PAPER

CHARACTERIZATION OF VOLATILE COMPOUNDS IN FIVE BLUEBERRY VARIETIES USING PURGE AND TRAP COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

The volatile composition of five blueberry varieties from two different regions was analysed by dynamic headspace (purge and trap, P&T) coupled to gas chromatography-mass spectrometry (GC-MS). Under the optimized conditions, the P&T method was successfully validated, showing good linearity, high accuracy, good reproducibility and a low limit of detection. A total of 80 volatiles were identified, including 19 esters, 30 alcohols, 18 aldehydes, 7 ketones and 6 other compounds. Furthermore, a spider web diagram was constructed to compare the flavour profiles of these blueberries, and the obtained results demonstrated that blueberries from different locations have different flavour profiles.

Keywords: aroma active compounds, blueberry, GC-MS, P&T, volatile compounds

1. INTRODUCTION

Blueberries have been recognized by the scientific community and consumers for their health-promoting potential (SILVA *et al.*, 2017). The history of blueberry cultivation in China is approximately 20 years old, and Chinese blueberries were mainly introduced from the United States and Japan. There are three main types of blueberries: highbush (*Vaccinium corymbosum*), lowbush (*Vaccinium angustifolium*), and rabbiteye (*Vaccinium virgatum*). Highbush blueberries can be further divided into northern highbush and southern highbush blueberries (DU and ROUSEFF, 2014). Northern highbush and lowbush blueberries are the predominant varieties in the Greater Khingan Range. Southern highbush and rabbiteye blueberries are generally grown to the south of the Yangtze River (HE and WU, 2010).

In addition to being rich in vitamins and anthocyanins, blueberries are rich in volatile compounds such as ethyl acetate, butyl acetate, and 1-nonanal. Flavour and aroma are two of the most important fruit quality characteristics and ultimately determine consumer acceptability and purchase decisions (DU and ROUSEFF, 2014). Volatile compounds are important contributors to fruit aroma, which is one of the main characteristics that determine blueberry organoleptic quality and style (SUN *et al.*, 2013). Different proportions of volatile components determine the overall aromatic properties (LV and LIN, 2015). People realized the importance of volatile compounds with regards to aroma approximately 50 years ago. However, due to equipment being less advanced, studies of blueberry aroma are still very limited. The volatile compounds of highbush blueberries were analysed by PARLIAMENT and KOLOR in 1975, and 18 individual components were identified by mass spectrometry, infrared analysis and gas chromatographic retention times (PARLIMENT and KOLOR, 1975). HALL *et al*. (1970) used gas-liquid chromatography (GLC) to examine the aromatic composition of lowbush blueberries. Acetaldehyde, methyl acetate, ethyl acetate and ethyl alcohol were reported as the major aromatic compounds. Currently, with the emergence of detection techniques with high sensitivity and accuracy, such as gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), more volatile compounds at relatively low concentrations and thresholds are expected to be detected.

Purge and trap (P&T), also known as dynamic headspace, has been widely used for the preconcentration of volatile compounds (LARRETA *et al.*, 2008). With P&T, an inert gas is purged throughout the sample in the same way as which we breathe, making this technique suitable for correlation with organoleptic studies (AZNAR and ARROYO, 2007). It can be applied to solid or liquid matrices (MURAT *et al.*, 2012). Compared to SPME, the high recovery of very volatile compounds and the low dispersion associated with the use of a totally automated system are the main advantages of P&T-GC-MS-based methods (SORIA *et al*., 2009).

In this study, five blueberry varieties from two major blueberry production areas were identified (i) by purge and trap coupled to gas chromatography-mass spectrometry (P&T-GC-MS). To provide a representative analysis of the blueberry volatiles, we first (ii) optimized this method and evaluate its correctness and then (iii) drew a spider web diagram to compare the flavour profiles of these blueberries.

2. MATERIALS AND METHODS

2.1. Plant materials

All the samples were purchased from the Hulun Buiroroqen Pristine Production Co. Ltd. Blueberries were squeezed into juice, diluted three-fold, and filtered for analysis.
In this work, a total of five blueberry taxa were used to study volatiles. These taxa included two wild blueberries and three cultivated blueberries (Table 1).

Table 1. Blueberry taxa in this study*.

