Food Chemistry 119 (2010) 1127–1134

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Volatile composition and odour-activity value of thornless ‘Black Diamond’ and ‘Marion’ blackberries

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ARTICLE INFO

Article history:
Received 4 February 2009
Received in revised form 19 May 2009
Accepted 17 August 2009

Keywords:
Marion
Marionberry
Black Diamond
Blackberry
Stir bar sorptive extraction (SBSE)
Solid-phase extraction (SPE)
Microvial insert thermal desorption

ABSTRACT

‘Black Diamond’ is a recently developed thornless blackberry cultivar with large fruit size, high yield, and good processed fruit quality that has rapidly become an industry standard. The flavour of ‘Black Diamond’ fruit is not the same as ‘Marion’, which is regarded by the industry as having the ideal flavour. In order to understand the aroma differences, the volatile composition of ‘Marion’ and ‘Black Diamond’ was analysed using stir bar sorptive extraction-gas chromatography–mass spectrometry (SBSE-GC–MS) and solid-phase extraction (SPE)-microvial insert thermal desorption-GC–MS for two growing seasons. Although seasonal variations were present, the overall volatile profile in ‘Marion’ and ‘Black Diamond’ were very similar, but the concentrations of some aroma compounds varied greatly. Odour-activity value (OAV) indicated that furaneol, linalool, β-ionone, and hexanal could be most important in ‘Marion’, while in ‘Black Diamond’, the most important compounds were linalool, β-ionone, furaneol, and 2-heptanol. The major difference between the cultivars for aroma compounds was that ‘Marion’ had a 5 times higher OAV of furaneol than ‘Black Diamond’, while ‘Black Diamond’ had a 3 times higher OAV of linalool than ‘Marion’. The chemical analysis results matched with the descriptive sensory evaluation that ‘Marion’ had more berry, fruity, strawberry aroma while ‘Black Diamond’ had more floral aroma.

1. Introduction

Blackberries are widely distributed around the world and their commercial popularity has increased rapidly in the past 10 years (Strik, Clark, Finn, & Bañados, 2007). In addition to being a rich source of vitamins and dietary fibre, blackberries are rich in phenolic compounds such as anthocyanins, flavonols, flavanols, ellagitannins, gallotannins, proanthocyanidins, and phenolic acids (Seeram et al., 2006). Numerous studies have demonstrated that these phenolic compounds have many biological functions such as antioxidant, anticancer, anti-neurodegenerative, and anti-inflammatory activities (Seeram et al., 2006). The new discoveries of health benefits of blackberries have spurred the rapid increase in blackberry consumption in the fresh, processed, and nutraceutical markets.

The Pacific Northwest of America is the leading blackberry production region in the world and ‘Marion’, commonly marketed as “marionberry”, has been the predominant blackberry cultivar grown since the 1960s. ‘Marion’ has become so important because it has outstanding processed fruit quality and has an outstanding reputation for its flavour. However, the ‘Marion’ plants are thorny and the thorns can end up in the fruit when the berries are machine harvested. The thorns in the product can be dangerous for the consumer and a liability for the processor. One of the top research priorities for the Pacific Northwest blackberry industry has been the development of thornless cultivars with flavour characteristics similar to ‘Marion’.

Towards this goal, three thornless blackberries were recently released including ‘Black Diamond’, ‘Black Pearl’, and ‘Nightfall’ (Finn, Yorgey, Strik, Martin, & Qian, 2005b; Finn, Yorgey, Strik, Martin, & Qian, 2005c; Finn et al., 2005a). Based on the number of plants sold, ‘Black Diamond’ has been the most commonly planted cultivar since 2005 with 55% more plants of it planted than ‘Marion’ (P. Moore, pers. comm.). As these plants grow and reach maturity, they are becoming a significant proportion of the fruit harvested in the Pacific Northwest. The outstanding characteristics of ‘Black Diamond’ include large, uniformly shaped and firm fruit, high yield, good processed fruit quality, excellent adaptation to machine harvesting, and good disease and winter injury tolerance (Finn et al., 2005a). While ‘Black Diamond’ has been predominantly planted to supply the processed market, it is also grown to supply fruit for regional, wholesale fresh markets.

‘Black Diamond’ fruit has a flavour appealing to many consumers. However, ‘Black Diamond’ and ‘Marion’ have different flavour profiles, and ‘Marion’ flavour is preferred by some consumers. ‘Marion’ has typical sweet, caramel, fresh fruity flavour; while

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doi:10.1016/j.foodchem.2009.08.024

'Black Diamond' has raspberry and fresh, fruity aroma (Kurnianta, 2005).

Although blackberry has been widely planted, the study of blackberry flavour is still very limited. The early studies focused on the volatiles constituents of blackberry (Georgilopoulos & Gallois, 1987; Georgilopoulos & Gallois, 1988; Gulan, Veek, Scanlan, & Libbey, 1973; Scanlan, Bills, & Libbey, 1970), and very diverse compounds have been identified. 2-Heptanol, *p*-cymen-8-ol, 2-heptanone, 1-hexanol, α-terpineol, pulegone, 1-octanol, isoborneol, myrtenol, 4-terpineol, carvone, elemicine, and nonanal have been identified as the major volatiles. Furfural and its derivatives were also identified to be the abundant in some blackberries (Turemis, Kafkas, Kafkas, Kurkcuoglu, & Baser, 2003).

Aroma compounds in blackberry have been studied using gas chromatography–olfactometry (GC–O) technique. Klesk and Qian (2003a), Klesk and Qian (2003b) studied aroma compounds in 'Thornless Evergreen' and 'Marion' using dynamic headspace GC–O and aroma extract dilution analysis technique. Ethyl 2-methylbutanoate, ethyl 2-methylbutanoate, 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, 4,5-dimethyl-3-hydroxy-2-(5H)-furanone, and 5-ethyl-3-hydroxy-4-methyl-2-(5H)-furanone, dimethyl trisulphide, linalool, and methional were identified as the major aroma compounds in blackberries.

Qian and Wang (2005) further compared the odour-activity values (OAV: ratio of concentration to its sensory threshold) of 'Marion' and 'Thornless Evergreen' from three growing seasons. They found large seasonal variation was present for both cultivars. They further identified linalool, α-ionone, and β-ionone to be also important to the aroma of 'Marion'.

