

Aroma Extract Dilution Analysis of Cv. Marion (*Rubus* spp. *hyb*) and Cv. Evergreen (*R. laciniatus* L.) Blackberries

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Cultivar Marion and Evergreen blackberry aromas were analyzed by aroma extract dilution analysis. Sixty-three aromas were identified (some tentatively) by mass spectrometry and gas chromatography–retention time; 48 were common to both cultivars, and 27 have not been previously reported in blackberry fruit. A comparison of cultivars shows that both have comparable compound types and numbers but with widely differing aroma impacts, as measured by flavor dilution (FD) factors. Ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, hexanal, furanones (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, and 5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone), and sulfur compounds (thiophene, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, 2-methylthiophene, and methional) were prominent in Evergreen (FD 512–2048). Except for ethyl 2-methylpropanoate, these same compounds were also prominent in Marion, but the FD factors varied significantly (FD 8–256) from Evergreen. The aroma profile of blackberry is complex, as no single volatile was unanimously described as characteristically blackberry.

KEYWORDS: Marion; Evergreen; blackberry aroma; AEDA; GC/O; GC-MS

INTRODUCTION

Blackberries are popular fruits because of their flavor and nutritional content; they have been used as food and medicine for hundreds of years (1). Used fresh and processed into a variety of food products, blackberries are extensively cultivated; yet, their aroma compositions have not been detailed as compared to the aromas of other small fruits such as raspberry and strawberry (2–6). Independent analyses of blackberries examined anthocyanins (1), sugars and acids (7, 8), and volatile and bound volatile compounds in fresh or processed blackberries (9–17). Most of the studies investigated volatile compounds (9–14) in the Evergreen cultivar. A total of 147 volatiles have been reported in fresh blackberries (18), but very few studies were about aroma active compounds, and few compounds were specifically described as “blackberry-like”.

The Marion cultivar blackberry (*Rubus* spp. *hyb*) has a flavor greatly preferred by consumers; consequently, it has replaced cv. Thornless Evergreen (*Rubus laciniatus* Willd.) as the predominant cultivar planted in the Pacific Northwest (19, 20). Consumer preference for the Marion has stimulated research to correlate quantifiable blackberry flavor characteristics to berry genetic makeup, to breed new thornless blackberry cultivars with Marion flavor. Because the aroma differences between Marion and Evergreen have been only subjectively described (20), the purpose of this investigation was to identify, rank, and compare

the odor active compounds in the two cultivars using aroma extract dilution analysis (AEDA) and gas chromatography–mass spectrometry (GC-MS).

MATERIALS AND METHODS

Chemicals. Authentic aroma standards were obtained as follows: butyl acetate, limonene, octyl acetate, octyl formate, 2-heptanone, and 2-undecanone were from K&K Laboratories (Jamaica, NY). Methyl hexanoate and octanol were from Eastman (Rochester, NY). Acetaldehyde, acetic acid, β -ionone, butanoic acid, *l*-carvone, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone, dimethyl disulfide, dimethyltrisulfide, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylpropanoate, eugenol, 2-heptanol, hexanal, hexanoic acid, *t*-2-hexenal, linalool, *p*-methylacetophenone, 3-methylbutanal, methyl butanoate, 2-methylbutanoic acid, nonanal, *t*-2-nonenal, octanol, 1-octen-3-ol, 1-octen-3-one, and phenethyl alcohol were from Aldrich Chemical Co. Inc. (Milwaukee, WI). Diacetyl and methional were from Sigma Chemical Co. (St. Louis, MO).

Blackberry Samples. Marion and Evergreen blackberries were grown in Woodburn, OR, from 5 to 10 year old plants. The fruits (both machine and hand-harvested) were washed, graded, individually quick frozen (IQF), and stored at $-18\text{ }^{\circ}\text{C}$. One box of each cultivar (13.6 kg, frozen for 5 months) was transported on ice to the laboratory, where they were stored at $-23\text{ }^{\circ}\text{C}$. Samples had been frozen for 9 months when analyzed.

