, RIL[i]	0.06aA	0.15b	0.05aA	0.09	0.75aB	0.17b	0.16cB	0.36	<0.01*
	Aldehydes[c]		**0.17**	**0.15**	**0.1**	**0.14**	**0.55**	**0.41**	**0.15**	**0.37**	
925	2-methylbutanal	MS, RI					0.02	0.02	0.01	0.01	
929	3-methylbutanal	MS, RI					0.02	0.01	0.01	0.01	
1098	hexanal	MS, RI	0.08	0.05	0.06	0.06	0.06	0.08	0.03	0.06	0.77
1119	2-methyl-2-butenal	MS, RIL[j]					0.02	0.01	0.01	0.01	
1237	*t*-2-hexenal	MS, RI	0.09A	0.09A	0.04	0.07	0.39aB	0.28aB	0.07b	0.25	<0.01*
1514	*t, t*-2,4-heptadienal	MS, RIL[k]					0.03	0.01	0.01	0.02	
	Ketones[c]		**0.35**	**0.6**	**0.36**	**0.44**	**1.38**	**0.61**	**0.59**	**0.84**	
918	2-butanone	MS, RI					0.01	0.01	0.01	0.01	
992	2-pentanone	MS, RI					0.03b	0.01a	0.01a	0.02	
1006	3-methyl-3-buten-2-one[T]	MS					0.34	0.27	0.36	0.32	
1200	2-heptanone	MS, RI	0.04A	0.06A	0.05A	0.05	0.91bB	0.30aB	0.17aB	0.46	<0.01*
1309	acetoin	MS, RI	0.14bA	0.05aA	0.02a	0.07	0.06bB	0.01aB	0.01a	0.03	<0.01*

Table 2.1 (continuted) 'Marion' and 'Thornless Evergreen' blackberry volatiles (ppm)

DB-Wax RI	Compound	Basis of identification	'Marion' Growing season[a] 2002	2001	1999	ppm[b]	'Thornless Evergreen' Growing season[a] 2002	2001	1999	ppm[b]	Main effect of cultivar (p-value)
1417	2-nonanone	MS, RI	0.01	0.01	0.03	0.02					
1608	2-undecanone	MS, RI	0.13a	0.42b	0.23ab	0.26					
1894	α-ionone	MS, RI	0.01a	0.02b	0.01a	0.01					
1978	β-ionone	MS, RI	0.02a	0.04b	0.02a	0.03					
	Terpenes and terpenoids[c]		**0.27**	**1.6**	**0.74**	**0.86**	**9.56**	**8.59**	**7.76**	**8.73**	
1029	α-pinene	MS, RI	0.02a	0.07b	0.04a	0.04	1.54	2.83	2.29	2.22	<0.01
1075	camphene	MS, RI	0.01a	0.03b	0.02a	0.02	0.13	0.15	0.16	0.15	<0.01
1129	β-pinene	MS, RI					0.05	0.12	0.11	0.09	
1192	α-phellandrene	MS, RI					0.04	0.07	0.06	0.05	
1213	limonene	MS, RI	0.03a	0.07b	0.04ab	0.04	0.29	0.41	0.33	0.34	<0.01
1231	sabinene	MS, RI					0.90b	0.15a	0.06a	0.37	
1262	γ-terpinene	MS, RI	0.01	0.02	0.01	0.01	0.02ab	0.03a	0.02b	0.02	<0.01
1288	*p*-cymene	MS, RI					0.13a	0.23b	0.17ab	0.18	
1301	α-terpinolene	MS, RI	0.06a	0.19b	0.07a	0.11	0.22ab	0.28a	0.13b	0.21	<0.01
1489	*cis*-sabinene hydrate	MS, RIL[l]					0.02a	0.04b	0.01a	0.02	
1496	linalool oxide	MS, RI					0.01			0.01	
1545	camphor	MS, RIL[f]					0.02	0.02	0.02	0.02	
1562	linalool	MS, RI	0.08aA	1.03bA	0.49abA	0.53	0.26bB	0.11aB	0.14aB	0.17	<0.01*
1566	theaspirane (B)	MS, RI	0.01	0.02	0.01	0.01					
1614	4-terpineol	MS, RIL[f]					0.23	0.2	0.26	0.23	
1618	β-caryophyllene	MS, RI	0.003a	0.02b	0.01a	0.01					
1659	1-terpineol	MS, RI					0.01	0.01	0.01	0.01	
1665	myrtenal	MS, RI					0.01	0.01	0.01	0.01	
1717	1,8-menthadien-4-ol[T]	MS					0.03b	0.01a	0.02a	0.02	

Table 2.1 (continued) 'Marion' and 'Thornless Evergreen' blackberry volatiles (ppm)

DB-Wax RI	Compound	Basis of identification	'Marion' Growing season[a] 2002	2001	1999	ppm[b]	'Thornless Evergreen' Growing season[a] 2002	2001	1999	ppm[b]	Main effect of cultivar (p-value)
1721	α-terpineol	MS, RI					1.47b	0.99a	0.93a	1.13	
1728	*l*-borneol	MS, RI					0.56b	0.23a	0.38a	0.39	
1766	*t,t*-α-farnesene	MS, RIL[m]	0.04ab	0.05a	0.02b	0.04					
1791	citronellol	MS, RI					0.01a		0.01a	0.01	
1827	nopol[T]	MS					1.42a	1.21ab	1.00b	1.21	
1831	myrtenol	MS, RI					0.14			0.14	
1881	*p*-cymen-8-ol	MS, RIL[m]	0.01aA	0.10bA	0.03aA	0.05	1.51B	1.17B	1.35B	1.34	<0.01*
2040	perilla alcohol	MS, RIL[l]					0.32	0.12	0.09	0.18	
2137	p-cymen-α-ol[T]	MS,RIL					0.22	0.20	0.20	0.21	
	Esters[c]		**0.32**	**0.67**	**0.21**	**0.4**	**1.11**	**0.3**	**0.39**	**0.59**	
905	ethyl acetate	MS, RI	0.13aA	0.24bA	0.08a	0.15	0.29bB	0.07aB	0.08a	0.15	0.96*
1062	ethyl 2-methylbutanoate	MS, RI					0.01			0.01	
1088	butyl acetate	MS, RI					0.01			0.01	
1251	ethyl hexanoate	MS, RI	0.01ab	0.02aA	0.01b	0.02	0.02b	0.01aB	0.01a	0.01	0.07*
1291	hexyl acetate	MS, RI	0.02aA	0.15bA	0.04a	0.07	0.04aB	0.03abB	0.01b	0.03	<0.01*
1354	*t*-2-hexenyl acetate	MS, RI	0.01aA	0.06bA	0.01a	0.03	0.05bB	0.02aB	0.01a	0.03	0.23*
1367	ethyl *t*-2-hexenoate	MS, RIL[n]					0.02			0.02	
1454	ethyl octanoate	MS, RI	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.02	<0.01
1490	octyl acetate	MS, RI					0.09b	0.02a	0.02a	0.04	
1540	ethyl 3-hydroxybutanoate	MS, RIL[o]					0.16b	0.03a	0.03a	0.07	
1647	ethyl decanonate	MS, RI	0.04aA	0.04a	0.02bA	0.03	0.05aB		0.05aB	0.03	<0.01*
1809	methyl salicylate	MS, RIL[k]	0.03aA	0.11b	0.03aA	0.06	0.19aB	0.07b	0.12cB	0.13	<0.01*
1866	ethyl dodecanoate	MS, RIL[o]	0.05	0.04	0.01	0.03					
2281	ethyl hexadecanoate	MS, RI	0.02A	0.02	0.01	0.01	0.15bB	0.01a	0.05a	0.07	<0.01*

Table 2.1 (continuted) 'Marion' and 'Thornless Evergreen' blackberry volatiles (ppm)

DB-Wax RI	Compound	Basis of identification	'Marion' Growing season[a] 2002	2001	1999	ppm[b]	'Thornless Evergreen' Growing season[a] 2002	2001	1999	ppm[b]	Main effect of cultivar (p-value)
	Phenols[c]		0.02	0.02	0.02	0.02	**2.35**	0.35	0.45	**1.27**	
2039	phenol	MS, RIL[h]					0.06	0.02	0.03	0.04	
2042	methyl eugenol[T]	MS					0.32			0.32	
2205	eugenol	MS, RI	0.02A	0.02A	0.02A	0.02	0.80bB	0.18aB	0.23aB	0.41	<0.01*
2264	elemicin	MS, RIL[p]					1.17b	0.15a	0.19a	0.5	

[a] means of triplicate samples

[b] means of 3 growing seasons; different small letters in the same cultivar and the same row indicate significant differences between seasons ($p < 0.05$)

[c] class row values are totals

[d] different capital letters for the same season and same row indicate siginificantly different between 2 cultivars ($p<0.05$)

[e] *, indicate significantly interaction beween cultivar and growing season ($p<0.05$)

[f] retention index from the literature, Umano and others (2000)

[g] from Jorgensen and others (2000)

[h] from Parada and others (2000)

[i] from Choi and Sawamura (2000)

[j] from Pino and Marbot (2001)

[k] from Vichi and others (2003)

[l] from Verzera and others (2000)

[m] from Buttery and others (2000)

[n] from Dregus and Engel (2003)

[o] from Pino and others (2001)

[p] from Kjeldsen and others (2003)

[T] Tentatively identified by MS only

The major acids found in 'Marion' and 'Thornless Evergreen' blackberries were even-numbered carbon acids, C_2 - C_{10}. Acids were the largest chemical class in 'Marion'. Concentrations of acids varied from season to season for both 'Marion' and 'Thornless Evergreen' blackberries. The dominated acids in 'Marion' were acetic and hexanoic acids which represented about 87% of total acids. Hexanoic acid was dominated in both 1999 and 2001 growing seasons, while acetic acid was dominated in 2002 growing season. The dominated acids in 'Thornless Evergreen' were hexanoic and 2/3-methylbutanoic acids which represented 80% of total acids. The acids were about 4 times higher in 2002 growing season than in 1999 and 2001 growing seasons. On average, the total acids in 'Marion' were twice as much as in 'Thornless Evergreen'. Most of these acids were probably derived from β-oxidation of fatty acids (Sanz, et al., 1997). During fruit ripening, fatty acids, more precisely acyl-CoA derivatives, are metabolized to shorter-chain acyl-CoAs by sequentially losing 2 carbons during each round of the β-oxidation cycle (Sanz, et al., 1997).