As a newly developed thornless cultivar, 'Black Diamond' has a very appealing flavour (Finn et al., 2005a), however, its flavour profile, especially as it compares to Marion', has never been investigated. As fruit of 'Black Diamond' become a more important constituent of the processed blackberry supply, understanding how it compares with the current standard 'Marion' will be critical. The objective of this study was to quantify volatile composition in 'Marion' and 'Black Diamond' and use OAV to compare the aroma of 'Marion' and 'Black Diamond' blackberries.

2. Materials and methods

2.1. Chemicals

Sources of volatile standards used in this study are listed in Tables 1 and 2. Methanol (HPLC grade) was from EM Science (Gibbstown, NJ), and dichloromethane (HPLC grade) was from Burdick and Jackson (Muskegon, MI). Standard solutions of 7-methyl-3-methylene-1,6-octadiene (myrcene), 3,7-dimethyl-1,3,6-octatriene (ocimene), and octanal were prepared in dichloromethane individually at a concentration around 10,000 mg/L, and all other standard solutions were prepared in methanol. Two internal standard mixtures were prepared. Internal standard mixture A was composed of 1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane (eucalyptol), 4-methyl-2-propan-2-ylphenol (isothymol), 4-heptanolide-4, 5-dihydro-5-propyl-2(3H)-furanone (γ-heptalactone), and 1-(2-hydroxy-5-methylphenyl)ethanone (2-hydroxy-5-methylacetophenone) with the concentrations of 3.4, 8.3, 7.6, and 3.5 mg/L; internal standard mixture B was composed of 2-hydroxy-5-methylacetophenone and 2-ethyl-3-hydroxy-4-pyranone (ethyl maltol), with the concentration of 70 and 248 mg/L, respectively.

Anhydrous sodium sulphate (99.9%, ACS certified) was supplied by Mallinckrodt (Mallinckrodt Baker, Phillipsburg, NJ). Fructose, glucose, and citric acid were from Lancaster (Ward Hill, MA); sucrose and malic acid were from Spectrum (Gardena, CA). Synthetic juice containing 3.0% fructose, 3.1% glucose, 0.17% sucrose, 0.8% citric acid, and 0.9% malic acid, was prepared according to the procedure described previously (Scherz & Senser, 1994).

2.2. Blackberry samples

Full ripe (shiny black) 'Black Diamond' fruits (°Brix 10.5 ± 0.5, TA 1.1 ± 0.1%) were hand harvested from plants growing in research plots at Oregon State University Lewis-Brown Farm in Corvallis, Oregon, between June and July of the 2003 and 2004 growing seasons; while 'Marion' (°Brix 12.0 ± 0.5, TA 1.6 ± 0.1%) were harvested in 2004 and 2006. The berries were individually quick frozen (IQF) and stored at −18 °C until analysis. During analysis, 100 g of IQF fruits were thawed in a refrigerator (1 °C), and equal weight of distilled water and 1% calcium chloride (final concentration) was added to inhibit enzymes. The sample was then pureed in a blender (Waring Products Div., Dynamics Corp. of America, New Hartford, CT). The puree was centrifuged for 20 min at 5000 rpm. The supernatant of juice was filtered through a Waterman No. 1 filter paper (particle retention > 11 μm), followed by a VWR 413 filter paper (particle retention > 5 μm). The fresh clear juice was used for stir bar sorptive extraction (SBSE) and solid-phase extraction (SPE) sample preparation.

2.3. Stir bar sorptive extraction GC–MS analysis

A 10 mL aliquot of blackberry juice was placed in a 20 mL vial to which 3 g of sodium chloride and 20 μL of internal standard solution A were added. A stir bar (Twister) coated with poly(dimethylsiloxane) (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc., Baltimore, MD) was used to extract volatile compounds. The stir bar was preconditioned with solvent (methanol: dichloromethane 1:1) according to the manufacture's instruction, then dried with air and conditioned for 30 min at 300 °C. The sample was extracted with the Twister bar for 2 h at a speed of 1000 rpm. After extraction, the Twister bar was rinsed with distilled water and placed into a sample holder for GC–MS analysis.

GC–MS analyses were performed using an Agilent 6890 gas chromatograph with a 5973 mass selective detector (Agilent, Santa Clara, CA). Samples were loaded into a Twister desorption unit (TDU) by a multi-purpose auto-sampler (Gerstel). A cooled injection system (CIS4) (Gerstel) was used in the GC–MS system. A CIS liner packed with 1-cm of Tenax sorbent (TA, 60/80, Supelco, Bellefonte, PA) was used during thermal desorption of samples.

The TDU had an initial temperature of 25 °C. After the sample was loaded, the TDU was heated at a rate of 300 °C/min to a final temperature of 250 °C and held for 1 min. The TDU injection was in splitless mode during thermal desorption, while the CIS4 was in a solvent vent mode with a venting flow of 60 mL/min for 4.7 min, at a venting pressure of 22.8 psi. After the solvent vent, the CIS4 was switched to splitless mode for 3.0 min, then changed to split mode with a venting flow of 60 mL/min. The initial temperature of the CIS4 was kept at −80 °C for 0.2 min then ramped at a rate of 10 °C/s to a final temperature of 250 °C and held for 10 min.

Compounds were separated with a DB-WAX column (30 m × 0.25 mm ID, 0.50 μm film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40 °C for a 2 min holding, then to 230 °C at a rate of 4 °C/min with 6 min holding. A constant helium column flow of 2.5 mL/min was used. A column splitter was used at the end of the column, 1 mL/min column flow was introduced to the MS, and the other 1.5 mL/min column flow was vented out. The MS transfer line and ion source temperature were 280 and 230 °C, respectively. Electron impact mass spectrometric data from *m*/*z* 35–350 were collected using a scan rate of 5.27/s, with an ionisation voltage of 70 eV.

Table 1
Chemical standards and quantification by stir bar sorptive extraction.