Extraction of Volatile Compounds. For each cultivar, 1 kg of IQF blackberries was thawed at room temperature in a single layer for 3 h. The berries were combined with 100 g of NaCl and 10 g of CaCl_2 in a commercial blender and blended by pulsing for a total of 3 min at

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Table 1. AEDA of Cv. Marion and Evergreen Blackberries (Stabilwax Column)

| RI | compd ^a | aroma descriptors this study | basis of identification ^b | FD factors | |
|------|--|-------------------------------------|--------------------------------------|------------|-----------|
| | | | | Marion | Evergreen |
| 811 | ethyl acetate ^f | floral, fruity | MS, RI | 16 | 2 |
| 910 | 3-methylbutanal | fresh grass, fruity, leaf | MS, RI | | 4 |
| 935 | dimethyl sulfide ^{r,T} | garlic bologna, cabbage | RIL | 16 | 128 |
| 965 | ethyl 2-methylpropanoate ^r | sweet, fruity, berry, floral | RI | | 256 |
| 973 | methyl butanoate | fruity, sweet | RI | 4 | |
| 998 | 2,3-butanedione (diacetyl) ^r | buttery | MS, RI | 2 | 2 |
| 1030 | thiophene ^{r,T} | garlic bologna, sulfury | RIL | 8 | 128 |
| 1038 | ethyl butanoate | fruity, banana | MS, RI | 4 | |
| 1053 | ethyl 2-methylbutanoate ^f | fruity | MS, RI | 4 | |
| 1065 | ethyl 3-methylbutanoate | fruit, sweet, banana | RI | | 64 |
| 1082 | butyl acetate | fruity, juicy | MS, RI | 2 | |
| 1103 | dimethyl disulfide ^r | vegetal | MS, RI | 2 | |
| 1106 | hexanal ^f | green, fresh | MS, RI | | 4 |
| 1161 | unk | plastic, fatty, waxy | | 32 | |
| 1191 | 2-heptanone ^f | fruity, banana, sweet, floral | MS, RI | 16 | 32 |
| 1202 | methyl hexanoate ^f | fruity, green, sweet | MS, RI | 8 | 4 |
| 1251 | ethyl hexanoate ^f | fruity, floral | MS, RI | 8 | |
| 1326 | 1-octen-3-one ^{r,f} | mushroom, earthy | RI | 8 | 16 |
| 1333 | 2-heptanol ^f | woody, earthy, vegetal, minty | MS, RI | 4 | 512 |
| 1369 | hexanol ^{f,T} | floral, spice | MS, RIL | 4 | |
| 1383 | dimethyl trisulfide ^r | vegetal, garlic | MS, RI | 2 | 16 |
| 1402 | nonanal ^f | floral, fruity | MS, RI | 2 | |
| 1467 | acetic acid ^f | acid, sour | MS, RI | 16 | 32 |
| 1476 | 1-octen-3-ol | mushroom | RI | 4 | |
| 1490 | methional ^f | potato, earthy, onion | RI | 32 | 512 |
| 1491 | octyl acetate | floral, sweet | MS, RI | 4 | |
| 1508 | theaspirane A ^{r,r,T} | floral, earthy, tea, green | MS, RIL | 4 | |
| 1550 | theaspirane B ^{r,r,T} | earthy, fruity, sweet | MS, RIL | 4 | |
| 1560 | linalool ^f | sweet, floral, berry, green | MS, RI | 4 | 128 |
| 1574 | octanol ^f | waxy, fruity | MS, RI | 2 | |
| 1622 | 2-undecanone ^f | floral, grn, pine, citrus | MS, RI | 8 | 128 |
| 1634 | unk | roasted peanuts | | 2 | |
| 1650 | butanoic acid ^{r,f} | rancid cheese, sour, pungent | MS, RI | 32 | |
| 1693 | 2-methylbutanoic acid ^{r,f} | rancid cheese, sour, acid | MS, RI | 32 | 128 |
| 1724 | unk | plastic curtain, waxy | | | 32 |
| 1749 | <i>l</i> -carvone | peppermint, fresh leaf | MS, RI | 8 | 4 |
| 1794 | <i>p</i> -methylacetophenone | fresh, green, floral, fruity | MS, RI | | 32 |
| 1851 | β -damascenone ^{r,T} | sweet, floral, grape, blackberry | MS, RIL | 4 | 32 |
| 1868 | hexanoic acid ^{r,f} | pungent, sour | MS, RI | 2 | 128 |
| 1875 | unk | waxy citrus, lemon, woody | | | 32 |
| 1905 | benzyl alcohol ^{r,T} | sweet, citrus, grass | MS, RIL | 2 | 2 |
| 1952 | phenethyl alcohol ^f | floral, perfume, peach | MS, RI | 8 | 64 |
| 1967 | unk | grass, pungent, green, floral | | | 8 |
| 2017 | cinnamic aldehyde ^T | sweet, spice, cinnamon | MS, RIL | 4 | |
| 2018 | unk | floral, grass, green | | | 8 |
| 2053 | 2,5-dimethyl-4-hydroxy-3-(2 <i>H</i>)-furanone ^{r,f} | fruity, sweet, caramel | RI | 8 | 1024 |
| 2078 | 2-ethyl-4-hydroxy-5-methyl-3-(2 <i>H</i>)-furanone ^{r,T} | cooked bramble, sweet caramel | RIL | 32 | 16 |
| 2114 | 4-hydroxy-5-methyl-3-(2 <i>H</i>)-furanone ^{r,T} | caramel, strawberry, cooked bramble | RIL | 32 | 8 |
| 2211 | 4,5-dimethyl-3-hydroxy-2-(5 <i>H</i>)-furanone ^{r,T} | spice, curry, fruity | RIL | 4 | 128 |
| 2246 | 5-ethyl-3-hydroxy-4-methyl-2-(5 <i>H</i>)-furanone ^{r,T} | roasted meat, cumin, maple syrup | RIL | 4 | 256 |
| 2294 | cinnamic alcohol ^{r,T} | floral, tea, sweet, fruity | RIL | 8 | 32 |