The alcohol levels in 'Marion' were relatively small. Except ethanol, most alcohols had concentrations less than 0.5 ppm. Seasonal variations were not obvious. In 'Thornless Evergreen', however, many alcohols were present at very high concentrations (>1.0 ppm). The dominant alcohols were 2-heptanol, octanol, and hexanol. Seasonal variations were observed for hexanol (ranging from 0.57 to 4.09 ppm), octanol (ranging from 0.66 to 6.64 ppm), and decanol (ranging from 0.24 to 1.54 ppm). In all cases, the concentrations of hexanol, octanol and

decanol were highest in 2002 growing seasons and lowest in 1999 growing seasons. On the other hand, seasonal variations were not obvious for 2-heptanol (range from 3.95 to 4.15 ppm), suggesting different metabolic pathway of 2-heptanol from hexanol, octanol and decanol in 'Thornless Evergreen' blackberry. It is possible that fatty acids serve as the precursor for hexanol, octanol and decanol. In addition to hexanol, several other C_6 alcohols (*t*-3-hexenol, *cis*-3-hexenol, *t*-2-hexenol, *cis*-2-hexenol) were also identified. These C_6 alcohols, typically gave green, leafy aromas, could be generated through lipoxygenase pathway of unsaturated linoleic and linolenic acids (Olias, et al., 1993, Pérez, et al., 1999, Stone, et al., 1975). In many types of fruits, this enzymatic oxidative degradation starts with acyl hydrolases, which produce polyunsaturated fatty acids from glycolipids, phospholipids, or triacylglycerols. Through the action of LOX and LOX isozymes, linoleic and linolenic acids are degraded and produce fatty acid hydroperoxides. Hydroperoxide lyase (HL) converts these fatty acid hydroperoxides to aldehydes and oxoacids, while alcohol dehydrogenase (ADH) acts on them to produce the corresponding alcohols (Sanz, et al., 1997).

Aromatic alcohols (benzyl alcohol, phenylethyl alcohol, 4-phenyl-2-butanol and cinnamic alcohol) were identified in both cultivars. 4-Phenyl-2-butanol had slightly higher concentration in 'Marion' while benzyl alcohol, phenylethyl alcohol and cinnamic alcohol were slightly higher in 'Thornless Evergreen'. It is possible that benzyl alcohol, phenylethyl alcohol and cinnamic alcohol share the same pathway, with phenylalanine as the common

precursor as in other fruits such as apples, kiwi, pineapple, strawberry, tomato, quince, passion fruit, and guava, among others (Leahy and Roderick, 1999, Rouseff and Leahy, 1995, Williams, 1993).

Aldehydes and ketones represented a small percentage of total volatiles in both 'Marion' and 'Thornless Evergreen' blackberries. The dominant aldehyde in 'Thornless Evergreen' was *t*-2-hexenal. *t*-2-Hexenal probably shares the same metabolic pathways of other C_6 compounds, e.g., lipoxygenase catalyzed degradation of unsaturated fatty acid, as *t*-2-hexenol was also the major unsaturated C_6 alcohols in 'Thornless Evergreen'. None of the aldehydes were large amounts in 'Marion'. 2-Heptanone and 3-methyl-3-buten-2-one were dominant ketones in 'Thornless Evergreen', while in 'Marion' 2-undecanone was large amount. α- and β-Ionones were also identified in 'Marion' but not in 'Thornless Evergreen'. Although the exact breeding process of 'Marion' is still a mystery, it has been suspected that raspberry was involved in the breeding process of 'Marion', and α- and β-ionones have been identified as the major volatile component in red raspberry (Klesk, et al., 2004).

Esters accounted about 4.6% of the total volatiles in 'Marion' while only about 2.2% in 'Thornless Evergreen'. The amounts of esters were not related to individual acid composition, and ethyl acetate was always dominated in both 'Marion' and 'Evergreen' blackberries. The amount of methyl salicylate was also large in 'Thornless Evergreen'. Esters could be produced from the enzymatic

actions on alcohols and acyl CoA's derived from both fatty acid and amino acid metabolism (Wyllie and Fellman, 2000).

Terpenes and terpenoids represented 10% of total volatiles of 'Marion' while 32% of total volatiles for 'Thornless Evergreen'. In 'Thornless Evergreen' blackberry, the most abundant terpenes and terpenoids were α-pinene, α-terpineol, nopol and *p*-cymen-8-ol, while 'Marion' had no single terpene or terpenoid in large quantities. High levels of terpenes and terpenoids are probably responsible for the piney, resinous, and citrus odor characters described for 'Thornless Evergreen'(Klesk and Qian 2003a, 2003b). In most fruits, terpenes and terpenoids are probably produced from carbohydrate metabolism through the isoprenoid pathway (Sanz , et al., 1997). Mevalonic acid (MVA) is considered to be the first precursor, which is then converted to isopentenyl diphosphate (IPP). A molecule of isopentenyl diphosphate can be isomerized to dimethylallyl diphosphate (DMAPP) by isopentenyl diphosphate isomerase. DMAPP and IPP can be condensed to form geranyl diphosphate (GPP). From GPP, volatile monoterpenes and terpenoids can be generated through the enzymatic reactions of hydrolysis, cyclizations, and oxidoreductions (Sanz , et al., 1997).

Since aroma profiles not only depend on volatile concentrations, but also their odor thresholds, odor activity values (OAVs, the ratios of volatile concentrations to thresholds) were calculated. Table 2.2 summarizes the OAVs of aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries, based on published odor thresholds. In 'Marion', 18 aroma compounds had OAVs greater

than 1.0, 4 compounds had OAVs between 0.5 and 1.0, and 8 others had OAVs between 0.1 and 0.5. The compounds with the most extreme values (OAV > 10) were ethyl hexanoate (1518.1), β-ionone (282.2), linalool (88.7), 2-heptanone (50.9), 2-undecanone (36.8), α-ionone (23.9), and hexanal (14.2). Except for hexanal, the odor descriptors of these compounds match published 'Marion' aromas such as floral, fruity, sweet, caramel-fruity, and woody (Klesk and Qian, 2003b). Although the OAVs for hexanal (OAV: 14.2), limonene (OAV: 4.4), and hexanoic acid (OAV: 1.8) imply their possible aroma contributions, their odor descriptors in 'Marion' aroma is lacked, which is likely due to human olfactory dynamics. Olfaction is thought to be a combinatorial approach to recognizing and processing odors with proteinaceous odorant receptors. This theory implies that odor response is characterized by inhibition, suppression, and synergistic effects between odorants (Malnic , et al., 1999). It is plausible then that the perceived aroma of 'Marion' is a function of these effects acting on any number of the identified aromas. Further, previous blackberry aroma studies identified 5 furanones, compounds with powerful sweet and caramel-fruity aromas (Klesk and Qian 2003a). Although not quantified in this study, these furanones are likely to be an important source of 'Marion' aroma characteristics, while inhibiting or suppressing other strong aromas.

Table 2.2 OAV[a] of aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries

DB-Wax RI	Compound	Aroma Descriptors[b]	Odor threshold[c] (ppm)	'Marion' (ppm)[d]	OAV	'Thornless Evergreen' (ppm)[d]	OAV
	Acids						
1471	acetic acid	vinegar	60	1.8	<0.1	0.04	<0.1
1642	butanoic acid	rancid, cheesy	1	0.19	0.2	0.07	<0.1
1688	2/3-methylbutanoic acid	rancid, cheesy	4.7/0.25	0.34	1.4	0.95	3.8
1874	hexanoic acid	rancid	1	1.77	1.8	1.12	1.1
2002	*t*-2-hexenoic acid	fatty, rancid	1	0.07	<0.1	0.26	0.3
2085	octanoic acid	sour, goaty	0.91	0.18	0.2	0.04	<0.1
2308	decanoic acid	rancid, soapy	1	0.3	0.3	0.12	0.1
	Alcohols						
955	ethanol	alcoholic	16	0.6	<0.1	0.05	<0.1
1045	2-butanol	alcoholic	16			0.01	<0.1
1060	2-methyl-3-buten-2-ol	herbaceous	100			0.1	<0.1
1113	2-methylpropanol	wine-like	16			0.1	<0.1
1142	2-pentanol	green, fusel oil	8.1			0.03	<0.1
1167	butanol	alcoholic	28	0.03	<0.1	0.17	<0.1
1181	1-penten-3-ol	green	0.4			0.06	0.2
1220	2/3-methylbutanol	wine-like	20/0.41	0.04	<0.1	0.02	<0.1
1272	3-methyl-3-buten-1-ol	herbaceous	unknown			0.05	
1344	2-heptanol	fruity, herbaceous	0.07	0.27	3.8	4.05	57.9
1378	hexanol	fruity	2.5	0.19	<0.1	1.92	0.8
1389	*t*-3-hexenol	green	1.55			0.04	<0.1
1410	*cis*-3-hexenol	green, leaf	0.1	0.12	1.2	0.17	1.7
1432	*t*-2-hexenol	green	0.1	0.06	0.6	0.42	4.2
1441	*cis*-2-hexenol	green	unknown			0.01	