Chemicals	Source, purity	Quantify ions	Qualify ions	Slope[a]	Intercept	R^2	Range[b] (μg/L)	Recovery	RSD (%)
*Eucalyptol	Aldrich, 99%	81	108, 154						
Methyl butanoate	Aldrich, ⩾98%	74	71, 87	0.18	+0.11	0.979	0.5–230	100	17.1
Ethyl butanoate	Aldrich, ⩾98%	71	43, 88	0.68	+0.02	0.995	0.5–220	100	3.5
Butyl acetate	Aldrich, ⩾98%	43	56, 73	0.99	+0.07	0.987	0.5–270	117	7.1
Hexanal	Aldrich, ⩾97%	56	44, 41	0.27	+0.05	0.978	0.5–150	104	11.4
Isoamyl acetate	Aldrich, ⩾95%	43	55, 70	1.47	+0.02	0.995	0.5–160	97	6.0
Myrcene	K&K Lab, N.Y.	93	69, 41	0.70	+0.01	0.989	0.5–230	102	15.7
α-Terpinene	TCI American, 90%	121	93, 136	0.72	−0.06	0.932	0.5–200	93	9.2
Limonene	Aldrich, ⩾97%	68	93, 136	0.78	−0.06	0.976	0.5–370	94	12.4
Methyl hexanoate	Aldrich, ⩾99%	74	87, 99	2.10	+0.11	0.965	0.5–190	95	4.0
2-Heptanone	Sigma–Aldrich, 99%	43	58, 71	2.04	+0.17	0.961	0.5–200	104	6.3
trans-2-Hexenal	Aldrich, ⩾95%	69	55, 41	0.16	+0.01	0.960	0.5–200	106	14.3
Ethyl hexanoate	Aldrich, ⩾98%	88	99, 101	1.66	−0.03	0.983	0.5–240	91	2.6
β-Ocimene	Fluka, ∼97%	93	91, 92	0.18	+0.01	0.978	0.5–130	99	10.5
Hexyl acetate	Aldrich, ⩾ 98%	43	56, 84	2.23	−0.02	0.993	0.5–220	102	6.1
α-Terpinolene	Aldrich, ⩾90%	121	93, 136	0.33	+0.08	0.966	0.5–200	105	11.8
Octanal	Aldrich, 99%	43	57, 41	0.84	+0.02	0.986	0.5–300	97	9.9
cis-3-Hexenyhl acetate	Aldrich, ⩾98%	67	43, 82	2.16	−0.03	0.989	0.5–180	92	2.3
trans-2-Hexenyl acetate	Bedoukian Research	43	67, 82	3.18	−0.10	0.957	0.5–180	88	1.5
2-Nonanone	Aldrich, ⩾99%	58	43, 71	2.71	+0.02	0.997	0.5–180	95	3.4
cis-3-Hexenol	Bedoukian Research	67	55, 82	0.07	+0.17	0.966	2–670	91	10.1
trans, *trans*-2,4-Hexadienal	Pfaltz and Bauer Inc.	81	39, 96	0.15	+0.01	0.940	0.5–90	91	12.5
cis-Linalool oxide	Fluka, ⩾97%	59	94, 111	0.16	+0.08	0.919	1–800	116	1.5
1-Octen-3-ol	Aldrich, ⩾98%	57	43, 72	1.94	+0.16	0.969	0.5–190	90	10.4
trans-Linalool oxide	Fluka, ⩾97%	59	94, 43	0.13	+0.07	0.934	1–800	116	1.4
Menthone	Aldrich	112	69, 139	0.66	−0.05	0.986	0.5–380	102	11.7
Theaspirane A	Aldrich, ⩾85%	138	82, 96	0.75	+0.03	0.926	0.5–190	98	3.5
trans, *trans*-2,4-Heptadienal	Fluka, ⩾97%	81	39, 53	1.11	+0.06	0.970	0.5–110	100	5.8
Theaspirane B	Aldrich, ⩾85%	138	96, 109	1.79	−0.04	0.997	0.5–190	117	3.1
2-Nonanol	K&K Lab, N.Y.	45	69, 55	3.33	+0.05	0.988	0.5–190	94	2.4
*Isothymol	TCI American, 99%	135	91, 150						
2-Heptanol	Aldrich, ⩾97%	45	55, 83	0.22	+0.05	0.902	1–800	102	7.8
Hexanol	Sigma–Aldrich, ⩾99%	56	55, 43	0.05	+0.01	0.909	1–780	97	2.3
trans-2-Hexenol	Compagnie Parento. Inc.	57	41, 82	0.01	+0.008	0.924	0.5–150	97	2.5
6-Methyl-5-heptan-2-ol	Aldrich, 99%	95	41, 69	1.46	+0.14	0.976	0.5–150	100	6.3
Linalool	Aldrich, ⩾97%	93	71, 41	0.17	+0.02	0.957	1–1300	107	3.6
Octanol	Eastman Chemical	56	55, 70	0.22	+0.22	0.991	2–350	96	2.1
2-Undecanone	Aldrich, 99%	58	43, 59	0.53	+0.01	0.998	0.5–240	86	3.6
4-Terpineol	TCI Japan	71	93, 111	0.27	+0.01	0.971	0.5–460	103	2.1
α-Terpineol	K&K Lab, N.Y.	59	93, 121	0.14	+0.004	0.989	0.5–250	112	2.6
α-Ionone	Fluke, 75–90%	121	93, 136	0.68	−0.005	0.966	0.5–180	92	1.5
Geraniol	Aldrich, 98%	69	41, 93	0.32	−0.01	0.989	0.5–400	107	4.3
β-Ionone	Aldrich, ⩾97%	177	43, 178	1.11	+0.005	0.971	0.5–220	97	0.4
*γ-Heptalactone	Aldrich, ⩾ 98%	85	41, 56						
γ-Octalactone	Pfaltz and Bauer Inc.	85	100	4.28	+0.001	0.998	0.5–190	100	4.4
δ-Octalactone	Lancaster, 98%	99	71, 70	0.54	−0.0004	0.999	0.5–220	113	7.9
γ-Decalactone	Aldrich, ⩾98%	85	128	30.3	−0.13	0.977	0.5–330	107	2.8
δ-Decalactone	Aldrich, ⩾98%	99	71, 55	3.93	−0.10	0.972	0.5–250	98	4.1
γ-Undecalactone	Aldrich, ⩾98%	85	128	37.6	−0.11	0.961	0.5–180	100	3.5
δ-Dodecalactone	TCI Japan	99	71, 55	8.39	−0.29	0.937	0.5–220	93	3.1
*2-Hydroxy-5-methylacetophenone	Aldrich, 98%	135	150, 107						
Acetophenone	Lancaster, 99%	105	77, 120	0.24	+0.03	0.945	0.5–240	95	8.9
Menthol	Fluka, ⩾99%	81	71, 95	0.42	+0.02	0.990	0.5–320	101	2.7
Nonanol	Eastman Chemical	56	55, 70	0.35	+0.001	0.999	0.5–300	99	3.5
Carvone	Aldrich, ⩾97%	82	54, 108	0.55	+0.02	0.996	0.5–280	102	2.5
Methyl salicylate	Lancaster, 98%	120	92, 152	0.71	+0.02	0.989	1–660	104	1.6
Citronellol	Aldrich	69	82, 95	0.22	−0.01	0.999	0.5–230	103	1.7
Nerol	Sigma, ∼98%	69	41, 93	0.43	−0.02	0.984	0.5–190	102	5.8
trans-β-Damascenone	Firmenich	121	105, 109	1.16	+0.001	0.997	0.5–250	97	2.6
Hexanoic acid	Aldrich, ⩾99.5%	60	41, 73	0.01	+0.05	0.928	1–1000	102	3.0
Phenethyl alcohol	Sigma–Aldrich, ⩾99%	91	92, 122	0.03	+0.01	0.974	0.5–220	100	5.4
4-Phenyl-2-butanol	Lancaster, 98%	117	91, 132	0.16	+0.04	0.959	0.5–440	104	7.7
Perilla alcohol	Aldrich, 96%	79	68, 93	0.10	+0.005	0.992	0.5–460	99	6.2
Cinnamaldehyde	Aldrich, ⩾99%	131	132, 103	0.24	+0.02	0.976	0.5–60	97	7.1
Benzenepropanol	TCI American, 99%	117	91, 118	0.06	+0.008	0.974	0.5–150	109	11.1
Octanoic acid	Aldrich	60	73, 55	0.19	+0.19	0.989	1–710	105	11.8
Eugenol	Aldrich, ⩾98%	164	149, 103	0.34	+0.02	0.985	0.5–470	99	8.7
Cinnamyl alcohol	TCI American, 97%	92	134, 105	0.02	+0.002	0.981	0.5–130	99	9.3

