Volatile Composition in Raspberry Cultivars Grown in the Pacific Northwest Determined by Stir Bar Sorptive Extraction−Gas Chromatography−Mass Spectrometry

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Twenty-nine volatile compounds in 'Chilliwack', 'Tulameen', 'Willamette', 'Yellow Meeker', and 'Meeker' raspberries were quantified using stir bar sorptive extraction (SBSE) paired with gas chromatography−mass spectrometry (GC–MS). Good correlation coefficients were obtained with most aroma-active compounds in raspberry, with quantification limits of 1 μg/kg. However, poor recoveries were observed for raspberry ketone and zingerone. Quantitative data showed that volatile concentrations varied for different cultivars. Large variations for α-ionone, β-ionone, geraniol, linalool, and (*Z*)-3-hexenol were observed in different raspberry cultivars. In addition, the volatile compositions in 'Meeker' raspberry grown at different locations also varied. The chiral isomeric ratios of raspberry ketone, α-ionone, α-pinene, linalool, terpinen-4-ol, δ-octalactone, δ-decalactone, and 6-methyl-5-hepten-2-ol were studied using a CyclosilB column. α-Ionone, α-pinene, δ-octalactone, and δ-decalactone had strong chiral isomeric preference, with more than 96% for one isomeric form. Much weaker chiral isomeric preference was observed for terpinen-4-ol, while linalool was almost a racemic mixture. Both growing locations and cultivars affect the isomeric ratio of linalool with a range of 37–51% for (*R*)-linalool.

KEYWORDS: Raspberry; volatile; aroma; SBSE; volatile quantification

INTRODUCTION

Raspberries including red raspberry and black raspberry are the most widely grown small fruits (*1, 2*). The Pacific Northwest, Oregon and Washington in the U.S., and southwestern British Columbia in Canada, are the primary regions in North America for raspberry production. Ninety-seven percent of the raspberries grown in Oregon and Washington are the red variety, and the majority of them are processed into a variety of food products. Many different varieties of red raspberry are grown in this region, with the most popular cultivars being 'Meeker', 'Willamette', and 'Tulameen'.

Aroma compounds in raspberry have been studied extensively. Many compounds including raspberry ketone, α-ionone, β-ionone, linalool, (*Z*)-3-hexenol, geraniol, nerol, α-terpineol, Furaneol, hexanal, β-ocimene, 1-octanol, β-pinene, β-damascenone, ethyl 2-methylpropanoate, (*E*)-2-hexenal, heptanal, and benzaldehyde have been identified to contribute raspberry aroma (*3–7*). Among them, α-ionone, β-ionone, geraniol, nerol,

linalool, and raspberry ketone could be particularly important to red raspberry aroma (*7–10*).

Fruit flavor is influenced by numerous factors including cultivar variation, climate, soil, ripeness, and many other variables (*1, 11–14*). Cultivar variations are common, although the significance of these variations depends on the cultivars and flavor compounds under discussion (*10, 12, 15*). Significant variations in raspberry ketone and β-ionone have been observed in 'Chilliwack', 'Meeker', 'Tulameen', and 'Willamette' red raspberries (*12*). Overall volatile concentration differences have also been reported with other cultivars (*11*). Volatile aroma compounds are secondary metabolites generated from carbohydrates, amino acids, and fatty acids (*16*). Variations in metabolic pathways could result in the variation of final concentrations of volatile aroma compounds in the fruit (*14*).

Flavor compositions in fruits can be dramatically influenced by the degree of ripening (*11*). Aroma volatiles generally change dramatically at the final stages of ripening (*15*). Some aroma volatiles increase with ripening and continue to increase into the overripe stage, whereas some compounds maintain at the constant levels into the overripe stage, or decrease after ripening (*15*) due to formation of bound glycosides or conversion to other compounds (*17*). Although these changes vary with cultivar, the general trend remains the same for the same plant (*15*).