^a *, not previously reported in blackberry; r, reported in red raspberry; T, tentative identification. ^b MS, mass spectral data; RIL, retention index from literature; RI, retention index from standards.

high speed. Calcium chloride was added to inhibit enzyme activity as described by Buttery and others (21). The pureed fruit was passed through a commercial stainless steel food mill to remove seeds. The seed pulp was batch-extracted three times with freshly distilled pentane: diethyl ether (1:1 v/v) while the seedless puree was extracted three times in a separatory funnel. The extracts were combined to yield a total volume of 880 mL. Nonvolatiles were removed from the organic extract using solvent-assisted flavor extraction (SAFE) at 50 °C under vacuum according to the method proposed by Engel and others (22). The organic SAFE extract was dried with anhydrous Na₂SO₄, concentrated to 1 mL by solvent distillation, and reduced to its final volume of 0.1 mL with a flow of nitrogen.

GC/O Analysis. 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 poly(ethylene glycol), 1 μm film thickness, Restek Corp., Bellefonte, PA) and a DB-5 column (30 m × 0.32 mm i.d., cross-linked phenyl-methyl polysiloxane, 1 μm film thickness, J&W Scientific, Folsom, CA). The 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, and the 2 μL sample injections were splitless. The oven temperature was programmed for a 2 min hold at 40 °C,

Table 2. AEDA of Cv. Marion and Evergreen Blackberries (DB5 Column)