RSD: relative standard deviation.

* Internal standard.

[a] Values for the slope in the equation R_{TC}/R_{IS} = slope(C_{TC}/C_{IS}) + intercept. R_{TC}: MS response of target compound; R_{IS}: MS response of internal standard; C_{TC}: concentration of target compound; C_{IS}: concentration of internal standard.

[b] Actual concentration range for standard calibration curve.

Table 2
Chemical standards and quantification by solid-phase extraction and microvial insert thermal desorption.

Chemicals	Source, purity	Quantify ions	Qualify ions	Slope[a]	Intercept	R^2	Range[b] (mg/L)	Recovery	RSD (%)
*2-Hydroxy-5-methylacetophenone	Aldrich, 98%	135	150, 77						
Mesifurane	Aldrich, ⩾97%	142	43, 71	0.32	−0.003	1.000	0.01–7	105	2.6
Butanoic acid	Aldrich, ⩾99%	60	73	0.45	−0.004	0.999	0.1–55	98	3.0
2-Methylbutanoic acid	Aldrich	74	57, 87	0.47	−0.01	0.998	0.1–57	99	4.0
γ-Hexalactone	Lancaster, 98%	85	57, 70	0.69	+0.01	0.999	0.03–23	93	2.5
Benzyl alcohol	Sigma–Aldrich, 99.8%	79	107, 108	0.45	+0.13	0.999	0.1–73	104	0.3
*Ethyl maltol	Aldrich, 99%	140	139, 97						
Furaneol	Fluka, ⩾99%	128	43, 57	1.25	−0.04	0.992	0.23–144	90	1.7

RSD: relative standard deviation.

* Internal standard.

[a] Values for the slope in the equation R_{TC}/R_{IS} = slope(C_{TC}/C_{IS}) + intercept. R_{TC}: MS response of target compound; R_{IS}: MS response of internal standard; C_{TC}: concentration of target compound; C_{IS}: concentration of internal standard.

[b] Actual concentration range for standard calibration curve.

2.4. Calibration curve and quantitative analysis

The individual standard solution was mixed and diluted with synthetic juice to obtain a range of concentrations (Table 1). Twenty millilitre of the internal standard A was added to the working solution and then extracted using a stir bar, as was done for the sample. Selective ion-monitoring (SIM) mass spectrometry was used to quantify the aroma active compounds (Table 1). Standard calibration curves were obtained through Chemstation software and were used to calculate the concentrations of volatile compounds in the samples. Triplicate analysis was performed for each sample.

OAVs were calculated by dividing the concentrations of aroma active compounds with their sensory thresholds in water from literature.

2.5. Solid-phase extraction-direct microvial insert thermal desorption quantification

The polar aroma compounds (Table 2) were determined using a solid-phase extraction-direct microvial insert thermal desorption technique described previously with some modification (Du & Qian, 2008). Each 10 mL of blackberry juice was passed through a preconditioned LiChrolut-EN cartridge (200 mg, 3 mL, from Merck, Darmstadt, Germany, preconditioned with 5 mL of methanol followed by 10 mL of distilled water) according to the manufacture's instruction. After the sample was loaded, the SPE cartridge was washed with 20 mL of distilled water, and then gently dried with 20 mL of air. The retained aroma compounds were eluted with 1 mL of methanol. Twenty millilitre of internal standard mixture B was added and the eluent was dried with anhydrous sodium sulphate. Twenty millilitre of the extract was loaded into a 200 μL glass insert and placed into the sample holder of the TDU for GC–MS analysis. The TDU, CIS4 and GC–MS conditions were the same as described previously in the SBSE-GC–MS section except that the TDU was heated at a rate of 100 °C/min to the final temperature, and the initial CIS4 temperature was kept at 25 °C.