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Table 1. Calibration Curve of Aroma-Active Compounds by SBSE–GC–MS[a]

| compound | quantifier ion | qualifier ion(s) | quantification range (μg/kg) | equation: conc = {(Rc/Rs)}/{M}*Cs | | correlation coefficient |
				M	Cs	
(Z)-3-hexenol[b]	67	41	1–750	0.0007368	0.1969	0.9869
4-isopropylbenzyl alcohol	135	150, 105	1–500	0.0001753	0.3922	0.9999
6-methyl-5-hepten-2-ol[b]	95	69, 41	1–500	0.008595	0.1969	0.9998
2-nonanol	45	69, 55	1–500	0.001183	0.3922	0.9925
hexanal[b]	56	44	1–750	0.004221	0.1969	0.9788
(E)-2-hexenal[b]	41	55	1–750	0.001553	0.1969	0.9814
(Z)-3-hexenyl-acetate[b]	67	43, 82	1–500	0.05638	0.1969	0.9937
ethyl hexanoate	88	99	1–1000	0.0009557	0.3922	0.9722
methyl nonanoate	74	87	1–750	0.001658	0.3922	0.9202
2-heptanone[b]	43	58	1–750	0.01405	0.1969	0.9968
2-nonanone[b]	58	43	1–750	0.04101	0.1969	0.9790
δ-octalactone	99	71	1–1000	0.00006914	0.3922	0.9839
δ-decalactone	99	71	1–1000	0.0005589	0.3922	0.9973
para-cymene	119	134	1–500	0.0005589	0.3922	0.9973
geraniol[b]	69	41	1–750	0.002575	0.1969	0.9925
α-ionone	121	93	1–500	0.002917	0.3922	0.9967
β-ionone	177	43	1–500	0.003951	0.3922	0.9994
limonene[b]	68	93	1–500	0.04214	0.1969	0.9998
linalool[b]	71	93, 80	1–500	0.01521	0.1969	0.9938
myrcene[b]	41	93	1–750	0.02903	0.1969	0.9929
nerol[b]	69	41	1–750	0.01468	0.1969	0.9923
α-phellandrene[b]	93	77	1–750	0.02431	0.1969	0.9971
α-pinene[b]	93	77	1–500	0.04191	0.1969	0.9961
sabinene[b]	93	77, 79	1–500	0.06246	0.1969	0.9926
α-terpinene[b]	121	93	1–750	0.02974	0.1969	0.9963
γ-terpinene[b]	93	77	1–750	0.03418	0.1969	0.9913
α-terpineol[b]	59	93	1–750	0.006199	0.1969	0.9970
terpinen-4-ol[b]	71	93	1–750	0.01035	0.1969	0.9899
terpinolene[b]	121	93	1–750	0.04046	0.1969	0.9988

[a] Rc, compound response; Rs, internal standard response; M, line slope; Cs, internal standard concentration. [b] Use cis-jasmone as internal standard (Cs = 0.1969); rest of the compounds use γ-nonalactone as internal standard (Cs = 0.3922).

Table 2. Sugar and Organic Acid Compositions for Raspberries Grown in Washington during 2005[a]

	'Meeker' site 1	'Meeker' site 2	'Meeker' site 3	'Meeker' site 4	**'Meeker' average**	**'Meeker' 95% range**	'Chilliwack'	'Tulameen'	'Willamette'	'Yellow Meeker'
fructose (g/100 g)	1.43	0.58	1.18	1.54	**1.18**	**0.39–1.97**	1.13	1.25	0.89	2.02
glucose (g/100 g)	1.23	0.44	0.96	1.35	**0.99**	**0.25–1.74**	0.75	1.01	0.67	1.75
sucrose (g/100 g)	0.17	ND	0.24	0.39	**0.20**	**0–0.49**	1.95	0.84	0.75	0.83
total sugar (g/100 g)	2.83	1.01	2.38	3.28	**2.38**	**0.59–4.17**	3.84	3.09	2.31	4.60
citric acid (g/100 g)	1.45	1.52	1.72	1.33	**1.51**	**1.07–1.94**	2.63	1.96	2.53	2.13
malic acid (g/100 g)	0.03	ND	0.07	0.08	**0.04**	**0–0.11**	0.02	0.02	ND	0.07
total acid (g/100 g)	1.48	1.52	1.79	1.40	**1.55**	**1.09–2.00**	2.65	1.98	2.53	2.20
sugar/acid ratio	1.91	0.67	1.33	2.34	**1.54**		1.45	1.57	0.91	2.09
°Brix	11.0	8.6	9.2	9.8	**9.6**	**7.6–11.6**	9.6	9.5	8.7	10.8
titratable acidity (citric acid %)	1.28	1.22	1.47	1.14	**1.28**	**1.00–1.56**	2.23	1.67	2.10	1.72
°Brix/TA ratio	8.54	7.00	6.22	8.56	**7.51**		4.30	5.64	4.15	6.27

[a] ND: not detected with detection limit of 0.01% for sugars and 0.01% for acids.