Taxa	Characters	Origin	Population
Wild blueberry	around the humus	Mohe area of Greater Khingan Range	WH-M
Wild blueberry	around the stones	Mohe area of Greater Khingan Range	WS-M
Cultivated blueberry	Bluecrop	Greater Khingan Range	CB-G
Cultivated blueberry	Powderblue	Greater Khingan Range	CP-G
Cultivated blueberry	Britewell	Yangzhou	CB-Y

*CB-G is northern highbush blueberry; CP-G and CB-Y are rabbiteye blueberries.

2.2. Chemicals

NaCl and n-alkanes (C_6-C_{22}) were purchased from Beijing Chemical Reagents Co. Ltd. (Beijing, China). Analytical grade 2-methylbutyraldehyde, ethyl acetate, 2-nonanone, linalool, and ethyl caprylate were purchased from Sigma-Aldrich Co. Ltd. (Shanghai, China).

2.3. Volatile compounds extracted by purge and trap

P&T was performed by an Eclipse 4660 purge and trap sample concentrator with a 4551A autosampler (OI Analytical Company, USA) and a #10 trap. Three millilitres of each juice sample was placed in a 5 mL purge tube. Nitrogen gas was utilized as a purge at 10 psi at 25°C.
The other analytical conditions were as follows:
Trap temperature: purge, 30°C; desorption, 190°C; transfer line, 110°C; and valve oven, 110°C.
Time: purge 11 min; desorption 1 min.

2.4. GC-MS conditions

Chromatographic analysis was performed in a GC-MS (QP2010 Ultra, Shimadzu Corporation, Japan) system equipped with a Rtx-5MS capillary column (0.25 mm×30 m×0.25 μm) (Restek, USA). Helium was used as the carrier gas at a linear velocity of 1.0 mL/min. The column temperature was held at 50°C for 5 min, increased to 180°C at a rate of 10°C/min, increased to 210°C for 5 min at a rate of 5°C/min, and finally increased to 280°C at a rate of 20°C/min. The mass selective was operated in the electron ionization mode at 70 eV and a scan range m/z of 45-400.

2.5. Identification of volatile compounds

Volatile compounds were identified by matching their mass spectra with those of the known compounds from the NIST 11/11s edition library.
The relative odour activity value (ROAV) was calculated to measure the contribution of each volatile compound towards the whole aroma profile and was calculated using the following equation (ZHUANG *et al.*, 2008; GU *et al.*, 2012). ROAVs were calculated by using Eq. (1):

$$\mathrm{ROAVi} = \frac{\mathrm{Ci\%}}{\mathrm{Cstan\%}} \times \frac{\mathrm{Tstan}}{\mathrm{Ti}} \times 100 \qquad (1)$$

where "stan" is the volatile compound that has the highest relative contents; ROAVi is the odour activity value of the compound in sample i; Ci is its content; and Ti is its odour threshold concentration. Compounds with a ROAV ≥1 significantly contribute to the aroma. (ZHUANG *et al.*,2016).

2.6. Statistical analysis

Significant differences in the volatile compounds of the five blueberry varieties obtained from duplicate analysis were determined by one-way ANOVA with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL). Statistically significant differences were determined at $p<0.05$. The OriginPro system (v8.5 SR6, OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Optimization of the P&T-GC-MS method

This study optimized the following P&T extraction parameters: sample volume, purge temperature and purge time.
Ethyl acetate, ethyl caprylate, 2-methylbutyraldehyde, 2-nonanone and linalool were used as standard compounds for optimization of the P&T-GC-MS method. As shown in Fig. 1, varying volumes of blueberry juice (3, 4, and 5 mL) were placed in the trapping apparatus flask and purged for 11 min at 25°C. For ethyl octanoate, ethyl acetate, and linalool, there was a considerable difference between the various sample volumes ($p<0.05$). For 2-methylbutyraldehyde and 2-nonanone, the relative percentages of these standard compounds in the 3 mL groups increased compared with the high sample quality group, but there was no significant difference ($p>0.05$). This study also showed that the number of volatile substances obtained from 3, 4, and 5 mL was 52, 50, and 49, respectively. The reason for this result may be that a high liquid level is too close to the top of the purge trap, so when a large amount of N_2 purifies the liquid, extra water could be purged into the trap, which can shorten the trap life in the same way as a longer purge time (DENG *et al.*, 2011).