Table 2.2 (continued) OAV[a] of aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries

DB-Wax RI	Compound	Aroma descriptors[b]	Odor threshold[c] (ppm)	'Marion' (ppm)[d]	OAV	'Thornless Evergreen' (ppm)[d]	OAV
1481	heptanol	fatty, pungent	0.33			0.08	0.3
1488	dl-6-methyl-5-hepten-2-ol	unknown	2	0.03	<0.1	0.05	<0.1
1538	2-nonanol	fruity, green	0.058	0.04	0.8	0.03	0.6
1573	octanol	sweet, rose-like	0.875	0.09	0.1	2.81	3.2
1676	nonanol	rose-orange	1	0.03	<0.1	0.26	0.3
1738	2-undecanol	fruity	0.041	0.15	3.6		
1787	decanol	fruity, floral, fatty	0.775	0.05	<0.1	0.73	0.9
1912	benzyl alcohol	Sweet, cherry	0.1	0.19	1.9	0.38	3.8
1950	phenylethyl alcohol	rose-like	1	0.06	<0.1	0.81	0.8
2029	4-phenyl-2-butanol	floral	unknown	0.05		0.03	
2331	cinnamic alcohol	floral	1	0.09	<0.1	0.36	0.4
	Aldehydes						
925	2-methylbutanal	green, malty	0.0013			0.01	11.5
929	3-methylbutanal	fresh grass, cocoa	0.00035			0.01	39.2
1098	hexanal	green, unripe fruit	0.0045	0.06	14.2	0.06	12.9
1119	2-methyl-2-butenal	fresh, fruity	0.5			0.01	<0.1
1237	*t*-2-hexenal	green, leaf	0.01	0.07	7.5	0.25	24.9
1514	*t,t*-2,4-heptadienal	fatty, green	0.049[e]			0.02	0.4
	Ketones						
918	2-butanone	acetone-like	80[f]			0.01	<0.1
992	2-pentanone	ethereal	0.01			0.02	2
1006	3-methyl-3-buten-2-one	unknown	unknown			0.32	
1200	2-heptanone	fruity	0.001	0.05	50.9	0.46	461.5

Table 2.2 (continued) OAV[a] of aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries

DB-Wax RI	Compound	Aroma descriptors[b]	Odor threshold[c] (ppm)	'Marion' (ppm)[d]	OAV	'Thornless Evergreen' (ppm)[d]	OAV
1309	acetoin	buttery	8	0.07	<0.1	0.03	<0.1
1417	2-nonanone	fruity	0.041	0.02	0.4		
1608	2-undecanone	orange	0.007	0.26	36.8		
1894	α-ionone	violet-like	0.0006	0.01	23.9		
1978	β-ionone	violet-like, fruity	0.0001	0.03	282.2		
	Terpines and terpinoids						
1029	α-pinene	pine, resinous	0.062	0.04	0.7	2.22	35.9
1075	camphene	terpene	1.98[g]	0.02	<0.1	0.15	<0.1
1129	β-pinene	woody, resinous	0.082			0.09	1.1
1192	α-phellandrene	sweet, rose-like	0.2			0.05	0.3
1213	limonene	lemon-like	0.01	0.04	4.4	0.34	34.4
1231	sabinene	woody	0.037			0.37	10
1262	γ-terpinene	fruity, lemon-like	unknown	0.01		0.02	
1288	*p*-cymene	carrot-like	0.0062			0.18	28.5
1301	α-terpinolene	sweet, piney	0.2	0.11	0.5	0.21	1.1
1496	linalool oxide	woody, floral	unknown			0.01	
1545	camphor	medicinal, woody	4.6			0.02	<0.1
1562	linalool	floral, citrus	0.006	0.53	88.7	0.17	28.1
1566	theaspirane (B)	ionone-like, fruity	unknown	0.01			
1614	4-terpineol	earthy, lilac	6.4			0.23	<0.1
1618	β-caryophyllene	terpeney, spicy	0.064	0.01	0.2		
1659	1-terpineol	woody, musty	unknown			0.01	

Table 2.2 (continued) OAV[a] of aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries

DB-Wax RI	Compound	Aroma descriptors[b]	Odor threshold[c] (ppm)	'Marion' (ppm)[d]	OAV	'Thornless Evergreen' (ppm)[d]	OAV
1665	myrtenal	spicy, cinnamon	unknown			0.01	
1717	1,8-menthadien-4-ol	unknown	unknown			0.02	
1721	α-terpineol	lilac	0.33			1.13	3.4
1728	l-borneol	pungent, minty	0.14			0.39	2.8
1766	*t,t* -α-farnesene	sweet, flowery	unknown	0.04			
1791	citronellol	sweet, floral	0.062			0.01	0.1
1827	nopol	camphoraceous	unknown			1.21	
1831	myrtenol	flowery, minty	0.007			0.14	19.7
1881	*p*-cymen-8-ol	musty	unknown	0.05		1.34	
2040	perilla alcohol	green, fatty	1.66[g]			0.18	0.1
2137	*p*-cymen-α-ol	unknown	unknown			0.21	
	Esters						
905	ethyl acetate	fruity, floral	25	0.15	<0.1	0.15	<0.1
1062	ethyl 2-methylbutanoate	fruity, pineapple	0.000091			0.01	103.4
1088	butyl acetate	fruity, pineapple	0.01			0.01	1.3
1251	ethyl hexanoate	fruity, banana	0.00001	0.02	1518.1	0.01	1184.2
1291	hexyl acetate	sweet, fruity	0.01	0.07	7.1	0.03	3
1354	*t*-2-hexenyl acetate	fruity, green	unknown	0.01		0.03	
1367	ethyl *t*-2-hexenoate	fruity, pineapple	unknown			0.01	
1454	ethyl octanoate	fruity, floral	0.005	0.01	1.6	0.02	4.6
1490	octyl acetate	fruity, floral	0.047			0.04	0.9
1540	ethyl 3-hydroxybutanoate	marshmallow	20[h]			0.07	<0.1

Table 2.2 (continued) OAV[a] of aroma compounds in 'Marion' and 'Thornless Evergreen' blackberries

DB-Wax RI	Compound	Aroma descriptors[b]	Odor threshold[c] (ppm)	'Marion' (ppm)[d]	OAV	'Thornless Evergreen' (ppm)[d]	OAV
1647	ethyl decanoate	fruity	0.122	0.03	0.3	0.05	0.4
1809	methyl salicylate	green	0.04	0.06	1.4	0.13	3.2
1866	ethyl dodecanoate	fruity	3.5	0.03	<0.1		<0.1
2281	ethyl hexadecanoate	waxy	2	0.01	<0.1	0.07	<0.1
	Phenols						
2039	phenol	medicinal	5.5			0.04	<0.1
2042	methyl eugenol	clove	0.775			0.32	0.4
2205	eugenol	clove, pungent	0.15	0.02	0.1	0.41	2.7
2264	elemicin	woody, floral	22[i]			0.5	<0.1

[a] Odor Activity Value(s)

[b] aroma descriptors from the literature (Bauer and others 1997; Burdock 2001;Klesk and Qian 2003a, 2003b; Lee and others 2001; Perez and others 2002; Pino and others 2001)

[c] thresholds in water from Van Gemert (1999) unless noted otherwise.

[d] means of 3 growing seasons

[e] Lillard and others (1962)

[f] Tan and Siebert (2004)

[g] Padrayuttawat and others (1997)

[h] Cullere and others (2004)

[i] Moshonas and Shaw (1978)

In 'Thornless Evergreen', thirty aroma compounds had OAVs greater than 1.0, 5 compounds had OAVs between 0.5 and 1.0, and twelve others had OAVs between 0.1 and 0.5. The compounds with the most extreme values (OAV > 10) were ethyl hexanoate (1184.2), 2-heptanone (461.5), ethyl 2-methylbutanoate (103.4), 2-heptanol (57.9), 3-methylbutanal (39.2), α-pinene (35.9), limonene (34.4), *p*-cymene (28.50), linalool (28.1), *t*-2-hexenal (24.9), myrtenol (19.7), hexanal (12.9), 2-methylbutanal (11.5), and sabinene (10.0). The odor descriptors of these compounds match published 'Thornless Evergreen' aromas such as spicy, green, herbaceous, fruity, and sweet (Klesk and Qian, 2003b).

CONCLUSIONS

Seasonal variations were observed for some volatile compounds in both 'Marion' and 'Thornless Evergreen' blackberries. In 'Marion', these compounds were mainly acids. In 'Thornless Evergree', seasonal variations were mainly noted for acids, alcohols, and a few terpenoids. These variations and magnitude of changes appear randomly with regards to growing seasons. Volatile compositions of 'Marion' and 'Thornless Evergreen' blackberries were different. 'Thoenless Evergreen' had many more volatiles than 'Marion'. While more acids were found in 'Marion', more alcohols, terpenes and terpenoids were found in 'Thornless Evergreen'. The OAVs reported in this study corroborate published aroma descriptions of the 2 cultivars; however, sensory recombination study is needed to confirm the results.

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CHAPTER III

IMPACT OF GROWING ENVIROMENT ON 'CHICKASAW' (*Rubus* L. subgenus *Rubus* Watson) BLACKBERRY AROMA EVALUATED BY GAS CHROMATOGRAPHY OLFACTOMETRY DILUTION ANALYSIS

YUANYUAN WANG, CHAD FINN AND MICHAEL QIAN

Journal of Agricultural and Food Chemistry

1155, 16th St. N.W., Washington, DC, 20036

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ABSTRACT

Aroma extract of 'Chickasaw' blackberry was separated with silica gel normal phase chromatography into six fractions. Gas chromatography olfactometry (GCO) was performed on each fraction to identify aroma-active compounds. Aroma extraction dilution analysis was employed to characterize the aroma profile of 'Chickasaw' blackberries from two growing regions of United States: Oregon and Arkansas. Comparative AEDA analysis showed that the berries grown in the two regions had similar aroma compositions, however, those odorants had various aroma impacts in each region. The compounds with high FD factors in Oregon's 'Chickasaw' were ethyl butanoate, linalool, methional, *trans, cis*-2,6-nonadienal, *cis*-1,5-octadien-3-one, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, whereas in the 'Chickasaw' grown in Arkansas, they were ethyl butanoate, linalool, methional, ethyl 2-methylbutanoate, β-damascenone, and geraniol.