Many aroma compounds in nature have chiral centers and can exist as enantiomeric forms. Different enantiomeric compounds could have quite different sensory thresholds (*18*). In some cases, different enantiomeric compounds may even have different aroma descriptors (*18–22*). Cultivar variation and agnominal factors could influence the enantiomeric ratio of aroma compounds, although one isomer is generally predominant, because some of the enzymes involved in the aroma formation are generally stereospecific (*22–24*).

Flavor extraction and quantification is historically a long and laborious process. Liquid–liquid extraction has been widely used for aroma compound identification and quantification, and the recovery is highly dependent upon the specific compounds and the solvent used for extraction. Headspace analysis, such as static and dynamic headspace, is only useful for compounds with high volatility (*7, 11, 15*). Solid-phase microextraction

(SPME) allows for simple sample preparation paired with minimal extraction time to establish a volatile spectrum; however, saturation and competitive adsorption need to be carefully addressed for quantification. Stir bar sorptive extraction (SBSE) is a new technique for volatile extraction; it has a higher sensitivity than SPME for nonpolar and some medium polar compounds and minimum competition and saturation effects due to the increased volume of absorbent phase (*25, 26*). The volatiles can be thermally desorbed onto the GC column. The high sensitivity and flexibility of SBSE for nonpolar and medium polar compounds makes it an effective and time-saving method for extracting trace volatile compounds from complex matrices (*26*). SBSE extraction coupled with gas chromatography–mass spectrometry (GC–MS) has been proved to be a valuable technique to quantify volatile aroma compounds in foods and beverages (*27*). The objective of this study is using the SB-

Table 3. Volatile Concentrations (μg/kg) in 'Meeker' Red Raspberry Grown at Different Sites in Washington during 2005

compound	Meeker site 1	Meeker site 2	Meeker site 3	Meeker site 4	range (95% confidence)
(Z)-3-hexenol	312 ± 7	260 ± 17	260 ± 15	279 ± 3	228–327
4-isopropylbenzyl alcohol	45 ± 2	59 ± 0	40 ± 2	65 ± 4	32–74
6-methyl-5-hepten-2-ol	70 ± 1	60 ± 2	62 ± 3	71 ± 0	55–76
2-nonanol	7 ± 1	9 ± 0	4 ± 0	9 ± 0	2–12
hexanal	56 ± 9	37 ± 4	40 ± 3	54 ± 4	27–66
(E)-2-hexenal	320 ± 17	280 ± 18	330 ± 19	311 ± 3	260–357
(Z)-3-hexenyl acetate	5 ± 0	7 ± 0	7 ± 0	9 ± 0	4–10
ethyl hexanoate	6 ± 1	12 ± 1	10 ± 1	10 ± 1	5–13
methyl nonanoate	ND	ND	1 ± 0	1 ± 0	0–1
2-heptanone	71 ± 9	94 ± 9	81 ± 3	80 ± 1	61–102
2-nonanone	20 ± 1	25 ± 1	15 ± 0	29 ± 1	11–34
δ-octalactone	810 ± 25	799 ± 9	700 ± 37	920 ± 25	636–978
δ-decalactone	783 ± 9	799 ± 2	710 ± 20	870 ± 11	666–917
para-cymene	12 ± 1	16 ± 1	12 ± 0	21 ± 1	8–23
geraniol	149 ± 8	126 ± 5	118 ± 5	156 ± 6	102–172
α-ionone	32 ± 2	30 ± 1	41 ± 1	47 ± 2	23–52
β-ionone	67 ± 4	71 ± 1	71 ± 2	89 ± 2	56–93
limonene	1 ± 0	1 ± 0	1 ± 0	2 ± 0	1–2
linalool	39 ± 1	37 ± 1	34 ± 1	42 ± 1	31–44
myrcene	5 ± 3	3 ± 0	4 ± 1	5 ± 1	1–8
nerol	29 ± 1	28 ± 1	21 ± 1	33 ± 2	19–37
α-phellandrene	60 ± 18	69 ± 3	52 ± 3	87 ± 4	26–100
α-pinene	27 ± 5	17 ± 1	14 ± 0	21 ± 1	11–27
sabinene	20 ± 2	25 ± 1	17 ± 1	29 ± 1	13–32
α-terpinene	14 ± 5	23 ± 1	18 ± 1	33 ± 1	4–39
γ-terpinene	4 ± 3	9 ± 1	7 ± 1	16 ± 1	0–18
α-terpineol	43 ± 1	48 ± 2	39 ± 2	54 ± 1	35–58
terpinen-4-ol	154 ± 4	160 ± 8	111 ± 5	175 ± 4	100–201
terpinolene	2 ± 0	2 ± 0	2 ± 0	4 ± 0	1–4