| RI | compd ^a | aroma descriptors this study | basis of identification ^b | FD factors | |
|------|--|--------------------------------------|--------------------------------------|------------|-----------|
| | | | | Marion | Evergreen |
| <500 | acetaldehyde ^{r,T} | grass, green | MS, RI | 1 | 8 |
| 516 | dimethyl sulfide ^{r,T} | garlic, onion | RIL | 16 | 8 |
| 557 | 2-methylpropanal ^{r,T} | wood, grass | MS, RIL | 1 | |
| 579 | 2,3-butanedione (diacetyl) ^{r,T} | buttery | MS, RI | 2 | 2 |
| 599 | acetic acid ^r | acetic acid, vinegar | RI | | 16 |
| 609 | methylethyl sulfide ^{r,T} | alliaceous, pungent | RIL | 16 | 8 |
| 610 | ethyl acetate ^r | fruity | RI | 1 | |
| 621 | unk | pungent | | 8 | 4 |
| 649 | 3-methylbutanal | vegetal, earthy | MS, RI | 1 | 1 |
| 661 | thiophene ^{r,T} | sour, green, earthy, onion | RIL | 4 | 2048 |
| 727 | dimethyl disulfide ^r | pungent, garlic, sulfury | MS, RI | 32 | 2048 |
| 753 | ethyl 2-methylpropanoate ^r | fruity | RI | | 2048 |
| 758 | 2-methylthiophene ^{r,T} | earthy, pungent | RIL | | 512 |
| 770 | ethyl butanoate | fruity | MS, RI | 2 | |
| 791 | hexanal ^r | green, fresh | MS, RI | 64 | 1024 |
| 808 | butanoic acid ^{r,T} | cheesy, pungent | RI | 1 | 2 |
| 848 | ethyl 2-methyl/3-methylbutanoate ^r | fruity, sweet, berry, banana | RI | 128 | 1024 |
| 854 | 2-methylbutanoic acid ^{r,T} | cheesy, sour, smelly | RIL | 32 | 16 |
| 874 | <i>t</i> -2-hexenal ^r | fruity, orange, green | MS, RI | 1 | 4 |
| 897 | methional ^r | baked potato | RI | 256 | 2048 |
| 906 | 2-heptanol ^r | peppermint, green, woody | MS, RI | 8 | 2 |
| 957 | unk | woody, floral, green | | | 128 |
| 959 | benzaldehyde ^{r,T} | fruity, berry, juicy | MS, RIL | 64 | 256 |
| 971 | 1-octen-3-one ^{r,T} | mushroom, earthy | MS, RI | 2 | 16 |
| 979 | dimethyl trisulfide ^r | green veggie, garlic | RI | 16 | |
| 980 | 1-octen-3-ol | woody, earthy, mushroom | RI | | 64 |
| 999 | hexyl acetate ^r | fruity | MS, RI | 1 | 1 |
| 1002 | ethyl hexanoate ^r | floral, fruity | MS, RI | 32 | 1 |
| 1033 | limonene ^r | overripe melon, green, tea | MS, RI | 4 | 2 |
| 1042 | <i>t</i> - β -ocimene ^{r,T} | sweet, floral, woody, perfume | RIL | 8 | 2 |
| 1045 | benzyl alcohol ^{r,T} | floral, fruity, rose | MS, RIL | 1 | 2 |
| 1072 | 2,5-dimethyl-4-hydroxy-3-(2 <i>H</i>)-furanone ^{r,T} | caramel, strawberry | RI | 32 | |
| 1087 | 4-hydroxy-5-methyl-3-(2 <i>H</i>)-furanone ^{r,T} | cotton candy, sweet | RIL | 4 | |
| 1096 | linalool ^r | fruity, green, sweet, watermelon | MS, RI | 16 | 8 |
| 1099 | α -terpinolene ^{r,T} | woody, sweet, earthy | MS, RIL | 1 | 64 |
| 1100 | nonanal ^r | watermelon, citrus, floral | MS, RI | 8 | 8 |
| 1104 | octyl formate ^r | fruity | RI | 1 | |
| 1112 | phenethyl alcohol ^r | fruity, floral, rose, sweet | MS, RI | | 32 |
| 1131 | 4,5-dimethyl-3-hydroxy-2-(5 <i>H</i>)-furanone ^{r,T} | roasted vegetables, sweet, caramel | RIL | 32 | 4 |
| 1136 | 2-ethyl-4-hydroxy-5-methyl-3-(2 <i>H</i>)-furanone ^{r,T} | floral, sweet, caramel | RIL | 2 | 2 |
| 1149 | neo-allo-ocimene ^{r,T} | citrus, vegetal, cucumber | MS, RIL | 16 | 4 |
| 1161 | <i>t</i> -2-nonenal ^r | watermelon, fresh vegetable, green | MS, RI | 1 | 64 |
| 1179 | <i>p</i> -methylacetophenone | floral, hot candy, sweet | RI | 2 | 8 |
| 1234 | <i>l</i> -carvone | anise, fennel | RI | 2 | |
| 1255 | 5-ethyl-3-hydroxy-4-methyl-2-(5 <i>H</i>)-furanone ^{r,T} | caramel, smoky | RIL | 1 | |
| 1290 | 2-undecanone ^r | wet grass, tea, floral, green | MS, RI | 4 | 16 |
| 1305 | theaspirane A ^{r,T} | warm spices, vegetal, pungent | MS, RIL | 2 | 1 |
| 1321 | unk | caramel, fruity, tea, green | | | 16 |
| 1324 | 4-vinylguaiaicol ^{r,T} | BBQ rub, spicy | RIL | 1 | |
| 1370 | eugenol ^r | woody, citrus, spicy | RI | 4 | 4 |
| 1392 | β -damascenone ^{r,T} | floral, berry, sweet, grape | MS, RIL | 8 | 4 |
| 1433 | unk | sweet, fruity, herbal, tea | | 2 | |
| 1451 | unk | floral, spice, perfume, fruit, juicy | | 4 | |
| 1496 | β -ionone ^r | floral, perfume, woody, spicy | MS, RI | 2 | 16 |
| 1543 | elemicin ^T | green tea, spicy, perfume | RIL | 1 | |