KEY WORDS: Aroma extraction dilution analysis; 'Chickasaw'; blackberry aroma; GCO; aroma fractionation

INTRODUCTION

Blackberries are a popular food in North America partly due to their unique flavor. Recent studies show that blackberries contain high levels of phenolic compounds and those polyphenolic compounds in fruits may reduce the risk of chronic diseases such as coronary heart disease, cancer, and diabetes (Heinonen, et al., 1998, Meskin, 2004, Scalbert and Williamson, 2000). Breeding programs, particularly those at the University of Arkansas for erect blackberries and the USDA-ARS in Oregon for trailing blackberries, have been very active to release new cultivars that meet the needs of the expanding commercial industry, including improved fruit quality. 'Chickasaw' (*Rubus* L.) is a high quality and productive blackberry that was first developed by the University of Arkansas in 1998(Clark and Moore, 1999). 'Chickasaw' ripens very early, has attractive, large, firm fruit and the fruit has a good flavor, typical for erect blackberries. Due to its positive traits, 'Chickasaw' has become a popular cultivar for the fresh fruit market.

Although sensorial qualities, in particular aroma, can largely influence consumers' acceptance and purchase preferences, the aroma composition of blackberry has been hardly studied. Most of early studies were focused on volatile compositions in blackberries(Georgilopoulos and Gallois, 1987a, Georgilopoulos and Gallois, 1987b, Georgilopoulos and Gallois, 1988, Honkanen and Hirvi, 1990a, Humpf and Schreier, 1991). Recently, the aroma profiles of 'Thornless Evergreen' and 'Marion' trailing blackberries were investigated (Klesk and Qian, 2003a, Klesk and Qian, 2003c, Qian and Wang, 2005). Studies showed

that the most important aroma compounds in 'Marion' are 2,3-butanedione, 2-heptanol, linalool, dimethyltrisulfide, 1-penten-3-one, methional, ethyl 2-methylbutanoate, benzaldehyde, and hexanal, while the most important aroma compounds in 'Thornless Evergreen' are 2,3-butanedione, *l*-carvone, β-pinene, methional, ethyl 2-methylpropanoate, thiophene, dimethyl disulfide, 2,5-dimethyl-4-hydroxy-3-(2*H*)- furanone, and 2-heptanol. Blackberries have a very wide range of aroma profiles. The aroma profiles of 'Thornless Evergreen' and 'Marion' blackberries would be expected to be quite different from 'Chickasaw' as they have a very different genetic background and are typically grown for different markets. The aroma profile has not been previously determined for 'Chickasaw' or for any other erect blackberry cultivar.

Aroma compounds in fruits can be generated from fatty acids, amino acids, and carbohydrates (Sanz, et al., 1997). Different climates and sites can affect the levels of precursors and activities of related enzymes, thereby impacting flavor development. The environmental influence on flavor formation has been examined in several small fruits, including strawberry, raspberry, blueberry, and grape (Belancic, et al., 1997, Bureau, et al., 2000, Forney, 2001, Forney, et al., 2000, Klesk, et al., 2004, Marais, et al., 1999, Moore, et al., 2002, Reynolds, et al., 1996), however, no work has been done on blackberries. The objectives of this study were to identify the aroma compounds in 'Chickasaw' blackberry using gas chromatography olfactometry (GCO), to compare the aroma profile in 'Chickasaw' blackberries grown in Oregon and Arkansas by aroma extraction

dilution analysis (AEDA) technique, and to elucidate the influence of growing environment on aroma quality.

MATERIALS & METHODS

Chemicals

1-Methyl-4-isopropenyl-1-cyclohexene (limonene), 2-heptanone, 2,6,6-trimethylbicyclo-[3,1,1]-2-heptene (α- pinene), 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (β- pinene), 2-nonenal, ethyl 2-methylpropanoate, and 2-undecanone were obtained from K&K Laboratories (Jamaica, N.Y.). Ethyl butanoate, ethyl pentanoate, ethyl *trans*-2-butenoate, ethyl hexanoate, ethyl propanoate, ethyl butanoate, butyl acetate, hexyl acetate, hexyl butanoate, *cis*-3-hexenyl acetate, ethyl octanoate, ethyl 3-hydroxyhexanoate, ethyl decanoate, ethyl benzoate,,methyl dodecanoate, methyl hexanoate, methyl butanoate, ethyl acetate, ethyl 2-methylbutanoate, ethyl 3-phenylpropanoate (ethyl dihydrocinnamate), ethanoic acid (acetic acid), butanoic acid, hexanoic acid, 2-methylbutanoic acid, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (β-ionone), 2-heptanol, hexanal, hepanal, octanal, nonanal, decanal, cyclohexane carbaldehyde (benzaldehyde), *trans, cis*-2,6-nonadienal, phenylacetaldehyde, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (β-damascenone), *trans*-2-hexenal, 3,7-dimethyl-1,6-octandien-3-ol (linalool), 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde (myrtenal), 7-methyl-3-methylene-1,6-octadiene (myrcene),

1-isopropyl-4-methylenebicyclo[3.1.0]hexane (sabinene), β-3,7-dimethyl-1,3,6-octatriene (β-ocimene), 1-isopropyl-4-methyl-1,4-cyclohexadiene (γ-terpinene), trans-3,7-dimethyl-2,6-octadienal (geranial), trans-3,7-dimethyl-2,6-octadien-1-ol (geraniol), 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol), 1,7,7-trimethylbicyclo[2.2.1]-2-heptanone (camphor), 2,2-dimethyl-3-methylene-bicyclo[2.2.1]heptane (camphene), 3-methylene-6-(1-methylethenyl)cyclohexane (β-phellandrene), 1-methyl-4-(1-methylethylidene)-cyclohexene (α-terpinolene), 2-pentanol, 3-pentanol, 1-penten-3-ol, 3-methylbutanol, hexanol, *cis*-3-hexenol, *trans*-3-hexenol, *trans*-2-hexenol, 1-octen-3-ol, heptanol, nonanol, octanol, phenylmethanol (benzyl alcohol), 2-phenylethanol (phenethyl alcohol), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol), and 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene (theaspirane) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, Wisc.). 4-Hydroxy-decanoic acid (γ-decalactone) was purchased from Pfaltz & Bauer (Waterbury, Conn.). Sodium chloride was obtained from Fisher Scientific (Fair Lawn, N.J.). Diethyl ether, pentane, and methanol were obtained from Honeywell International Inc. (Muskegon, Mich.), Malinckrodt Baker Inc. (Philipsburg, N.J.), and J.T. Baker Inc. (Phillipsburg, N.J.) respectively.

Blackberry samples

Fully ripe 'Chickasaw' blackberries were hand picked from plants growing at the Oregon State University North Willamette Research and Extension Center (OSU-NWREC; Aurora, Ore.) and the University of Arkansas Fruit Substation (Clarksville, Ark.) in July 2003. The fruit were immediately placed on ice and transported to the laboratory, where they were individually quick frozen (IQF) and stored at -23 °C. The samples from Arkansas were shipped on ice to the laboratory at Oregon State University and stored at -23 °C. The samples had been frozen for one month when analyzed.

Extraction of volatile compounds

One kilogram of IQF 'Chickasaw' samples from both locations were took out of the freezer and stand at room temperature until they were just soft enough to be blended (but still remain icy and cold) . The berries were blended in a glass blender jar (Waring Products Division, Dynamics Corp. of America, New Hartford, Conn.) for a total of 90 s. 10 g of calcium chloride was added to inhibit enzyme activity before blending. The puréed fruit was transferred to a 1-L Erlenmeyer flask covered with aluminum foil and extracted with 335 mL of freshly distilled pentane:diethyl ether (1:1 v/v) on a platform shaker (Innova 2300; New Brunswick Scientific, Edison, N.J.) at 125 rpm for 3 h. The solvent and juice were poured into a separatory funnel. The juice was drawn off and returned to the fruit; the organic phase was retained. The extraction procedure was repeated twice,

yielding a total volume of 880 mL solvent. Volatile compounds were recovered from the organic extract by using solvent-assisted flavor evaporation (SAFE) at 50 °C under vacuum (Engel, et al., 1999). The organic SAFE extract was dried with anhydrous Na_2SO_4, concentrated to 2 mL by solvent evaporation, and reduced to its final volume of 0.2 mL with a flow of nitrogen.

Gas chromatography olfactometry (GCO)

The analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactometer. Samples were analyzed on a Stabilwax column (30 m × 0.32 mm i.d. cross-linked polyethylene glycol, 1 μm film thickness, Restek Corp., Bellefonte, Penn.), and a DB-5 column (30 m × 0.32 mm i.d., cross-linked phenyl-methyl polysiloxane, 1μm film thickness, J&W Scientific, Folsom, Cal.). Column effluent was split 1:1 (by volume) into the FID and a heated sniffing port with a fused silica outlet splitter (Alltech Associates, Inc., Deerfield, IL). Injector and detector temperatures were 250 °C, the helium column flow rate was 2.0 mL/min at 25 °C, and the 2-μL sample was injected in the splitless mode. The oven temperature was programmed for a 2 min hold at 40 °C, then 40 to 100 °C at 5 °C/min, then 100 to 230 °C at 4 °C/min (10 min hold). Retention indices were estimated in accordance with a modified Kovats method (Van and Kratz, 1963).