Table 4. Volatile Concentration (μg/kg) in 'Chilliwack', 'Tulameen', 'Willamette', and 'Yellow Meeker' Cultivars Grown in Washington in 2005

compound	'Chilliwack'	'Tulameen'	'Willamette'	'Yellow Meeker'
(Z)-3-hexenol	170 ± 11	86 ± 12	160 ± 27	180 ± 10
4-isopropylbenzyl alcohol	3 ± 0	2 ± 0	2 ± 2	36 ± 5
6-methyl-5-hepten-2-ol	17 ± 1	29 ± 3	10 ± 0	55 ± 5
2-nonanol	ND	ND	2 ± 0	5 ± 1
hexanal	178 ± 8	70 ± 5	110 ± 30	150 ± 35
(E)-2-hexenal	400 ± 27	420 ± 36	250 ± 150	460 ± 200
(Z)-3-hexenyl acetate	10 ± 0	1 ± 0	15 ± 5	7 ± 1
ethyl hexanoate	2 ± 0	8 ± 0	3 ± 2	3 ± 2
methyl nonanoate	1 ± 0	1 ± 0	ND	ND
2-heptanone	61 ± 4	65 ± 4	90 ± 50	57 ± 24
2-nonanone	11 ± 0	2 ± 0	13 ± 1	20 ± 2
δ-octalactone	106 ± 4	600 ± 16	200 ± 40	390 ± 45
δ-decalactone	168 ± 3	510 ± 24	260 ± 24	470 ± 35
para-cymene	4 ± 0	3 ± 0	1 ± 1	12 ± 2
geraniol	38 ± 1	155 ± 3	48 ± 9	130 ± 7
α-ionone	81 ± 3	20 ± 1	54 ± 4	63 ± 6
β-ionone	115 ± 6	62 ± 2	73 ± 1	70 ± 3
limonene	1 ± 0.0	2 ± 0	1 ± 0	1 ± 1
linalool	14 ± 1	140 ± 13	20 ± 3	16 ± 1
myrcene	4 ± 3	6 ± 1	6 ± 4	10 ± 4
nerol	5 ± 0	23 ± 2	4 ± 1	19 ± 1
α-phellandrene	6 ± 3	20 ± 4	20 ± 4	50 ± 16
α-pinene	6 ± 0	4 ± 0	22 ± 5	30 ± 4
sabinene	4 ± 0	8 ± 1	4 ± 4	12 ± 10
α-terpinene	3 ± 0	10 ± 1	5 ± 2	15 ± 6
γ-terpinene	3 ± 0	2 ± 0	4 ± 2	13 ± 8
α-terpineol	8 ± 0	120 ± 12	17 ± 2	20 ± 2
terpinen-4-ol	23 ± 1	74 ± 7	46 ± 6	120 ± 52
terpinolene	1 ± 0	3 ± 0	1 ± 1	3 ± 0

SE–GC–MS technique to compare aroma-active compounds in raspberry cultivars grown in the Pacific Northwest.