^a*, not previously reported in blackberry; r, reported in red raspberry; T, tentative identification. ^b MS, mass spectral data; RIL, retention index from literature; RI, retention index from standards.

then 40–100 °C at 5 °C/min, then 100–230 °C at 4 °C/min (10 min hold). Retention indices (RI) were estimated in accordance with a modified Kovats method (23).

AEDA. Flavor dilution (FD) factors for the odor active compounds in each cultivar were determined using AEDA (24). Concentrated samples were serially diluted with 1:1 (v/v) pentane:diethyl ether (1 + 1). GC/O with two experienced panelists was then performed with 2 μ L injections of original samples and diluted extracts.

GC-MS Analysis. Analysis of the original concentrated AEDA samples was performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector. System software control and data management/analysis were performed through Enhanced ChemStation Software, G1701CA v. C.00.01.08 (Agilent Technologies, Inc., Wilmington, DE). Volatile separation was achieved with two fused silica capillary columns: a 30 m \times 0.32 mm i.d. Stabilwax (cross-linked poly(ethylene glycol)) column with a 1 μ m

Table 3. AEDA Summary of Cv. Marion and Evergreen Blackberries

| cultivar | compd ^a | | cultivar | compd ^a |
|-----------|--|--------------|-----------|--|
| | | Acids | | |
| both | acetic acid ^f | | both | hexanoic acid ^{f,r} |
| both | butanoic acid ^{f,r} | | both | 2-methylbutanoic acid ^{f,r} |
| | | Alcohols | | |
| both | benzyl alcohol ^f | | both | linalool ^f |
| both | cinnamic alcohol ^f | | Marion | octanol ^f |
| Evergreen | heptanol ^f | | both | 1-octen-3-ol |
| both | 2-heptanol ^f | | both | phenethyl alcohol ^f |
| Marion | hexanol ^f | | | |
| | | Aldehydes | | |
| both | acetaldehyde ^{f,r} | | both | methional ^f |
| both | benzaldehyde ^f | | both | 3-methylbutanal |
| Marion | cinnamic aldehyde | | Marion | 2-methylpropanal ^f |
| both | hexanal ^f | | both | nonanal ^f |
| both | <i>t</i> -2-hexenal ^f | | both | <i>t</i> -2-nonenal ^f |
| | | Esters | | |
| Marion | butyl acetate | | Evergreen | ethyl 2-methylpropanoate [*] |
| both | ethyl acetate ^f | | both | hexyl acetate ^f |
| Marion | ethyl butanoate | | Marion | methyl butanoate |
| both | ethyl hexanoate ^f | | both | methyl hexanoate ^f |
| both | ethyl 2-methylbutanoate ^f | | Marion | octyl acetate |
| Evergreen | ethyl 3-methylbutanoate | | Marion | octyl formate [*] |
| | | Furanones | | |
| both | 2,5-dimethyl-4-hydroxy-3-(2 <i>H</i>)-furanone ^{f,r} | | both | 5-ethyl-3-hydroxy-4-methyl-2-(5 <i>H</i>)-furanone [*] |
| both | 4,5-dimethyl-3-hydroxy-2-(5 <i>H</i>)-furanone [*] | | both | 4-hydroxy-5-methyl-3-(2 <i>H</i>)-furanone [*] |
| both | 2-ethyl-4-hydroxy-5-methyl-3-(2 <i>H</i>)-furanone [*] | | | |
| | | Hydrocarbons | | |
| both | limonene ^f | | both | <i>t</i> - β -ocimene [*] |
| both | neo-allo-ocimene [*] | | both | α -terpinolene ^f |
| | | Ketones | | |
| both | 2,3-butanedione (diacetyl) ^{f,r} | | both | β -ionone ^f |
| both | <i>t</i> -carvone | | both | <i>p</i> -methylacetophenone |
| both | β -damascenone ^f | | both | 1-octen-3-one ^{f,r} |
| both | 2-heptanone ^f | | both | 2-undecanone ^f |
| | | Phenols | | |
| Marion | elemicin | | Marion | 4-vinylguaiacol ^{f,r} |
| both | eugenol ^f | | | |
| | | Sulfur | | |
| both | dimethyl disulfide [*] | | both | methylethyl sulfide [*] |
| both | dimethyl sulfide [*] | | both | 2-methylthiophene [*] |
| both | dimethyl trisulfide [*] | | both | thiophene [*] |
| | | Theaspiranes | | |
| both | theaspirane A ^{f,r} | | Marion | theaspirane B ^{f,r} |