Aroma extract fractionation/GCO

For a better GC resolution and identification, one kilogram of Oregon 'Chickasaw' blackberries were extracted using the same procedure as described previously. The aroma extract was concentrated to 1 mL. The concentrated sample was fractionated by adsorption chromatography on silica gel (EM Science, 35-70 mesh, 60 Å) described by Qian and Reineccius (Qian and Reineccius, 2002) with some modification. 8 g of silica gel was packed into a 1.5 cm ID × 30 cm glass column. The column was washed with methanol and equilibrated in pentane. The sample (1 mL) was applied to the column and fractionated by eluting with 70 mL of each following solvent: pentane (fraction 1), pentane and diethyl ether (fraction 2, 95/5; fraction 3, 90/10; fraction 4, 50/50), diethyl ether (fraction 5), and methanol (fraction 6) at a flow rate of 1.5 mL/min. Each fraction was concentrated to 2 mL by solvent evaporation, and reduced to its final volume of 0.2 mL with a flow of nitrogen. Fractions were analyzed by GCO at the same conditions and methods as for the AEDA analysis. The odor intensities of odorants were evaluated on a 9 - point intensity scale by a trained judge. This GCO analysis was done in triplicate for each fraction.

Aroma extract dilution analysis (AEDA)

Two experienced panelists were used for Aroma Extract Dilution Analysis (Grosch, 1994, Schieberle and Grosch, 1987). The aroma concentrate was diluted

sequentially at 1:1 ratio. Each dilution was analyzed until aroma can be no longer detected. Flavor dilution (FD) values were calculated based on the last dilution.

Gas chromatography – mass spectroscopy (GCMS)

The original concentrated samples as used for AEDA analysis (2-μL splitless injections) were analyzed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 Mass Selective Detector (MSD). System software control and data management/analysis was performed through Enhanced ChemStation Software, G1701CA v. C.00.01.08 (Agilent Technologies, Inc., Wilmington, Del.). Volatile separation was achieved with the same Stabilwax and DB-5 columns used in the AEDA analyses. A constant helium column flow rate was set at 2 mL/min and the same GC oven temperature programming was set as for the AEDA analysis. Injector, detector transfer line, and ion source temperatures were 250, 280, and 230 °C, respectively. Electron impact mass spectrometric data from m/z 35-300 was collected using a scan rate of 5.27 /s, with an ionization voltage of 70 eV. Retention indices were estimated in accordance with a modified Kovats method (Van and Kratz, 1963). Compound identifications were made by comparing mass spectral data from the Wiley 275.L (G1035) Database (Agilent), and confirmed by comparing Kovats retention indices (RI) to those of the standards or RIs reported in the literature.

RESULTS AND DISCUSSION

Aroma fractionation of Oregon 'Chickasaw' blackberries

The aroma extract of 'Chickasaw' blackberries is very complex. To increase the resolution of gas chromatography and facilitate the identification of aroma compounds, silica gel normal phase chromatography was applied on the aroma extract of the Oregon samples. Six fractions were obtained and GCO and GCMS were performed on each fraction. In the GCO experiment, one experienced judge was chosen to evaluate the intensity of odorants from each fraction on a 0-9 scale. Although this GCO experiment cannot provide a quantitative estimation of their odor intensity due to limited panelists, it can provide information about the aroma quality of the compounds, as well as positive mass spectrometry identification. The results of fractionation were summarized in Table 3.1.

A total of eighty-four compounds were isolated and identified from the six fractions, including nineteen esters, eighteen terpenes and terpenoids, fifteen alcohols, thirteen aldehydes, four ketones, four acids, four lactones, two furans, two sulfur-containing compounds, one pyrazine, and two miscellaneous compounds. The highest recoveries for terpenes were achieved in fractions 1 eluted with pentane, but terpenoids were found in fractions 2, 3 and 4, which are the more polar fractions (pentane/diethyl ether, 95:5v, 90:10v, and 50:50v). Except for ethyl 3-hydroxybutanoate and ethyl 3-hydroxyhexanoate, which were found in fraction 4, esters were mostly recovered in fraction 2 and 3. Aldehydes and ketones were

generally found in fraction 3 and 4. In general, alcohols were eluted in fraction 5 with diethyl ether. Acids and furans were mostly recovered in polar fraction 6 with methanol.

Based on their odor intensity, ethyl butanoate, linalool, *cis*-1,5-octadien-3-one, 2-methylthiophene, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (odor intensity = 8, 7, 8, 7, 8, 8 respectively) were detected as the most potent aroma compounds in the Oregon samples. Besides, ethyl propanoate, butyl acetate, ethyl *trans*-2-butenoate, ethyl hexanoate, ethyl *trans*-2-hexenoate, camphene, myrcene, γ-terpinene, hexanal , *trans*-2-hexenal, decanal, undecanal, *trans, cis*- 2,6-nonadienal, 2-undecanone, β-damascenone, *trans*-2-hexenol, 1-octen-3-ol, γ-decalactone, and γ-hexalactone also are important odorants to Oregon 'Chickasaw' blackberry flavor with a odor intensity around 5. Although the odor intensities of those odorants are not conclusive, it is very likely that those aroma compounds could be important to 'Chickasaw' aroma.

Table 3.1 Odor-active compounds in aroma fractions for Oregon-grown 'Chickasaw' blackberries

RI on		Odorant	Odor quality	Odor intensity					
Polar	Apolar			Fr[e]1	Fr2	Fr3	Fr4	Fr5	Fr6
938	718	dimethyl sulfide[c]	sulfur, cabbage-like			4			
965	725	ethyl propanoate[a]	fruity		3	5			
965	754	ethyl 2-methylpropanoate[a]	fruity		4				
989	728	methyl butanoate[a]	fruity, sweet		2	3			
1023	932	2,6,6-trimethylbicyclo-[3,1,1]-2-heptene (a-pinene)[a]	resinous	4					
1042	803	ethyl butanoate[a]	fruity, apple-like		5	8			
1051	848	ethyl 2-methylbutanoate[a]	fruity, sweet		3				
1061	946	2,2-dimethyl-3-methylenc-bicyclo[2.2.1]heptane (camphene)[a]	green, terpene-like	5					
1074	814	butyl acetate[a]	fruity, juicy		3	5			
1088	800	hexanal[a]	green, grassy			5	4		
1106	932	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (β-pinene)[a]	woody, resinous	4					
1110		3-pentanol[a]	green				2		
1122	718	2-pentanol[a]	cut grass, green				2		
1139	903	ethyl pentanoate[a]	fruity		3				
1167	844	ethyl *trans*-2-butenoate[a]	fruity, green			4~5			
1168	991	7-methyl-3-methylene-1,6-octadiene (myrcene)[a]	resinous, balsamic	5					
1169	680	1-penten-3-ol[a]	green				4		
1186		2-methylthiophene[d]	french fries, cooked potato			7			
1189	924	methyl hexanoate[a]	fruity, sweet		4				
1190	902	heptanal[a]	oily, fatty			3	2		
1198	1023	3-methylene-6-(1-methylethenyl)cyclohexane (β-phellandrene)[a]	mint, herbaceous	2					
1198	892	2-heptanone[a]	floral, fruity			4	2		
1199	1028	1-methyl-4-isopropenyl-1-cyclohexene (limonene)[a]	piney, herbaceous	4					
1213	1027	1-isopropyl-4-methylenebicyclo[3.1.0]hexane (sabinene)[a]	green, fatty, woody	2					
1218	786	3-methylbutanol[a]	green, pungent				2		
1221	851	*trans*-2-hexenal[a]	green, leafy				5		

Table 3.1 (continued) Odor-active compounds in aroma fractions for Oregon-grown 'Chickasaw' blackberries

RI on		Odorant	Odor quality	Odor intensity					
Polar	Apolar			Fr[e]1	Fr2	Fr3	Fr4	Fr5	Fr6
1240	1001	ethyl hexanoatea[a]	apple-like, fruity, sweet		2	5			
1240	1038	*trans*-β-3,7-dimethyl-1,3,6-octatriene *(trans*-β-ocimene)[a]	woody	2					
1247	1057	1-isopropyl-4-methyl-1,4-cyclohexadiene (γ-terpinene)[a]	woody, oil, barney	5					
1278	1086	1-methyl-4-(1-methylethylidene)-cyclohexene (α-terpinolene)[a]	woody, herbaceous		2				
1294	1003	octanal[a]	floral, fruity, fatty			5			
1317	1008	*cis*-3-hexenyl acetate[a]	green, grass		4				
1331	905	2-heptanol[a]	mushroom				2		
1364	875	hexanol[a]	fruity				3		
1374	853	*trans*-3-hexenol[a]	green				2		
1395	986	*cis*-1,5-octadien-3-one[d]	piney, green				8		
1398	1105	nonanal[a]	floral, citrus			4			
1414	1192	hexyl butanoate[a]	fruity, melon-like		2~3	4			
1417	868	*trans*-2-hexenol[a]	walnut, green				5		
1440	1200	ethyl octanoate[a]	fruity, fatty, apricot		2~3				
1462	985	1-octen-3-ol[a]	fatty, mushroom				5		
1465		3-isopropyl-2-methoxypyrazine[d]	beany, vegetable			3			
1476	716	ethanoic acid (acetic acid)[a]	acid, sour						6
1480	977	heptanol[a]	green, fruity				2		
1500	1298	2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene (theaspirane B)[a]	fruity, floral		3				
1503	1206	decanal[a]	soapy, citrus			3	5		
1509	1141	1,7,7-trimethylbicyclo[2.2.1]-2-heptanone (camphor)[a]	soapy, fatty				2		
1527	958	cyclohexane carbaldehyde (benzaldehyde)[a]	berry, fruity			2			
1540	964	ethyl 3-hydroxybutanoate[b]	fruity				2~3		
1560	1102	3,7-dimethyl-1,6-octandien-3-ol (linalool)[a]	floral			7	6		
1570		*trans*-2-nonenal[b]	green, fruity				2		