MATERIALS AND METHODS

Chemicals. Hexanal, (E)-2-hexenal, (Z)-3,7-dimethyl-2,6-octadien-1-ol (nerol), 5-isopropyl-2-methylcyclohexa-1,3-diene (α-phellandrene), 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one (α-ionone), 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (β-ionone), 4-methyl-iso-propyl-benzene (*para-c*ymene), ethyl hexanoate, 5-decanolide (δ-decalactone), γ-nonalactone, and 2,6-dimethyl-2,7-octadien-6-ol (linalool) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). (Z)-3-Hexenol and (Z)-3-hexenyl acetate were obtained from Bedoukian Research (Danbury, CT). 4-Isopropyl-1-methyl-1-cyclohexen-4-ol (ter-pinen-4-ol) was obtained from TCI Japan (Tokyo, Japan). 4-(*p*-Hydroxyphenyl)-2-butanone (raspberry ketone) was obtained from Acros Organics (Geel, Belgium). Methyl nonanoate was obtained from Eastman Blue (Rochester, NY). 5-Octanolide (δ-octalactone) was obtained from Alfa Aesar (Ward Hill, MA). 3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one (*cis*-jasmone) was purchased from Pfalz & Bauer (Waterbury, CT). 1-Isopropyl-4-methyl-1,4-cyclohexadiene (γ-ter-pinene), (E)-3,7-dimethyl-2,6-octadiene-1-ol (geraniol), 3-cyclohexene-1-methanol (α-terpinene), 1-methyl-4-(1-methylethylidene-1)-cyclo-hexene (terpinolene), 2-nonanol, 4-(4-hydroxy-3-methyoxyphenyl)-2-butanone (zingerone), 1-isopropyl-4-methylenebicyclo [3.1.0]hexane (sabinene), 6-methyl-5-hepten-2-ol and 4-isopropyl-benzyl-alcohol, 2-heptanone, 2-nonanone, 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene), 1-methyl-4-isopropenylcyclohex-1-ene (limonene), 2-(4-me-thylcyclohex-3-enyl)propan-2-ol (α-terpineol), and 7-methyl-3-methylene-1,6-octadiene (myrcene) were obtained from K&K Laboratories (Jamaica, NY).

Raspberry Samples. Ripe 'Chilliwack', 'Tulameen', 'Willamette', and 'Yellow Meeker' raspberries were hand harvested from plots in Lynden, WA, in July 2005, when the fruits were fully ripe. The fruits were transported, chilled, to the laboratory, where they were individually quick frozen (IQF) and stored at -38 °C until analyses were performed. 'Meeker' red raspberries from other Washington sites were obtained commercially in 2005; the raspberries were stored at -38 °C until analyses.

°Brix and Titratable Acidity. °Brix was analyzed at room temperature using a PAL-1 pocket refractometer (Atago U.S.A., Inc., Bellevue, WA). Titratable acidity was measured by mixing 7 mL of juice sample with 50 mL of boiled water and titrating with 0.1 N NaOH to an end point of pH 8.1, and reported as percentage of citric acid.

Sugar and Organic Acid Analysis. One hundred grams of red raspberries was thawed at room temperature for 3 h. The berries were blended with 50 g of boiling distilled water at high speed in a blender

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Table 5. Isomeric Ratio[a] of Some Chiral Compounds in Raspberries Grown in Washington

	(R)-α-ionone	(S)-α-ionone	(R)-α-pinene	(S)-α-pinene	(R)-linalool	(S)-linalool	(R)-terpinen-4-ol	(S)-terpinen-4-ol	(S)-δ-octalactone	(R)-δ-octalactone	(S)-δ-decalactone	(R)-δ-decalactone	6-methyl-5-hepten-2-ol 1	6-methyl-5-hepten-2-ol 2
'Meeker' site 1	98.1	1.9	100	0	41.4	58.6	20.7	79.3	98.1	1.9	96.8	3.2	94.7	5.3
'Meeker' site 2	96.8	3.2	100	0	40	60	20.9	79.1	96.3	3.7	97.7	2.3	94.8	5.2
'Meeker' site 3	97.8	2.2	100	0	42.1	57.9	20.8	79.2	96.5	3.5	97.8	2.2	95.5	4.5
'Meeker' site 4	97.5	2.5	100	0	37.4	62.6	20	80	96.4	3.6	97.9	2.1	93.9	6.1
'Meeker' average	97.6	2.4	100	0	40.2	59.8	20.6	79.4	96.9	3.1	97.5	2.5	94.7	5.3
wild-type 'Meeker' 95% range	96.5–98.6	1.4–3.5	100–100	0–0	36.3–44.1	55.9–63.7	19.8–21.3	78.7–80.2	94.3–99.4	0.6–5.7	96.6–98.5	1.5–3.4	93.5–96.0	4.0–6.5
'Chilliwack'	97.1	2.9	100	0	45.5	54.5	20.1	79.9	100	0	99.4	0.6	86.7	13.3
'Tulameen'	97.1	2.9	100	0	48.8	51.2	17.6	82.4	100	0	100	0	86.1	13.9
'Willamette'	97.7	2.3	100	0	51.2	48.8	19.6	80.4	100	0	99.7	0.3	77.4	22.6
'Yellow Meeker'	98.7	1.3	100	0	45.7	54.3	19.6	80.4	100	0	99.1	0.9	96.3	3.7

[a] Isomer designation is based on retention order on the column and literature reference.

for 30 s. This mixture was then placed in a boiling water bath for 5 min to deactivate enzymatic activity. The berry mixture was then centrifuged at 2000 rpm for 20 min, and the supernatant was collected for further analysis.