The standard solution was mixed and diluted in methanol to create a serial of concentration (Table 2). Twenty millilitre of internal standard B was added to each standard solution. A 20 μL of the standard solution was injected. The calibration curves were obtained from Chemstation software and used for calculation of volatiles in sample.

2.6. Recovery and reproducibility study

A known amount of the standards was added to freshly prepared 'Marion' juice at the same concentration as the middle level for the calibration curve, Tables 1 and 2. The spiked juice samples were analysed using the SBSE and SPE methods as was described previously. Triplicate analysis was performed for each method. The recovery of individual compound was determined by comparing the measured difference (concentration of standards in spiked juice – concentration of compounds in unspiked juice) with spiked amount of standards. The reproducibility of the method was determined by calculating the relative standard deviation (RSD) of the triplicate analysis for 'Marion' juice (Tables 1 and 2).

2.7. Statistical analyses

The S-PLUS Version 7.0 software (Insightful Corp., Seattle, WA) was used to test the variances of volatile concentration from two growing seasons and cultivars. A *t*-test was conducted to test the growing season variance (triplicate data for each growing season) and cultivar variance (mean for each growing season for each cultivar).

3. Results and discussion

3.1. SBSE GC–MS quantification

Quantifying aroma compounds in a complex matrix has always been a challenging task. Distillation, liquid–liquid extraction, dynamic headspace sampling, and solid-phase microextraction (SPME) have been used to isolate volatile compounds from blackberry samples (Georgilopoulos & Gallois, 1987, 1988; Ibáñez, López-Sebastián, Ramos, Tabera, & Reglero, 1998; Klesk & Qian, 2003b), however, these methods are often tedious or lack of reproducibility and sensitivity for quantitative analysis.

SBSE uses a heavy coating of PDMS polymer to extract volatiles directly from sample solution. It has no affinity for sugar, organic acids and pigments, and high sensitivity for volatile compounds, particularly for semi-volatile compounds, which make it an effective and time-saving method for extracting trace volatile compounds from complex matrix (David, Tienpont, & Sandra, 2003). SBSE extraction coupled with gas chromatography–mass spectrometry (SBSE-GC–MS) has been used to quantify volatile compounds in fruits such as strawberries (Kreck, Scharrer, Bilke, & Mosandl, 2001), raspberries (Malowicki, Martin, & Qian, 2008a; Malowicki, Martin, & Qian, 2008b), snake fruit (Wijaya, Ulrich, Lestari, Schippel, & Ebert, 2005), and grapes (Caven-Quantrill & Buglass, 2006; Luan, Mosandl, Gubesch, & Wuest, 2006).

In this study, approximate 70 volatile compounds were quantified with the SBSE method. The calibration curve obtained in the synthetic juice had squared regression coefficients were higher than 0.9 (Table 1). For most of compounds, the method detection limit ranged from 0.2 to 1 μg/kg. The recovery of the standard from

Table 3
Volatile constituents in 'Marion' and 'Black Diamond' blackberries (μg/kg, average ± SD).