Table 3.1 (continued) Odor-active compounds in aroma fractions for Oregon-grown 'Chickasaw' blackberries

RI on		Odorant	Odor quality	Odor intensity					
Polar	Apolar			Fr[e]1	Fr2	Fr3	Fr4	Fr5	Fr6
1570	1150	*trans,cis*-2,6 nonadienal[c]	cucumber, green			5	3		
1591		undecanal[b]	fresh floral fruity			4~5			
1601	1295	2-undecanone[a]	floral, fruity			4~5			
1619	1195	6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde (myrtenal)[a]	spicy, cinnamon				4		
1621		1-methyl-4-isopropyl-1-cyclohexen-4-ol (4-terpineol)[b]	floral , herbal				3		
1645	1397	ethyl decanoate[a]	fruity			4			
1648	815	butanoic acid[a]	sour, rancid						3
1649	1043	phenylacetaldehyde[a]	lilac, floral				4		
1663	1170	ethyl cyclohexane carboxylate (ethyl benzoate)[a]	fruity, tea		4				
1672	1185	nonanol[a]	fatty, green, oily, floral				3~4		
1690	1129	ethyl 3-hydroxyhexanoate[a]	floral, fruity				2		
1700	876	2/3-methylbutanoic acid[a]	cheesy, sour						3
1729	1271	*trans*-3,7-dimethyl-2,6-octadienal (geranial)[a]	tea, mint, citrus			3~4			
1804	1526	methyl dodecanoate[a]	sweet, floral		2				
1819	1383	1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (β-damascenone)[a]	berry, sweet, floral			5			
1862	1269	*trans*-3,7-dimethyl-2,6-octadien-1-ol (geraniol)[a]	rosy-like, floral				4		
1866	1006	hexanoic acid[a]	sour, acid., rancid						3
1890		phenylmethanol (benzyl alcohol)[a]	watermelon fruity floral				3		
1936	1136	2-phenylethanol (phenethyl alcohol)[a]	rosy				4		
1986	1486	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (β-ionone)[a]	dry fruit, floral				2		
2012	1550	3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol)[a]	woody, floral				5		
2064		2,5-dimethyl-4-hydroxy -3(2H)-furanone (furaneol)[a]	caramel, cooked sugar					3	8

Table 3.1 (continued) Odor-active compounds in aroma fractions for Oregon-grown 'Chickasaw' blackberries

RI on		Odorant	Odor quality	Odor intensity					
Polar	Apolar			Fr[e]1	Fr2	Fr3	Fr4	Fr5	Fr6
2100		2-ethyl-4-hydroxy-5-methyl -3(2H)-furanone (homofuraneol)[d]	sweet, caramel					2	8
2149	1472	5-hexyldihydro-2[3H]-furanone (γ-decalactone)[a]	candy, sweet					5	
	858	*cis*-3-hexenol[a]	cut grass				4		
	1049	ethyl *trans*-2-hexenoate[a]	sweet, green, vegetable			5			
	1066	5-ethyldihydro-2[3H]-furanone (γ-hexalactone)[b]	sweet, fruity					6	
	1080	octanol[a]	soapy, fatty				3~4		
	1242	6,6-dimethyl-2-oxymethlybicyclo[1.1.3]hept-2-ene (myrtenol)[a]	woody, balsamic				4		
	1257	4-octanolide (γ-octalactone)[a]	coconut, sweet					3~4	
	1295	5-octanolide (δ-octalactone)[b]	fruity, sweet					3	
	1369	*trans*-2-undecenal[b]	fatty, waxy, green			2			
	1559	1,2,3-trimethoxy-5-allylbenzene (elemicin)[b]	sweet, woody		2				

[a] Identified by comparing it with the standard on the basis of retention index (RI) , GC-MS, and odor quality.

[b] As for footnote *a* but no RI from standards available, the RI was compared with that from literatures.

[c] As for footnote *a* but no GC-MS data available.

[d] As for footnote *a* but no GC-MS data and RI from standards available, the RI was compared with that from literatures.

[e] Fraction

Comparative AEDA of 'Chickasaw' blackberries from Oregon and Arkansas States

Although the two samples selected for this study were from the same blackberry cultivar, the two climates during fruit ripening were quite different. During June and July in Clarksville, Arkansas, the temperatures during the day (mean July max. = 33.6°C) and night (mean July min. = 20.1°C) are warm, the relative humidity is generally very high, there can be substantial precipitations (mean July cumulative precipitation=73.7 mm) and while the sunlight can be quite intense, there is much greater cloud cover than in Oregon. In contrast during July at the OSU-NWREC in Oregon, the temperatures are moderate during the day (mean July max. = 26.8°C), cool at night (mean July min. = 11.7°C), the relative humidity is very low, there is very little precipitation (mean July cumulative precipitation=16.5 mm) and the days are very clear and sunny. Due to the difference in growing environment, the fruits developed distinctive aromas. In preliminary sensory evaluations (7 expert panelists), berries from Oregon were characterized as having cut grass, green, fruity, citrus, and watermelon aroma. However, Arkansas berries were judged to be less green and fruity, but described as having cinnamon, piney, floral, sweet, and caramel smelling.

The odor-active volatiles in the samples from the two regions were analyzed by AEDA(Grosch, 1994, Schieberle and Grosch, 1987). When the data from polar (Table 3.2) and non polar columns (Table 3.3) are combined, a total of 87 odorants were detected in the flavor dilution range of 2-2048, 77 of which could be

identified. Sixty-five odorants were identified in Oregon samples, while 68 were identified in the Arkansas sample. Of these, 56 were common to both.

Judging by flavor dilution (FD) factors, esters were found to be the most important chemical class in 'Chickasaw' blackberries from both states; they were responsible for intense fruity and floral notes in the fruit samples. A total of 20 odor-active esters were detected, all the 20 esters were found in Oregon samples and 16 in Arkansas samples. Thirteen of these esters were considered to be important odorants with high FD factors ranging from 64 to 2048. Of these, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, methyl hexanoate, and ethyl *trans*-2-hexenoate (FD =2048, 64, 512, 128, and 128 respectively in Oregon fruit; FD = 2048, 2048, 512, 256, 64 respectively in Arkansas fruit) were found in both. With the exception of ethyl 2-methylbutanoate, the other four esters were considered to contribute equally (FD factors ± one dilution are considered equivalent) to the aroma profile in the berry samples from both states. Ethyl butanoate was found to be the most important volatile in both samples (FD = 2048 in both), and it actively contributes to the desirable fruity flavor of blackberries due to its small molecular weight and high volatility. Apart from ethyl butanoate, six more esters, ethyl propanoate, methyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate, and ethyl dihydrocinnamate show high FD factors in Oregon berry samples, while two more esters, *cis*-3-hexenyl acetate and ethyl benzoate show high FD factors in Arkansas samples. Comparing the numbers and the magnitude of FD factors of these odorants in the two samples, the esters in

Oregon might play a bigger role than in Arkansas for the overall aroma, which was supported by the sensory evaluation.

There were eighteen odor-active terpenes and terpenoids detected in both berries. Eight of them show high FD factors. Linalool (FD = 2048 in both), geraniol (FD = 256 in Oregon, FD = 2048 in Arkansas), and α-pinene (FD= 512 in Oregon, FD = 256 in Arkansas) were the most potent odorants in both samples. Linalool and geraniol, characterized as floral, rose-like odor, could be related to the floral notes of 'Chickasaw' blackberries, whereas α-pinene might be associated with the piney, woody, and resinous smell. Additionally, limonene and citronellol (FD = 512 and 256) show high FD factors in Oregon, while γ-terpinene, γ-cadinene, and *allo*-ocimene (FD = 256, 256, 512 respectively) show high FD factors in Arkansas. Among these five compounds, while citronellol is sweet, fruity smelling, the other terpenes were described as having piney, mint, and woody notes. The fact that Arkansas has more potent piney smelling compounds is in good agreement with the results from sensory evaluation, which suggested that 'Chickasaw' from Arkansas had stronger piney and woody notes than those from Oregon.