Sugar Analysis. The juice was diluted in a 1:2 ratio with acetonitrile to precipitate the pectin. One and a half milliliters of the supernatant was collected for sugar analysis. Twenty microliters of sample was injected onto a Shimadzu HPLC (Shimadzu Corp., Kyoto, Japan) system equipped with a Restek ultra amino column (3 μm, 200 × 4.6 mm, Bellefonte, PA) and a refractive index (RI) detector. The mobile phase was 81% (v/v) acetonitrile–water solution with a flow rate of 1.2 mL/min. The column was maintained at 30 °C. Each sample was run in triplicate.

Organic Acid Analysis. The juice was diluted in a 1:2 ratio with 0.005 M sulfuric acid, and 6 mL of the sample was passed through a C_{18} Sep-Pak Cartridge (200 mg, Waters Corp., Milford, MA) that had been conditioned with 10 mL of methanol, water, and dried with air prior to use. The first 4 mL of filtrate was discarded, and the last 2 mL was collected for analysis.

Ten microliters of sample was injected onto a Shimadzu HPLC system equipped with a Bio-Rad Aminex ion exclusion column (HPX-87H, 300 × 7.8 mm, Richmond, CA) maintained at 30 °C. A mobile phase of 0.005 M sulfuric acid was programmed at a constant flow rate of 0.4 mL/min. A UV–vis spectrophotometric detector at a wavelength of 210 nm was used for detection. Each sample was run in triplicate.

Extraction of Volatile Compounds. One hundred fifty grams of red raspberries was thawed for 3 h. The berries were blended with 1% $CaCl_2$ and 10% NaCl in a commercial blender for 30 s. The calcium chloride was added to inhibit enzyme activity, and the sodium chloride was added to increase sensitivity (*28*). The mixture was centrifuged at 2000 rpm for 20 min, and the supernatant was collected.

A stir bar sorptive extraction (SBSE) stir bar (1 cm long, 0.32 mm OD, 0.5 mm film thickness) with a polydimethylsiloxane (PDMS) phase was used for the extraction of volatile compounds. The stir bar was cleaned with 80% acetonitrile in methanol overnight, allowed to air-dry for 1 h, and then conditioned for 45 min at 300 °C with 50 mL/min nitrogen flow. Ten grams of juice samples was weighed into 20 mL clear glass vials (I-Chem, New Castle, DE) with polytetrafluoroethylene septum caps, and 10 μL of internal standard mixture in methanol was added. The juice was extracted at room temperature for 1 h at 1000 rpm. All samples were analyzed in triplicate.

Gas Chromatography–Mass Spectrometry (GC–MS). The analysis of volatile compounds was carried out by using an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Techonologies, Inc., Wilmington, DE) and a Gerstel MPS-2 multipurpose TDU autosampler with a CIS-4 cooled injection system (Gerstel USA, Baltimore, MD). The analytes were thermally desorbed at the TDU in splitless mode, ramping from 35 to 300 °C at a rate of 700 °C/min, and held at the final temperature for 3 min. The CIS-4 was cooled to −80 °C with liquid nitrogen during the sample injection, then heated at 10 °C/s to 250 °C for 3 min. Solvent vent mode was used during the injection with a split vent purge flow of 50 mL/min beginning at 3 min. The helium column flow was 2.0 mL/min. Separation was achieved using a ZB-FFAP column (30 m × 0.32 mm ID, 0.5 μm film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40 °C for 2 min, then ramped to 180 °C at a rate of 6 °C/min, then increased to 240 °C at a rate of 4 °C/min and held at the final temperature for 20 min. Standard EI mode was used at 70 eV. The total mass ion chromatogram was obtained from 35 to 350 amu. System software control and data management/analysis were performed through Enhanced ChemStation Software (Agilent Techonologies, Inc.). Compounds were identified through mass spectra and retention index comparison with the pure standards.