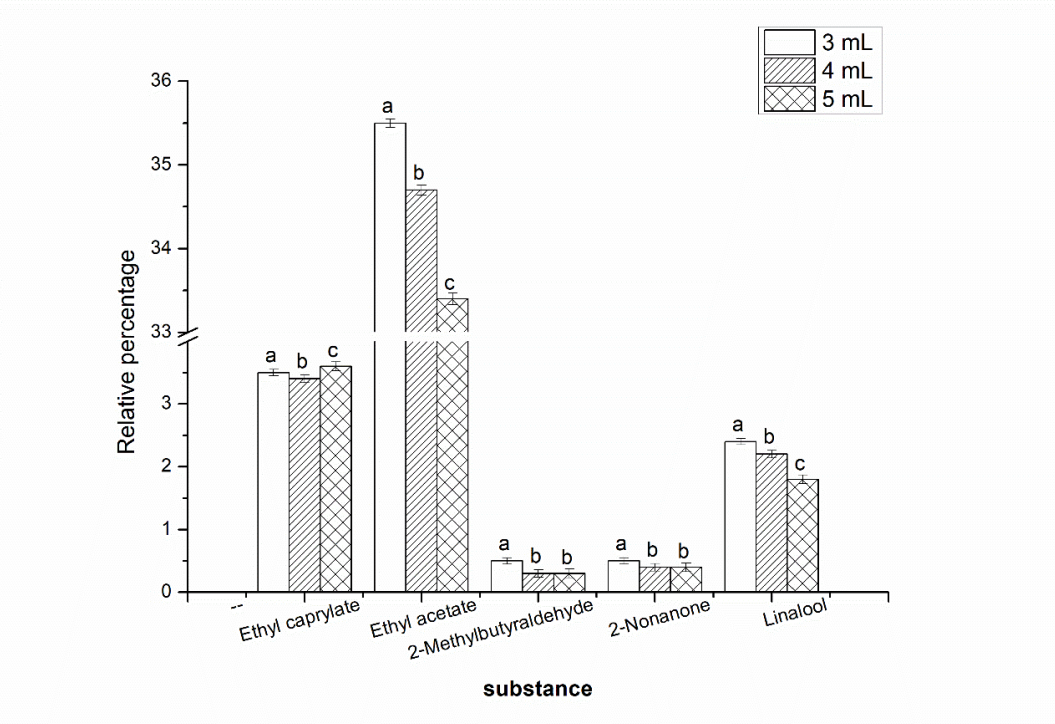

Figure 1. Effect of sample volume on the extraction efficiency; purge temperature = 25°C, purge time = 11 min.

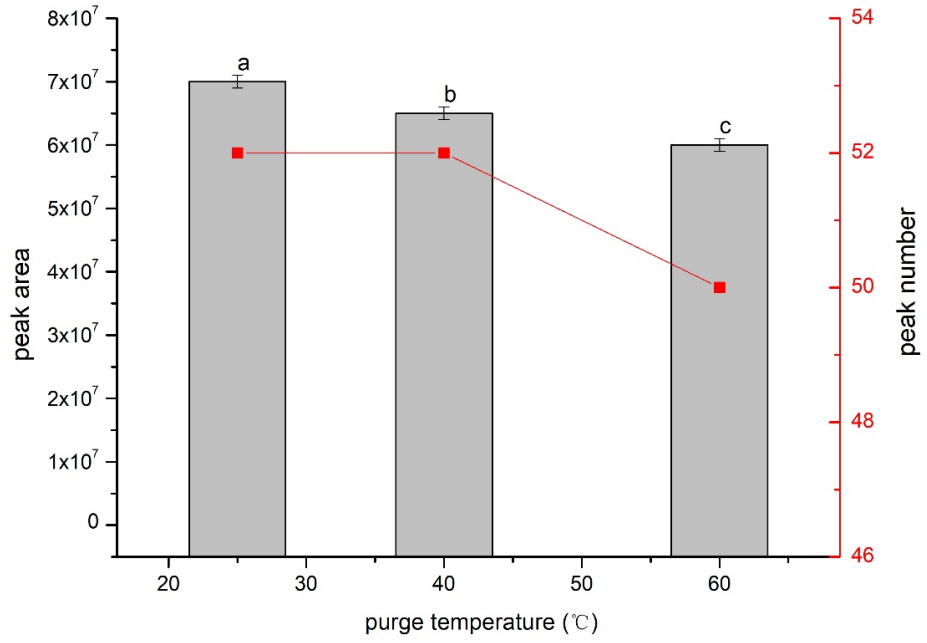

Figure 2. Effect of purge temperature on the extraction efficiency. Sample volume = 3 mL, purge time = 11 min.