RI	Compounds	Odour threshold[a]	Marion		Mean	OAV	Black Diamond		Mean	OAV
			2004	2006			2003	2004		
	Esters		*106*	*145*	*125*		*147*	*167*	*157*	
1017	Methyl butanoate	43	ND	ND	ND	NA	6.3 ± 0.8	23 ± 1	15	0.3
1043	Ethyl butanoate	18	1.3 ± 0.02	1.3 ± 0.04	1.3	0.07	34 ± 2	39 ± 2	36	2.0
1085	Butyl acetate	10	1.3 ± 0.05	ND	0.7	0.07	0.4 ± 0.04	18 ± 1	9.2	0.9
1187	Isoamyl acetate	30	1.3 ± 0.04	1.3 ± 0.1	1.3	0.04	3.8 ± 0.7	1.2 ± 0.1	2.5	0.08
1197	Methyl hexanoate	50	20 ± 1	6.3 ± 0.8	13	0.3	15 ± 1	20 ± 1	17	0.3
1244	Ethyl hexanoate	5	13.9 ± 0.5	22 ± 1	18	3.6	66 ± 2	50 ± 2	58	11.7
1274	Hexyl acetate	10	34 ± 1	26 ± 1	30	3.0	5.1 ± 0.1	6.3 ± 0.4	5.7	0.6
1314	*cis*-3-Hexenyl acetate	210	10.1 ± 0.7	8.9 ± 0.9	9.5	0.05	1.3 ± 0.04	1.3 ± 0.07	1.3	0.006
1330	*trans*-2-Hexenyl acetate	320	8.9 ± 0.05	21 ± 1	15	0.05	10.1 ± 0.2	2.5 ± 0.1	6.3	0.02
1755	Methyl salicylate	40	15 ± 1	58 ± 7	36	0.9	5.1 ± 0.1	6.3 ± 0.3	5.7	0.1
	Aldehydes		*94*	*703*	*397*		*74*	*63*	*68*	
1092	Hexanal	20	68 ± 1	680 ± 80	373	18.7	65 ± 6	51 ± 2	58	2.9
1222	*trans*-2-Hexenal	10	13 ± 1	16 ± 1	14	1.4	ND	ND	ND	NA
1287	Octanal	30	ND	ND	ND	NA	3.8 ± 0.4	ND	1.9	0.06
1385	*trans*, *trans*-2,4-Hexadienal	60	2.5 ± 0.1	2.5 ± 0.1	2.5	0.04	ND	ND	ND	NA
1477	*trans*, *trans*-2,4-Heptadienal	49	3.8 ± 0.5	2.5 ± 0.4	3.2	0.06	ND	4 ± 0.2	2.0	0.04
2042	Cinnamaldehyde	160	6.3 ± 0.1	2.5 ± 0.1	4.4	0.03	5.1 ± 0.5	7.6 ± 0.6	6.3	0.04
	Ketones		*103*	*199*	*151*		*38*	*41*	*39*	
1192	2-Heptanone	140	33 ± 1	167 ± 9	100	0.7	14 ± 1	19 ± 1	16	0.1
1374	2-Nonanone	41	14 ± 1	20 ± 2	17	0.4	ND	ND	ND	NA
1570	2-Undecanone	41	14 ± 2	8.8 ± 0.6	11.4	0.3	ND	ND	ND	NA
1617	Acetophenone	65	39 ± 5	ND	20	0.3	24 ± 1	22 ± 1	23	0.3
	Terpenoids		*1532*	*1143*	*1338*		*6850*	*3463*	*5156*	
1169	Myrcene	15	25 ± 4	110 ± 10	68	4.5	180 ± 10	58 ± 7	119	7.9
1176	α-Terpinene	85	5.1 ± 0.5	25 ± 2	15	0.2	24 ± 1	10 ± 2	17	0.2
1202	Limonene	200	6.3 ± 0.7	6.3 ± 0.6	6.3	0.03	62 ± 4	22 ± 2	42	0.2
1237	*cis*-β-Ocimene	34	1.3 ± 0.2	1.2 ± 0.1	1.3	0.04	220 ± 20	16.5 ± 0.1	118	3.5
1276	a-Terpinolene	200	3.8 ± 0.7	5 ± 1	4.4	0.02	11 ± 2	6.3 ± 0.2	8.8	0.04
1425	*cis*-Linalool oxide	100	ND	ND	ND	NA	150 ± 10	250 ± 20	200	2.0
1432	p-Menthone	170	16 ± 1	3.8 ± 0.1	9.9	0.06	3.8 ± 0.5	ND	1.9	0.01
1451	*trans*-Linalool oxide	190	30 ± 5	11 ± 1	20	0.1	44 ± 5	23 ± 1	33	0.2
1532	Linalool	6	1260 ± 20	870 ± 20	1064	177	5060 ± 90	2680 ± 90	3870	645
1577	4-Terpineol	340	14 ± 1	6 ± 1	10	0.03	6.3 ± 0.03	ND	3.2	0.01
1623	Menthol	920	3.8 ± 0.6	2.5 ± 0.3	3.2	0.003	2.5 ± 0.05	ND	1.3	0.001
1684	α-Terpineol	330	94 ± 1	56 ± 2	75	0.2	730 ± 10	255 ± 9	493	1.5
1706	Carvone	6.7	3.8 ± 0.5	3.8 ± 0.7	3.8	0.6	1.3 ± 0.03	2.5 ± 0.2	1.9	0.3
1769	Citronellol	30	2.5 ± 0.3	2.5 ± 0.5	2.5	0.08	44.3 ± 0.1	13.9 ± 0.4	29	1.0
1810	Nerol	290	3.8 ± 0.3	3.8 ± 0.3	3.8	0.01	52 ± 1	19 ± 1	35	0.1
1863	Geraniol	40	30 ± 1	20 ± 1	25	0.6	191 ± 8	68 ± 2	130	3.2
	Norisoprenoids		*30*	*46*	*38*		*84*	*72*	*78*	
1464	Theaspirane A	NA	16 ± 1	23 ± 2	19	NA	39 ± 6	27 ± 2	32	NA
1500	Theaspirane B	NA	11 ± 1	16 ± 2	14	NA	26 ± 2	19 ± 1	23	NA
1810	β-Damascenone	10	ND	ND	ND	NA	2.5 ± 0.05	3.8 ± 0.6	3.1	0.3
1844	α-Ionone	0.6	1.2 ± 0.1	1.3 ± 0.1	1.3	2.0	ND	2.5 ± 0.2	1.3	2.2
1936	β-Ionone	0.1	0.6 ± 0.1	5.1 ± 0.6	2.8	28	12.7 ± 0.4	10 ± 1	11.3	113
	Alcohols		*3756*	*3760*	*3756*		*3050*	*2185*	*2618*	
1326	2-Heptanol	70	354 ± 5	597 ± 9	476	6.8	1250 ± 30	890 ± 40	1069	15
1355	Hexanol	2500	644 ± 6	457 ± 9	550	0.2	265 ± 6	278 ± 5	272	0.1
1379	*cis*-3-Hexenol	100	81 ± 5	165 ± 9	123	1.2	49 ± 1	35 ± 2	42	0.4
1400	*trans*-2-Hexenol	100	320 ± 30	450 ± 40	385	3.8	550 ± 20	295 ± 9	424	4.2
1446	1-Octen-3-ol	1	1.2 ± 0.03	1.3 ± 0.2	1.2	1.2	ND	ND	ND	NA
1461	6-Methyl-5-hepten-2-ol	2000	16 ± 2	20.3 ± 0.4	18	0.009	8.9 ± 0.2	11 ± 1	10	0.005
1509	2-Nonanol	58	40 ± 1	19.0 ± 0.4	30	0.5	20.2 ± 0.6	25.3 ± 0.4	22.7	0.4
1543	Octanol	130	18 ± 1	11.9 ± 0.1	15	0.1	110 ± 10	105 ± 4	109	0.8
1830	Nonanol	1000	3.8 ± 0.1	1.2 ± 0.2	2.5	0.003	15.2 ± 0.3	11.4 ± 0.6	13.3	0.01
1885	Benzyl alcohol	100	1970 ± 50	1850 ± 40	1909	19	490 ± 60	320 ± 20	408	4.1
1920	Phenethyl alcohol	1000	65 ± 6	49 ± 5	57	0.06	150 ± 20	80 ± 10	115	0.1
2010	4-Phenyl-2-butanol	NA	61 ± 8	34 ± 5	48	NA	24 ± 1	19 ± 1	43	NA
2022	Perillyl alcohol	1660	9 ± 1	5.1 ± 0.9	7	0.004	13.9 ± 0.5	6.3 ± 0.9	10	0.006
2064	3-Phenylpropanol	NA	28 ± 3	ND	14	NA	ND	1.3 ± 0.1	1.3	NA
2303	Cinnamyl alcohol	1000	145 ± 2	100 ± 2	123	0.1	104 ± 1	108 ± 4	106	0.1
	Lactones		*152*	*75*	*113*		*212*	*37*	*126*	
1674	γ-Hexalactone	260	80 ± 2	16 ± 1	48	0.2	110 ± 2	ND	56	0.2
1912	γ-Octalactone	7	3.8 ± 0.1	1.3 ± 0.02	2.5	0.4	6.3 ± 0.2	8.9 ± 0.1	7.6	1.1
1967	δ-Octalactone	400	43 ± 6	39 ± 1	41	0.1	73 ± 7	ND	37	0.09
2031	γ-Decalactone	10	2.5 ± 0.05	2.5 ± 0.06	2.5	0.3	7.6 ± 0.1	11.4 ± 0.1	9.5	1.0
2079	δ-Decalactone	100	16.5 ± 0.2	10 ± 1	13	0.1	11.4 ± 0.1	12.7 ± 0.4	12	0.1
2142	γ-Undecalactone	60	1.3 ± 0.03	1.2 ± 0.01	1.2	0.02	1.2 ± 0.01	1.3 ± 0.06	1.2	0.02
2294	δ-Dodecalactone	160	5.1 ± 0.1	5.1 ± 0.1	5.1	0.03	2.5 ± 0.1	2.5 ± 0.04	2.5	0.02