Volatile Compound Quantification. An internal standard solution was prepared in methanol containing 784 mg/L and 395 mg/L of γ-nonalactone and *cis*-jasmone, respectively. An aliquot (5 μL) of the internal standard mixture was then added to 10 mL of water to yield a final concentration of 392 and 197 μg/L, respectively. Pure volatile standards were divided into four groups for sample preparation and analysis. Standard stock solutions of 2000 mg/L of each compound

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were prepared in methanol. The stock and the internal standard solution (5 μL) were then used to create working standard with concentrations of 1000, 750, 500, 100, 50, 5, and 1 μg/L in water. Quantification was carried out using selective mass ions to avoid interference between coeluted compounds as listed in **Table 1**. Quantification curves were built by plotting the selected ion abundance ratio of target compounds with their respective internal standards against the concentration ratio, and an individual quantification curve was used to quantify the concentrations of aroma-active compounds in the samples.

Chiral Analysis. The samples were extracted using the same procedure as described previously; however, internal standards were not added. Separation was achieved using a Cyclosil B column (30 m × 0.25 mm ID, 0.25 μm film thickness, Agilent). The oven temperature was programmed at 40 °C for 2 min, then increased to 90 °C at a rate 6 °C/min, then to 135 °C at a rate of 1 °C/min, increased to 220 °C at a rate of 10 °C/min and held at the final temperature for 5 min. Isomeric ratio was determined using the relative total mass ion abundance of the compound.

Statistics. A 95% 'Meeker' confidence interval, constructed using the average for each of the 'Meeker' values, with two standard deviations, was used as the variation range for 'Meeker' from different sites.

RESULTS AND DISCUSSION

Sugars and Organic Acids, °Brix, and Titratable Acidity. All of the raspberry cultivars were grown in Washington state during 2005. **Table 2** listed sugar and organic acid, °Brix, and titratable acidity in raspberry samples. Variations for °Brix, titratable acidity, sugar, and acid composition were observed in 'Meeker' fruits obtained from different sites. Sugars and organic acids are strongly affected by fruit ripening and climate variations (*1, 12, 14*). Higher temperatures and lower humidity generally produce fruit with higher sugar and lower acid contents (*1*).

The major sugars in 'Meeker' raspberry were fructose and glucose, and the ratio of fructose to glucose was around 1 to 1.1. 'Meeker' raspberry had a low concentration of sucrose. Significantly higher amounts of sucrose were found in other raspberry cultivars with sucrose being the major sugar in 'Chilliwack'. 'Yellow Meeker' had much higher fructose, glucose, and sucrose than did 'Meeker'. Sugar composition was studied before for some raspberries, and a wide range has been reported (*29*). Although the cultivars and locations were different, the sugar composition in this study was generally in agreement with the values reported previously (*29*).

The major organic acid identified in the raspberries was citric acid. The average citric acid content in 'Meeker' raspberries was 1.51% with a range from 1.33% to 1.72%. As compared to 'Meeker', most other cultivars had higher acid concentrations. 'Chilliwack' had the highest amount of citric acid, followed by 'Willamette'. The only exception was 'Tulameen', which had a citric acid content slightly above that of the 'Meeker'. A very small amount of malic acid was found in these raspberries. The concentrations of citric and malic acids in these cultivars were in agreement with those reported previously in the literature (*29*).

The sugar to acid ratio is considered to be very important to the flavor perception of fruits. For most of the cultivars, the high acids were balanced with high sugars, leading to a sugar/acid ratio that was very close to the 'Meeker' value. 'Willamette' is the exception; the sugar/acid ratio was considerably lower than the average 'Meeker' value. A low sugar/acid ratio can lead to a "tart" sensory perception.

Volatile Quantification. Raspberry ketone, α-ionone, β-ionone, (Z)-3-hexenol, geraniol, linalool, and many other compounds have been identified to contribute to raspberry aroma (*7, 10, 11, 30, 31*).