Fig. 2 showed the effect of purge temperature on the extraction efficiency. The purge temperature varied between 25-60°C with 3 mL of sample volume for 11 min. The amount of total volatiles detected in blueberries gradually decreased as the purge temperature increased from 25°C to 60°C, probably due to the amount of water that reached the trap and decreased the sensitivity; therefore, ambient temperature was maintained in all the experiments (CAMPILLO *et al.*, 2004).
The effect of purge time on the sensitivity is shown in Fig. 3. The purge time was varied between 8 and 14 min with 3 mL of sample volume at 25°C. Finally, a value of 11 min was chosen as the optimal time, since 8 and 14 min led to a slight decrease in the peak area and total number. Eight minutes is too short mainly because the volatile substances are not fully blown out. Indeed, 14 min decreased the signals because a flow of N_2 that was too long could move the volatiles from the trap before desorption and reduce the final signal (CAMPILLO *et al.*, 2004).

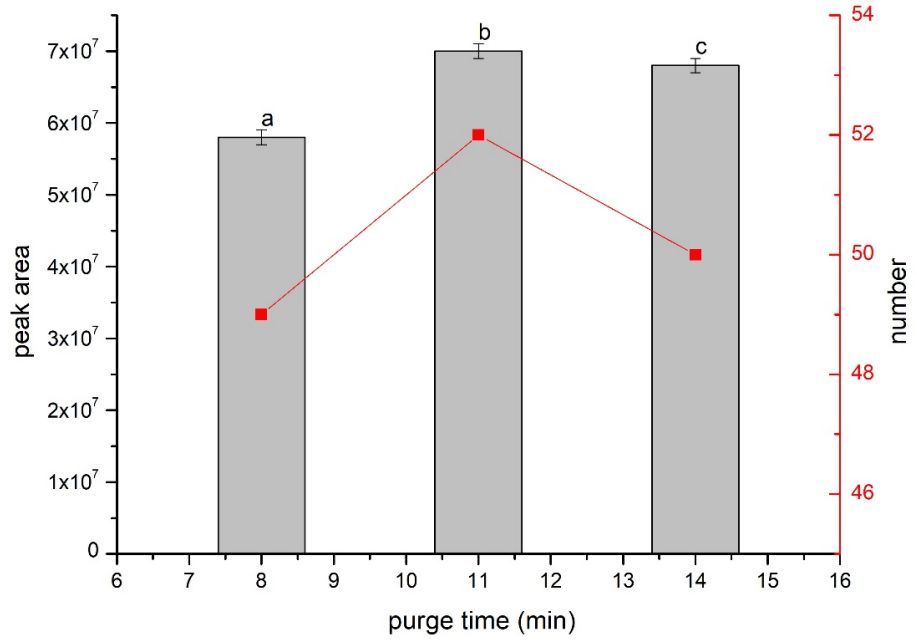

Figure 3. Effect of purge time on the extraction efficiency. Sample volume = 3 mL, purge temperature = 25°C.

Verification and quantitative analysis (of P&T-GC-MS method)

Once the final purge conditions were selected, these five aroma standards were detected. Table 2 shows the results from method validation: linearity, recovery, reproducibility, LOD and LOQ.

Linearity

The linearity of the method was evaluated by analysing a series of aromatic standards. Linearity was found in the concentration range between 5 and 160 μg/L, with high

reproducibility and accuracy. Regression analysis of the experimental data points showed a linear relationship with excellent regression coefficients (r^2>99%) for 2-methylbutyraldehyde, ethyl acetate, 2-nonanone, linalool, and ethyl caprylate.

Table 2. Performance parameters of the P&T method for the volatile compounds in blueberry.