Table 3.2 AEDA of Oregon and Arkansas grown 'Chickasaw' blackberries (Stabilwax Column)

			FD factors	
RI	**Compound**	**Descriptors**	**OR**	**AR**
874	ethyl acetate[a]	fruity	8	2
898	dimethyl sulfide[c]	cabbage, sulfur	4	8
955	ethyl propanoate[a]	fruity	16	16
966	ethyl 2-methylpropanoate[a]	fruity	32	16
976	2,3-butanedione (diacetyl)[c]	buttery	16	16
995	methyl butanoate[a]	sweet, fruity	512	-
1038	2,6,6-trimethylbicyclo-[3,1,1]-2-heptene (a-pinene)[a]	piney, resinous	4	8
1056	ethyl butanoate[a]	fruity, apple	2048	512
1070	ethyl 2-methylbutanoate[a]	fruity, sweet	32	32
1075	butyl acetate[a]	fruity, juicy	8	-
1098	hexanal[a]	green	128	16
1125	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (β-pinene)[a]	woody, resinous	2	8
1161	ethyl pentanoate[a]	fruity	32	-
1176	1-penten-3-ol[a]	green, pungent	32	-
1181	7-methyl-3-methylene-1,6-octadiene (myrcene)[a]	balsamic, plastic	8	8
1193	2-heptanone[a]	fruity, banana	-	16
1208	methyl hexanoate[a]	fruity, apple	128	256
1213	heptanal[a]	oily, fatty	-	4
1226	1-methyl-4-isopropenyl-1-cyclohexene (limonene)[a]	piney, herbaceous	128	32
1237	3-methylbutanol[a]	pungent, green	16	-
1248	*trans*-2-hexenal[a]	green, leaf	16	512
1258	ethyl hexanoate[a]	apple, fruit	32	16
1269	1-isopropyl-4-methyl-1,4-cyclohexadiene (γ-terpinene)[a]	oily, woody	4	256
1319	octanal[a]	citrus-like, fruity	32	8
1338	*cis*-3-hexenyl acetate[a]	green, grass	32	256
1344	2-heptanol[a]	woody vegetal	4	-

Table 3.2 (continued) AEDA of Oregon and Arkansas grown 'Chickasaw' blackberries (Stabilwax Column)

			FD factors	
RI	**Compound**	**Descriptors**	**OR**	**AR**
1408	*cis*-1,5-octadien-3-one[d]	green, grassy	1024	512
1441	hexyl butanoate[a]	apple peel	16	8
1464	ethyl octanoate[a]	fruity, apricot	16	8
1475	3-isopropyl-2-methoxypyrazine[d]	earthy, beany	2	-
1486	ethanoic acid (acetic acid)[a]	acid, sour	64	128
1499	3-methylmercaptopropionaldehyde (methional)[c]	cooked potato	2048	2048
1540	2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene (theaspirane B)[a]	floral	16	8
1578	3,7-dimethyl-1,6-octandien-3-ol (linalool)[a]	floral, citrus	2048	2048
1601	2-undecanone[a]	sweet, fruity	128	32
1608	*trans, cis*-2,6-nonadienal[c]	cucumber	128	64
1641	Unknown	floral, fruit, watermelon	128	-
1645	1-methyl-4-isopropyl-1-cyclohexen-4-ol (4-terpineol)[b]	earthy, woody	-	8
1663	butanoic acid[a]	rancid, acid	16	256
1706	2/3-methylbutanoic acid[a]	cheesy, sour	128	128
1725	*trans*-3,7-dimethyl-2,6-octadienal (geranial)[a]	sweet, floral	-	8
1745	(R)-5-isopropenyl-2-methyl-2-cyclohexenone (*l*-carvone)[a]	herbaceous, mint	8	8
1788	γ-cadinene[a]	woody	16	256
1803	3,7-dimethyl-6-octen-1-ol (citroncllol)[a]	green, cucumber	32	16
1817	*cis*-3,7-dimethyl-2,6-octadien-1-ol (nerol)[a]	floral, rose	-	4
1846	Unknown	Chinese medicine, herbaceous	-	2
1850	1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (β-damascenone)[a]	rose, floral, sweet	32	128
1879	*trans*-3,7-dimethyl-2,6-octadien-1-ol (geraniol)[a]	sweet, rose, candy	-	8
1884	hexanoic acid[a]	sour	8	2
1915	phenylmethanol (benzyl alcohol)[a]	floral	8	256

Table 3.2 (continued) AEDA of Oregon and Arkansas grown 'Chickasaw' blackberries (Stabilwax Column)

RI	Compound	Descriptors	FD factors	
			OR	AR
1961	2-phenylethanol (phenethyl alcohol) [a]	rosy	16	4
1967	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (β-ionone)[a]	dry fruit, berry	4	4
1995	Unknown	rosy, floral	-	32
2045	3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol) [a]	floral, woody	-	16
2063	2,5-dimethyl-4-hydroxy -3(2H)-furanone (furaneol) [a]	sweet, caramel	2048	32
2086	2-ethyl-4-hydroxy-5-methyl -3(2H)-furanone (homofuraneol)[d]	sweet, berry	32	8
2187	4-allyl-2-methoxyphenol (eugenol) [a]	clove, honey	-	4
2218	5-decanolide (δ-decalactone) [d]	coconut, peach, dairy	128	8
2264	Unknown	herbaceous, woody	128	2

[a] Identified by comparing it with the standard on the basis of retention index (RI) , GC-MS, and odor quality.

[b] As for footnote *a* but no RI from standards available, the RI was compared with that from literatures.

[c] As for footnote *a* but no GC-MS data available.

[d] As for footnote *a* but no GC-MS data and RI from standards available, the RI was compared with that from literatures.

Table 3.3 AEDA of Oregon and Arkansas grown 'Chickasaw' blackberries (DB5 Column)

RI	Compound	aroma descriptors	FD factors	
			OR	AR
709	2,3-butanedione (diacetyl)[c]	buttery	512	8
716	ethanoic acid (acetic acid)[a]	sour, vinegar	128	32
725	dimethyl sulfide[c]	cabbage-like	-	8
730	2-pentanol[a]	pungent, plastic, green	2	-
744	ethyl propanoate[a]	fruity	128	-
754	methyl butanoate[a]	sweet, fruity	16	4
779	ethyl 2-methylpropanoate[a]	fruity	512	512
800	hexanal[a]	green, grassy	512	16
807	ethyl butanoate[a]	fruity, apple	1024	2048
814	butyl acetate[a]	fruity	64	-
817	butanoic acid[a]	sour, rancid, pungent	32	128
823	ethyl 2-hydroxypropanoate (ethyl lactate)[a]	pungent, rubbery	-	8
823	Unknown	rubbery, buttery	-	8
854	ethyl 2-methylbutanoate[a]	fruity, apple	64	2048
870	2/3-methylbutanoic acid[a]	pungent, cheesy, sour	64	8
882	*cis*-3-hexenol[a]	green, grassy	-	8
899	heptanal[a]	oily, fatty	16	64
907	3-methylmercaptopropionaldehyde (methional)[c]	cooked potato	2048	2048
944	2,6,6-trimethylbicyclo-[3,1,1]-2-heptene (a-pinene)[a]	piney, woody, resinous	512	256
963	cyclohexane carbaldehyde (benzaldehyde)[a]	fruity, berry	256	8
982	*cis*-1,5-octadien-3-one[d]	green, resinous	2048	256
1003	ethyl hexanoate[a]	fruity, sweet, pineapple	512	32
1009	Unknown	hay, dry woody	-	4
1019	3-methylene-6-(1-methylethenyl)cyclohexane (β-phellandrene)[a]	mint, cool	-	8
1022	hexyl acetate[a]	sweet, fruity	256	-
1022	phenylacetaldehyde[a]	lilac, floral	-	4

Table 3.3 (continued) AEDA of Oregon and Arkansas grown 'Chickasaw' blackberries (DB5 Column)

RI	Compound	aroma descriptors	FD factors OR	FD factors AR
1027	1-methyl-4-isopropyl-benzene (*p*-cymene)[a]	pungent, solvent	16	-
1039	Unknown	fruity, sour,	-	4
1047	1-methyl-4-isopropenyl-1-cyclohexene (limonene)[a]	piney, herbaceous, mint	512	8
1052	ethyl *trans*-2-hexenoate[a]	green, vegetable, fruity	128	64
1062	2,5-dimethyl-4-hydroxy -3(2H)-furanone (furaneol)[a]	sweet, strawberry	512	512
1084	2-ethyl-4-hydroxy-5-methyl -3(2H)-furanone (homofuraneol)[d]	sweet, candy, caramel	128	8
1099	1-methyl-4-(1-methylethylidene)-cyclohexene (α-terpinolene)[a]	woody, earthy, herbaceous	-	8
1106	3,7-dimethyl-1,6-octandien-3-ol (linalool)[a]	floral, citrus	2048	2048
1068	octanol[a]	waxy, fatty	-	8
1112	nonanal[a]	floral	-	128
1121	*allo*-3,7-dimethyl-1,3,6-octatriene (*allo*-ocimene)[a]	Chinese medicine, herbaceous	-	512
1140	2-phenylethanol (phenethyl alcohol)[a]	sweet, rose	128	8
1157	ethyl 3-hydroxyhexanoate[a]	fruity	4	8
1164	*trans,cis*-2,6-nonadienal[c]	cucumber, green	1024	16
1174	ethyl cyclohexane carboxylate (ethyl benzoate)[a]	fruity, musty, tea	32	32
1180	1-methyl-4-acetyl benzene (*p*-methylacetophenone)[d]	sweet, hot candy	8	2
1189	1-methyl-4-isopropyl-1-cyclohexen-4-ol (4-terpineol)[b]	woody, earthy, musty	2	4
1214	6,6-dimethyl-2-oxymethlybicyclo[1.1.3]hept-2-ene (myrtenol)[a]	medicinal, woody	16	8
1237	3,7-dimethyl-6-octen-1-ol (citronellol)[c]	sweet, fruity	256	2
1246	(R)-5-isopropcnyl-2-methyl-2-cyclohexenonc (*l*-carvone)[a]	herbaceous, caraway	16	4
1275	*trans*-3,7-dimethyl-2,6-octadien-1-ol (geraniol)[a]	sweet, rose	256	2048
1315	2-undecanone[a]	rose, iris	64	4
1324	Unknown	herbaceous, floral	-	16
1360	ethyl 3-phenylpropanoate (ethyl dihydrocinnamate)[a]	sweet, floral	64	8

Table 3.3 (continued) AEDA of Oregon and Arkansas grown 'Chickasaw' blackberries (DB5 Column)

RI	Compound	aroma descriptors	FD factors	
			OR	AR
1394	1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (β-damascenone) [a]	rose, floral, berry, sweet	64	1024
1405	ethyl decanoate[a]	fruity	2	-
1411	3-methoxy-4-hydroxy-cyclohexane carbaldehyde[a] (3-methoxy-4-hydroxybenzaldehyde, vanillin) [b]	vanilla, ice cream	-	256
1470	5-hexyldihydro-2[3H]-furanone (γ-decalactone) [a]	sweet, coconut	16	64
1493	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (β-ionone) [a]	dry fruit	8	16
1504	5-decanolide (δ-decalactone) [b]	sweet, peach	16	64
1565	1,2,3-trimethoxy-5-allylbenzene (elemicin) [b]	spicy, woody, sweet	4	2
1751	benzyl benzene carboxylate (benzyl benzoate) [b]	sweet, balsamic	4	8
1892	Unknown	sweet, woody, caramel	4	8
1926	Unknown	floral, wine, perfume	-	16

[a] Identified by comparing it with the standard on the basis of retention index (RI) , GC-MS, and odor quality.