Different proportions of these compounds give rise to the perceived aroma difference of cultivars, growing areas, and growing conditions. However, quantitative data about the concentration of these aroma-active compounds in raspberry are rare. In this study, a total of 29 compounds were quantified. These compounds were selected on the basis of their previously reported importance to raspberry aroma as well as their representation to various chemical classes including alcohol, aldehyde and ketone, ester, and terpene and tepene alcohol. Calibration curves were constructed for the selected compounds using pure compounds and internal standards. Chromatographic conditions were selected to optimize resolution for the quantified compounds; quantifying and qualifying ions were selected to eliminate interference from coeluting compounds while providing good sensitivity. As shown in **Table 1**, good correlation coefficients (greater than 0.99) were achieved for most of the compounds, and the quantification limit for most of the compounds was at 1 μg/kg. The quantification of raspberry ketone and zingerone was also attempted; however, the PDMS phase has very low recoveries for these two compounds, and thus the quantification of these two compounds by the PDMS–SB-SE technique was not reliable.

The concentrations of selected volatile compounds in 'Meeker' raspberry from different growing sites were listed in **Table 3**. Volatile concentration varied from site to site with standard deviation of 10–20% for most of the compounds investigated. Similar to sugar and acid, flavor variations between sites and years can be influenced by temperature, soil variation, and the intensity of sunlight available during the growing and ripening seasons (*1, 14*). Year to year variations and location variations have been shown for the raspberry ketone, alcohols, aldehydes, esters, ketones, and terpene concentrations in red raspberries (*12*). It is found that year to year variations are more significant than the location variations (*12*). Areas with warmer and drier summers generally produce more aromatic fruit (*1*). As shown in **Table 3**, volatile concentration variations from different sites were much less than the variations observed for sugar and acids.

There were considerable differences between 'Meeker' red raspberry and other raspberry cultivars studied (**Table 4**). α-Ionone, β-ionone, geraniol, linalool, (Z)-3-hexenol, and the raspberry ketone are considered to be particularly important in differentiating red raspberries (*4, 7, 11*). When a 95% confidence was applied, 'Chilliwack' appeared to be the cultivar that was the most different from 'Meeker'. All four cultivars studied varied considerably from 'Meeker' for α-ionone, although 'Tulameen' and 'Willamette' were only just outside the range. 'Chilliwack' was the only cultivar to vary from 'Meeker' in β-ionone. 'Chilliwack' and 'Wilamette' varied for geraniol. All cultivars varied from 'Meeker' for linalool and (Z)-3-hexenol.

Chiral Analysis. Natural flavor molecules are generally found with one enantiomer predominating; synthetic flavor molecules are generally found in racemic mixtures. Analysis of chiral compounds is a useful method for analyzing products produced with raspberries or raspberry flavor (*23*).

Several chiral isomers were separated under experimental conditions, while only one isomeric form was observed for the raspberry ketone. This result supported a previous literature report that raspberry ketone only has one isomer present in natural raspberry fruit (*23*). The isomeric ratios of α-ionone, α-pinene, linalool, terpinen-4-ol, δ-octalactone, and δ-decalactone were displayed in **Table 5**. Most of the compounds demonstrated a much higher percentage of one isomer over another, particularly α-ionone, α-pinene, and the δ-lactones.

Raspberry Volatile by SBSE

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More than 96% of the isomeric form of α- ionone was (*R*)-form, which was consistent with the literature report (*23*), although 100% (*R*)-form has been reported (*24*). Similarly, more than 96% of isomeric lactones were the (*S*)-form, which was also in agreement with the literature (*23*). Linalool was nearly a racemic mixture, with a slightly higher percentage of the (*S*)-isomer. Terpinen-4-ol was a 20/80 mixture favoring the (*S*)-isomer.

Little variation was seen among 'Meeker' grown at different locations for the isomeric compounds studied; however, there was considerable variation between 'Meeker' and the other cultivars. The largest variations between cultivars were observed for linalool and 6-methyl-5-hepten-2-ol. Linalool showed a more racemic mixture for non-'Meeker' cultivars, particularly 'Tulameen' and 'Willamette'. 6-Methyl-5-hepten-2-ol had more than 94% of the isomer 1 for 'Meeker' cultivar, while the percentage of isomer 1 was much lower for non-'Meeker' cultivars, particularly 'Willamette'. Although 6-methyl-5-hepten-2-ol had a very high sensory threshold, and typically will not contribute to aroma, the consistent high isomeric ratio in 'Meeker' and lower isomeric ratio in other cultivars could be used to distinguish 'Meeker' raspberry from other cultivars. Overall, 'Chilliwack' red raspberries showed the most variation from 'Meeker' for volatile aroma compounds, and 'Yellow Meeker' raspberries showed the least variation from 'Meeker' raspberries.

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