(*continued on next page*)

Table 3 (*continued*)

RI	Compounds	Odour threshold[a]	Marion		Mean	OAV	Black Diamond		Mean	OAV
			2004	2006			2003	2004		
	Furanones		*1695*	*2033*	*1864*		*420*	*310*	*364*	
1567	Mesifurane	16	35 ± 1	13 ± 1	24	1.5	ND	ND	ND	NA
2060	Furaneol	5	1660 ± 80	2020 ± 60	1840	368	420 ± 30	310 ± 50	364	73
	Acids		*5417*	*4790*	*5103*		*11,318*	*5566*	*8439*	
1607	Butanoic acid	1000	787 ± 8	160 ± 20	473	0.5	3760 ± 90	3120 ± 90	3440	3.4
1655	2-Methylbutanoic acid	250	1160 ± 90	830 ± 20	998	4.0	114 ± 2	180 ± 20	146	0.6
1856	Hexanoic acid	1000	3370 ± 90	3580 ± 90	3473	3.5	7250 ± 90	2070 ± 14	4658	4.7
2080	Octanoic acid	910	100 ± 10	220 ± 20	159	0.2	194 ± 8	196 ± 2	195	0.2

OAV: odour-activity values. ND: not detectable. NA: not apply.

[a] Thresholds in water from reference (van Gemert, 2003) unless noted otherwise.

the spiked blackberry juice ranged from 85% to 115%, indicating that the matrix effect was not significant at the level of precision of the method. The reproducibility of the method was compound dependent, and more than half of the compounds had relative standard deviation (RSD) below 5%. Some compounds such as methyl butanoate and *trans*-2-hexenal had high RSD, which might be caused by a low concentration, or a lower affinity to stir bar. However, SBSE failed to quantify some very polar compounds such as the short chain acids, benzyl alcohol, and furaneol. Overall, SBSE GC–MS effectively quantified the majority of the compounds found in these blackberries.

3.2. Solid-phase extraction-direct microvial insert thermal desorption quantification

SPE was used to extract furaneol and other polar compounds in berry juice. Although C18 and XAD-2 sorbents have been widely used for volatile extraction (Edwards & Beelman, 1990; Lukic, Banovic, Persuric, Radeka, & Sladonja, 2006), LiChrolut-EN has been found to give higher recoveries for polar analytes than C18 and XAD-2 sorbents (Jesus Ibarz, Ferreira, Hernandez-Orte, Loscos, & Cacho, 2006). LiChrolut-EN sorbent has been used for the analysis of a wide range of volatile compounds in wine (Campo, Cacho, & Ferreira, 2007; Ferreira, Jarauta, Lopez, & Cacho, 2003; Ferreira, Jarauta, Ortega, & Cacho, 2004; Ferreira, Ortin, & Cacho, 2007; Lopez, Aznar, Cacho, & Ferreira, 2002; Pineiro, Palma, & Barroso, 2004). Our previous study (Du & Qian, 2008) has also verified that LiChrolut-EN can effectively extract furaneol from blackberry juice, and had much less retention for pigments and other non-volatiles than HLB and C18 columns. The furaneol can be completely eluted out from the SPE column with methanol, which can be directly analysed on GC–MS using an automated large volume microvial insert thermal desorption technique without further purification and concentration.

In this study, SPE-microvial insert thermal desorption technique was used to compliment the SBSE technique to analyse furaneol, mesifurane, short chain acids, γ-hexalactone, and benzyl alcohol in 'Black Diamond' and 'Marion' (Table 2). For all of the compounds, the squared regression coefficients were higher than 0.9. The recovery of the standard from the spiked blackberry juice ranged from 90% to 105%, which indicated that the matrix effect was not significant for these compounds. In all cases, the RSD was below 5%.

3.3. Comparison of volatile constituent in 'Black Diamond' and 'Marion'

Since seasonal variation may exist, samples from two growing seasons were analysed (Table 3). Approximate 70 volatile compounds in 'Marion' and 'Black Diamond' were quantified in this study. Two thirds of the compounds analysed had significant ($p \leqslant 0.05$) seasonal variation.

Overall, the volatile profiles in both cultivars were very similar. However, the concentrations of some volatile compounds varied greatly. 'Black Diamond' had higher acid contents than 'Marion', especially for butanoic acid and hexanoic acid, while 'Marion' had more 2-methylbutanoic acid. Both cultivars had very high level of furaneol, and 'Marion' had five times more furaneol than 'Black Diamond' on average. 'Marion' also contained mesifurane, while mesifurane was not detected in 'Black Diamond'. Terpenoids were also abundant for both 'Marion' and 'Black Diamond'. The most abundant terpenoids in both cultivars were linalool and α-terpineol. 'Black Diamond' had 3–4 times more linalool than 'Marion'; it also had very high concentration of *cis*-linalool oxide, whereas *cis*-linalool was not detected in 'Marion'. Surprisingly, both cultivars had comparable level of *trans*-linalool oxide. 'Black Diamond' also had higher content of α-terpineol and geraniol, compared to 'Marion'. Alcohols were one of the most predominant volatile classes in both cultivars. The most abundant alcohols included 2-heptanol, hexanol, *trans*-2-hexenol, and benzyl alcohol. Both cultivars had similar alcohol content. Only a very small amount of norisoprenoids were found in 'Marion' and 'Black Diamond', and 'Black Diamond' had higher level of β-ionone. Both cultivars had low levels of aldehydes and ketones, although the levels in 'Marion' were much higher than in 'Black Diamond'. Overall, the levels of esters and lactones were very low.