^a *, not previously reported in blackberry; r, reported in red raspberry.

film thickness (Restek) and the other a 30 m \times 0.25 mm i.d. DB-5 (cross-linked phenyl-methyl polysiloxane) column with a 0.25 μ m film thickness (J&W Scientific). The helium column flow rate was 2.0 mL/min, and the 2 μ L sample injections were splitless. The oven temperature was programmed as for the GC/O analysis. Injector, detector transfer line, and ion source temperatures were 250, 280, and 230 $^{\circ}$ C, respectively. Electron impact mass spectrometric data from *m/z* 35–300 was collected at 5.27 scans/s, at an ionization voltage of 70 eV. RI were estimated in accordance with a modified Kovats method (23). Compound identifications were made by comparing aromas with authentic standards and Kovats RI, RI reported in the literature (25, among others), and/or mass spectral data from the Wiley 275.L (G1035) Database (Agilent Technologies, Inc.).

Further Identification of Some Aroma Compounds by GC-MS Analysis. To further clarify AEDA volatile composition, for each cultivar, 5 kg of IQF blackberries was thawed at room temperature in a single layer for 3 h. The berries were blended by pulsing for a total of 3 min at high speed in a commercial blender, and the purée was poured into a stainless steel pan. Concentrated pectolytic enzyme

(Vinozym FCE G, Novo Nordisk, Franklinton, NC) was prepared and thoroughly mixed into the purée. A total of 0.15 g of enzyme were added to the Marion purée, and 1.0 g was added to the more viscous Evergreen purée. The mixture was covered with aluminum foil and left to stand at room temperature overnight (15 h). Five hundred grams of NaCl was blended in, and the mixture was strained and extracted as for the GC/O analysis using CH₂Cl₂ (total volume 2400 mL). The extraction produced an emulsion that was broken with centrifugation for 20 min (1800 rpm, approximately 1000 g). The organic extract was then further prepared as for the GC/O analysis and reduced to its final volume of 0.2 mL with a flow of nitrogen. Analysis conditions and methods were identical to those used for the 1 kg samples, except that 5 μ L of sample was injected, and the oven temperature was programmed for a 2 min hold at 40 $^{\circ}$ C, then 40–230 $^{\circ}$ C at 1 $^{\circ}$ C/min (2 min hold).

RESULTS AND DISCUSSION

Tables 1 and 2 list Marion and Evergreen blackberry volatiles separated with polar and nonpolar columns. On the polar

column, a total of 51 aroma compounds were detected, with 45 of them identified. On the nonpolar column, 55 aromas were detected, and 51 of them were identified. Among these identified aromas, 12 were detected on the polar column only, while 17 were detected on the nonpolar column only. Combined data (Table 3) show that 63 odor active volatiles were detected and 48 were common to both cultivars. Marion contained 60 of 63 volatiles, and Evergreen contained 51.

The most significant (FD \geq 16) odor active volatiles in Marion determined on the nonpolar (DB-5) column were methional (FD = 256); ethyl 2-methylbutanoate (FD = 128); benzaldehyde and hexanal (FD = 64); 2-methylbutanoic acid, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone, 4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone, ethyl hexanoate, dimethyl disulfide, and 2-methylthiophene (FD = 32); and linalool, neo-allo-ocimene, dimethyl sulfide, dimethyl trisulfide, and methylethyl sulfide (FD = 16). In addition, 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone, 4-hydroxy-5-methyl-3-(2*H*)-furanone, and butanoic acid (FD = 32), ethyl acetate, acetic acid, and 2-heptanone (FD = 16) may also be important to Marion blackberry flavor, as they had high FD factors as determined on the polar (Stabilwax) column.