[b] As for footnote *a* but no RI from standards available, the RI was compared with that from literatures.

[c] As for footnote *a* but no GC-MS data available.

[d] footnote *a* but no GC-MS data and RI from standards available, the RI was compared with that from literatures.

A total of 9 aldehydes were identified in berry samples from the two locations. All the six aldehydes found in Oregon fruit were also identified in Arkansas fruit. Furthermore, Arkansas has three more odor-active aldehydes than Oregon. Seven aldehydes were reported having high FD factors. Hexanal, *trans,cis*-2,6-nonadienal, and benzaldehyde (FD = 516, 1024, 256, respectively in Oregon; FD = 16, 64, 8 respectively in Arkansas) showed higher FD factors in Oregon, while heptanal and *trans*-2-hexenal (FD = 16 and 16 in Oregon; FD = 64 and 512 in Arkansas) had higher FD factors in Arkansas. Furthermore, the Arkansas samples had two more potent aldehydes that were not present in Oregon samples, nonanal and vanillin (FD = 128 and 256).

There were seven odor-active ketones detected. Four of these ketones had a FD factor exceeding 64. All four of these were found in the Oregon samples but only two was found in the berries grown in Arkansas. *Cis*-1,5-octadien-3-one (FD = 2048 in Oregon, FD = 256 in Arkansas) was the most important ketone that showed high FD factors in both berries. Beta-damascenone, with rose-like, sweet, berry odor, also has high FD factors (FD = 64 in Oregon, FD = 1024 in Arkansas) in samples from both locations, but it shows significantly higher aroma impact in Arkansas than in Oregon. Additionally, 2-undecanone and 2,3-butanedione (FD = 128 and 512) also showed high FD factors in Oregon.

Eight alcohols, including six aliphatic and two aromatic alcohols were perceived in two aroma extracts, while only aromatic alcohols were detected as having high FD factors. Benzyl alcohol and phenethyl alcohol, both exhibiting

floral, rose-like aroma, were detected. Phenethyl alcohol had a higher FD factor in the Oregon grown fruit (128, FD = 8 in Arkansas), while benzyl alcohol had a higher FD factor in those from Arkansas (256, FD = 8 in Oregon). The aroma of other aliphatic alcohols was generally described as grass, green, and waxy.

Four odor-active short-chain fatty acids were found in berries from both regions. Generally, acids were not considered as impact aroma compounds due to their high odor threshold and it is not easy to detect their acid, cheesy smelling except when they are present in large quantities. The highest FD factors in acids were given to acetic and 2/3-methylbutanoic acids, both were 128 in the berries from the two states. Butanoic acid was also present in the Arkansas sample and had a high FD factor of 128.

Two sulfur-containing compounds, methional which has cooked potato notes, and the cabbage-like dimethyl sulfide, were detected. Both compounds have been detected as odor-active compounds in 'Marion' and 'Thornless Evergreen' blackberries recently (Klesk and Qian, 2003a, Klesk and Qian, 2003c). In this study, AEDA also revealed that methional was one of the most important odorant for 'Chickasaw' berries, with a FD factor of 2048 in samples from both locations. Methional is well known to be generated from the Strecker-degradation of amino acid methionine, while dimethyl sulfide was formed from the further degradation of methional. With an odor threshold of 0.2 ppb in water (Buttery, et al., 1971), methional might strongly influence the blackberry aroma. However, in the sensory evaluation of berry samples from the two locations, no descriptions related to

cooked potato were given by the panelists.

There were two furans, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (homofuraneol), and two lactones, δ-decalactone and γ-decalactone , identified in samples from both states. 2,5-dimethyl-4-hydroxy-3(2H)-furanone, was characterized as a key aroma compound in both berry samples (FD = 2048 in Oregon, FD = 512 in Arkansas), exhibiting an intense sweet, caramel, and strawberry-like aroma note to fruits. In Oregon-grown 'Chickasaw', homofuranel rendered additional sweet, caramel notes (FD = 128), but it was not considered as important odorant in Arkansas-grown fruit (FD = 8). The FD factors of δ-decalactone were close in the two samples (FD = 128 in Oregon, FD = 64 in Arkansas), while γ-decalactone show somewhat higher FD factors in Arkansas (FD = 64, FD = 16 in Oregon).

Four other compounds were identified in berry samples from the two locations: 3-isopropyl-2-methoxypyrazine, eugenol, elemicin, and theaspirane B. Because none of these showed a FD factor higher than 16, they were not considered as important odorants. Some unknown compounds also showed up on two stationary phase columns. They were not able to be identified due to the low quantity and coeluted with other compounds.

When comparing the numbers of odor-active volatile in each chemical class, the aroma compositions of the 'Chickasaw' samples from two locations were quite similar. However, these odorants show various aroma impacts in the samples from the two regions. On the basis of FD factors (FD $\geq$1024), the aroma

of 'Chickasaw' grown in Oregon can be primarily attributed to six compounds: ethyl butanoate, linalool, methional, *trans,cis*-2,6-nonadienal, *cis*-1,5-octadien-3-one, and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. Additionally ethyl 2-methylpropanoate, methyl butanoate, ethyl hexanoate, 2,3-butanedione, α-pinene, limonene, and hexanal are also important volatiles in the Oregon-grown fruit (FD ≥512). In the samples from Arkansas, the most potent aromas are ethyl butanoate, linalool, methional, ethyl 2-methylbutanoate, β-damascenone, and geraniol. Besides, ethyl 2-methylpropanoate, *allo*-ocimene, *trans*-2-hexenal, and 2,5-dimethyl-4-hydroxy-3(2H)-furanone were detected as potent aroma compounds in 'Chickasaw' grown in Arkansas (FD ≥512).

CONCLUSIONS

Results showed that flavor formation in 'Chickasaw' blackberries was strongly influenced by their growing environment. The most potent aroma compounds in Oregon-grown 'Chickasaw' are ethyl butanoate (fruity, apple-like), linalool (floral, perfume), methional (cooked potato), *trans,cis*-2,6-nonadienal (green, cucumber), *cis*-1,5-octadien-3-one (green, grass), and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (sweet, strawberry-like), while in Arkansas-grown 'Chickasaw', they are ethyl butanoate, linalool, methional, ethyl 2-methylbutanoate (fruity), β-damascenone and geraniol (sweet, rose-like). Although AEDA is a widely used screening method to identify the important odorants, there are many variations associated with human olfactometry. To

further explain and identify the difference between samples of the same genotype grown in two different regions, quantitative studies are required.

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CHAPTER IV

GENERAL CONCLUSION

Volatile compositions of 'Marion' and 'Thornless Evergreen' blackberries from three growing seasons were analyzed using gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS). Seasonal variations were observed for some volatile compounds in both 'Marion' and 'Thornless Evergreen' blackberries. In 'Marion', these compounds were mainly acids. In 'Thornless Evergreen', seasonal variations were mainly noted for acids, alcohols, and a few terpenoids. These variations and magnitude of changes appear random with regards to growing seasons.

Based on the average volatile levels on three seasons, volatile compositions of 'Marion' and 'Thornless Evergreen' blackberries were very different. 'Thornless Evergreen' had many more volatiles than 'Marion'. While more acids were found in 'Marion', more alcohols, terpenes and terpenoids were found in 'Thornless Evergreen'. It was generally observed that the most abundant volatiles in 'Marion' blackberry were acetic, 2/3-methylbutanoic, hexanoic and decanoic acids, ethanol, and linalool, whereas the most abundant volatiles in 'Thornless Evergreen' were 2-heptanol, hexanol, octanol, α-pinene, nopol, and *p*-cymen-8-ol. The compounds with the high odor activity values (OAV > 10) in 'Marion' were ethyl hexanoate, β-ionone, linalool, 2-heptanone, 2-undecanone, α-ionone, and hexanal. The compounds with the high odor activity values (OAV >10) in

'Thornless Evergreen' were ethyl hexanoate, 2-heptanone, ethyl 2-methylbutanoate, 2-heptanol, 3-methylbutanal, α-pinene, limonene, *p*-cymene, linalool, *t*-2-hexenal, myrtenol, hexanal, 2-methylbutanal, and sabinene. The OAVs reported in this study corroborate published aroma descriptions of the 2 cultivars; however, sensory recombination study is needed to confirm the results.

The aroma profile was characterized for 'Chickasaw' blackberries from two growing regions of the United States: Oregon and Arkansas. These results showed that the flavor formation was largely influenced by their growing environments. Although some common compounds were found in the two berries grown in different regions, those odorants had various aroma impacts in each region. The most potent aroma in Oregon-grown 'Chickasaw' are ethyl butanoate (fruity, apple-like), linalool (floral, perfume), methional (cooked potato), *trans,cis*-2,6-nonadienal (green, cucumber), *cis*-1,5-octadien-3-one (green, cut grass), and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (sweet, strawberry-like), while in Arkansas-grown 'Chickasaw', they are ethyl butanoate, linalool, methional, ethyl 2-methylbutanoate (fruity), β-damascenone (rose-like, berry), and geraniol (sweet, rose-like). AEDA is a widely used screening method to identify the important odorants, however, there are many variations associated with human olfactometry. To further explain and identify the difference between samples of the same genotype grown in two different regions, quantitative studies are required.

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