3.4. Odour-activity value of 'Black Diamond' and 'Marion' and correlation with sensory attributes

An informal descriptive sensory evaluation of blackberry puree was conducted to compare the aroma attributes of 'Marion' and 'Black Diamond'. One of major differences between 'Marion' and 'Black Diamond' was that 'Marion' had higher honey, caramel, strawberry, and cooked jam aroma. This result is consistent with the sensory attributes of 'Marion' and 'Black Diamond' reported earlier (Kurnianta, 2005) (Fig. 1).

Odour-activity value was further calculated to relate volatile composition to aroma attributes of 'Marion' and 'Black Diamond'. Typically, odorants with high OAVs are more likely to be important, although aroma synergy and suppression exist. In 'Marion', the compounds with high odour-activity values (OAV > 10) were furaneol, linalool, β-ionone, hexanal, and benzyl alcohol. In 'Black Diamond', the compounds with OAVs > 10 were linalool, β-ionone, furaneol, 2-heptanol and ethyl hexanoate (Table 3).

Furaneol has an aroma of fruity, berry and strawberry. Furaneol had an OAV of 368 in 'Marion', which was 5 times higher than in 'Black Diamond'. This result was in agreement with sensory evaluation that 'Marion' had higher overall fruity, berry, strawberry aroma than 'Black Diamond'. Linalool and β-ionone could be very

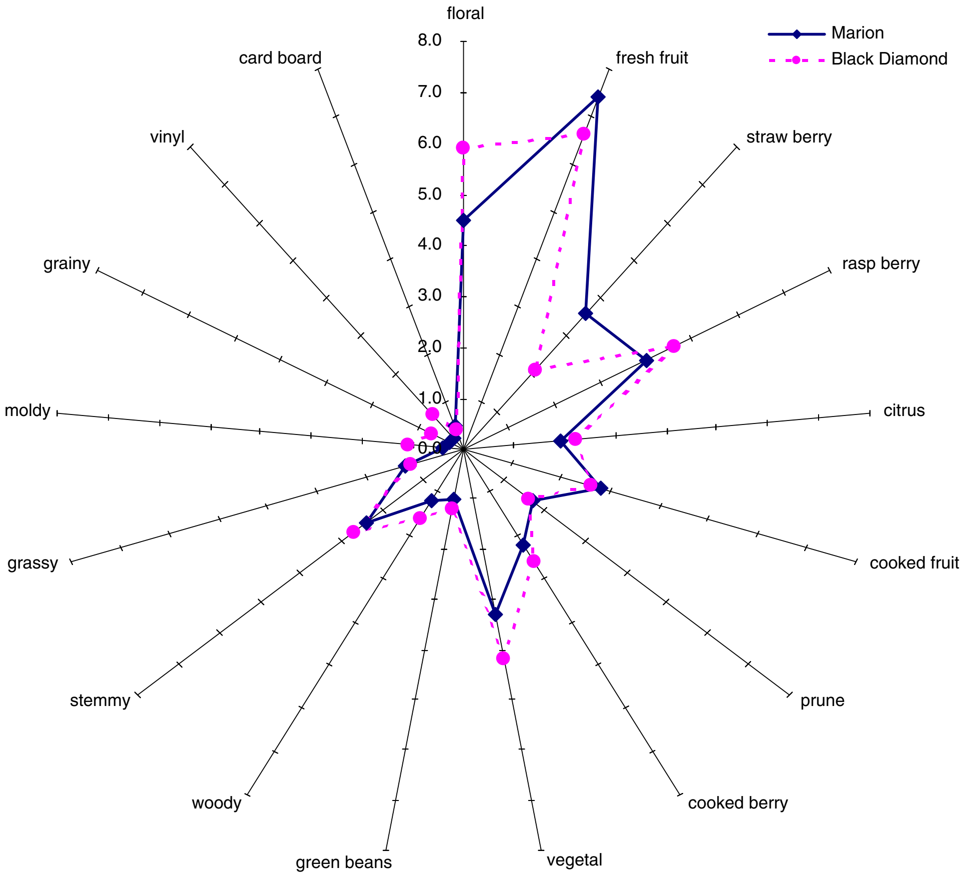

Fig. 1. Descriptive sensory evaluation of 'Marion' and 'Black Diamond'.

important odorants for both cultivars. The OAV of linalool in 'Black Diamond' was 4 times higher than 'Marion'. Linalool has a floral odour. Sensory evaluation demonstrated that 'Black Diamond' was more floral than 'Marion'.

Meanwhile, 'Marion' had higher OAVs of hexanal, which could contribute to a higher fresh fruit aroma in 'Marion'. In addition, 'Marion' also had a 5 times higher OAV of benzyl alcohol than 'Black Diamond', which could also contribute to the higher fresh fruit aroma in 'Marion'. These results could explain the higher fresh fruity aroma observed by descriptive sensory analysis. However, 'fresh fruit' is a term more difficult to describe and correlate with chemical compounds.

Since furaneol was one of the major volatile differences between 'Marion' and 'Black Diamond', furaneol was added to 'Black Diamond' juice to assess whether it would be perceived to be more similar to 'Marion'. Informal descriptive sensory evaluation demonstrated that 'Black Diamond' juice fortified with furaneol enhanced fruity, strawberry, cooked jam notes and suppressed the vegetal and earthy notes in 'Black Diamond'.

4. Conclusions

Quantification results indicated that the volatile profiles in 'Marion' and 'Black Diamond' very similar, although the concentrations of some aroma compounds varied greatly. OAVs indicated that in 'Marion', the compounds with high odour-activity values (OAV > 10) were furaneol, linalool, β-ionone, and hexanal; In 'Black Diamond', the compounds with OAV > 10 were linalool, β-ionone, furaneol, and 2-heptanol. The major difference between the cultivars for aroma compounds was that 'Marion' had a 5 times higher OAV of furaneol than 'Black Diamond', while 'Black Diamond' had much higher OAV of linalool than 'Marion'. These results matched with the descriptive sensory evaluation that 'Marion' had more berry, fruity, strawberry aroma while 'Black Diamond' had more floral aroma.

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