Many significant (FD \geq 16) odor active volatiles in Evergreen were identified on the DB-5 column. The most important aroma compounds included methional, ethyl 2-methylpropanoate, thiophene, and dimethyl disulfide (FD = 2048); hexanal and ethyl 2-methylbutanoate (FD = 1024); 2-methylthiophene (FD = 512); benzaldehyde (FD = 256); heptanol (FD = 128); 1-octen-3-ol, *t*-2-nonenal, and α -terpinolene (FD = 64); phenethyl alcohol (FD = 32); and 1-octen-3-one, 2-undecanone, acetic acid, 2-methylbutanoic acid, and β -ionone (FD = 16).

In addition, as determined on the polar (Stabilwax) column, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone (FD = 1024); 2-heptanol (FD = 512); ethyl 2-methylpropanoate and 5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone (FD = 256); thiophene, linalool, 2-undecanone, hexanoic acid, 4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone and dimethyl sulfide (FD = 128); ethyl 3-methylbutanoate (FD = 64); cinnamic alcohol, 2-heptanone, *p*-methylacetophenone, and β -damascenone (FD = 32); and 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone, 1-octen-3-one, and dimethyl trisulfide (FD = 16) may also be important to Evergreen blackberry flavor.

The cultivars have comparable compound types and numbers but with widely differing aroma impacts, as measured by FD factors. Fresh Marion blackberry aroma has been described as floral, fruity, sweet, caramel-fruity, and woody, while fresh Evergreen aroma is spicy, green, herbaceous, fruity, and sweet. However, there are no prominent corresponding compositional differences between the cultivars within a volatile class. Both cultivars contain the same numbers of odor active acids, furanones, hydrocarbons, ketones, and sulfur compounds. The Marion contains one more theaspirane (theaspirane B); two more alcohols (hexanol, octanol), aldehydes (cinnamic, 2-methylpropanal), and phenols (elemicin, 4-vinylquaiacol); and five more esters (methyl butanoate, ethyl butanoate, butyl acetate, octyl acetate, and octyl formate) than Evergreen. The Evergreen has one alcohol (heptanol) and two esters (ethyl 2-methylpropanoate, ethyl 3-methylbutanoate) not present in Marion. Of 27 newly reported volatiles, three organic acids, two aldehydes, five furanones, two hydrocarbons, two ketones, six sulfur compounds, and one theaspirane are shared by the cultivars. This relatively large number of new volatiles is probably due to the extraction and analytical methods used. It is thought that some portion of Marion aroma is due to its hybrid pedigree, which

contains at least five bramble species, including raspberry (20). However, although 35 volatiles in this study have been previously reported in red raspberry (12, 18, 26, 27), 30 of them are common to both Marion and Evergreen; only four are unique to Marion. Five volatiles out of 63 were described with aroma descriptors specific to bramble fruit (berry, blackberry); no single compound was unanimously described as characteristically blackberry.

AEDA is a suitable method to screen potent odorants in blackberry, and results indicate that characteristic blackberry aroma is apparently a complex formulation of volatiles. Marion and Evergreen blackberries have many potent odorants in common, but qualitative aroma comparisons consistently note the more floral, caramel-fruity, sweet aroma of Marion as compared to the spicy, herbaceous, less fruity aroma of Evergreen. Because a FD factor is the ratio of an odorant's concentration in an initial GC/O extract to its concentration in the most dilute extract that still allows detection, the value is a relative measure (28) and does not conclusively determine that one cultivar contains more of a given aroma compound than another. Because the aroma profile of a food is, among others, a function of volatile concentrations and odor thresholds, the next step in identifying specific aroma differences between Marion and Evergreen is the quantification of each aroma with a high FD factor and calculation of its odor activity value (OAV), the ratio of the aroma concentration to its odor threshold in air. OAVs are better measures of which aroma compounds contribute to a cultivar's aroma and of the differences in cultivar aroma profiles.

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