Compounds	Linearity (r^2)	Recovery (%)	C.V. (%)	LOD (µg/L)	LOQ (µg/L)
2-Methylbutyraldehyde	0.9982	117.01	4.3418	0.78	2.60
Ethyl acetate	0.9971	91.52	1.7276	0.90	3.00
2-Nonanone	0.9957	103.47	2.8291	1.02	3.40
Linalool	0.9933	99.33	5.4841	1.06	3.50
Ethyl caprylate	0.9964	106.18	7.1420	1.29	4.30

Recovery

Recoveries ranged between 96% and 120%, indicating that the accuracy of the method meets the experimental requirements and that the results are reliable.

Reproducibility

Reproducibility was evaluated by using the coefficient of variation (CV%) for replicate analyses. The CV% values obtained are shown in Table 3. CV% values were found to be <8% in the case of relative proportions (HAKALA *et al.*, 2002). The smallest CV% was found for ethyl acetate (1.73%), and the largest was found for ethyl octanoate (7.14%). In the range of esters, as the carbon number increases, the coefficient of variation also increased. As shown above, the P&T-GC-MS technique was reproducible enough to allow for comparative comparison studies of the volatiles of different varieties (HAKALA *et al.*, 2002).

Determination of the limit of detection (LOD) and the limit of quantification (LOQ). The LOD was calculated as the concentration required to obtain a signal that was three times higher than that of the baseline signal (PINO and OUERIS, 2010). Detection limits were below 1.29 μg/L for all volatiles. The LOQ can also be estimated as the concentration of analyte producing a signal that is 10 times that of the noise (S/N = 10) (PINO and OUERIS, 2010).

From the above results, good linearity, high accuracy, very good repeatability and a low limit of detection were achieved (DENG *et al.*, 2011). There were also good recoveries and reproducibility. This method can be applied for research on the volatiles in blueberries.

In conclusion, 3 mL of sample purged at 25°C for 11 min were selected as the best extraction conditions for the P&T methodology developed in this study.

Table 3. Analysis of volatile compounds from different blueberry varieties.

No.	RI	t_R (min)	Compounds	Relative content (%)				
				WH-M	WS-M	CB-G	CP-G	CB-Y
			Esters					
1	487	1.603	Methyl acetate				0.14±0.01	
2	584	1.644	Ethyl formate	9.9±0.50[a]	8.81±0.43[a]			9.54±0.51[a]
3	586	1.891	Ethyl acetate	60.94±3.05[a]	66.21±3.24[a]	4.89±0.22[c]	1.58±0.09[c]	17.36±0.95[b]
4	686	2.518	Ethyl propionate	0.48±0.02[a]	0.11±0.01[b]			
5	686	2.540	Propyl acetate	0.14±0.01[a]	0.10±0.01[a]			
6	686	2.620	Methyl butyrate	0.03±0.00[b]				0.84±0.03[a]
7	778	2.796	Isopentyl formate	0.24±0.02[c]	0.60±0.03[b]			1.00±0.04[a]
8	785	3.053	Ethyl butyrate	0.04±0.00[a]	0.04±0.00[a]			
9	785	3.247	Butyl acetate	9.80±0.50[a]	9.30±0.42[a]			5.09±0.27[b]
10	820	4.680	Ethyl isovalerate	1.78±0.10[b]	1.32±0.08[b]			12.66±0.68[a]
11	864	5.193	Amyl acetate	3.97±0.21[a]	3.80±0.24[a]			
12	869	6.330	Prenylacetate	0.02±0.00[b]				0.24±0.01[a]
13	869	6.414	Ethyl 3,3-dimethylacrylate	0.02±0.00				
14	983	7.614	Ethyl 2-hydroxy-3-methylbutanoate	0.04±0.00				
15	1029	8.875	Hexenyl acetate	0.16±0.01[a]	0.06±0.00[b]			
16	1043	8.900	Butyl pentanoate	0.01±0.00[b]	0.02±0.00[b]			0.17±0.01[a]
17	1047	9.117	Hexyl acetate	0.02±0.00[a]	0.01±0.00[a]			
18	1277	18.500	L-Bornyl acetate			0.10±0.01		
19	1294	19.949	2-Methylpropyl benzoate	0.07±0.00[c]	0.06±0.00[c]	0.40±0.02[a]	0.27±0.01[b]	
			Alcohols					
20	662	2.156	n-Butyl alcohol	0.10±0.00[b]				16.41±0.76[a]
21	788	2.275	Cyclopentanol					0.57±0.03
22	700	2.817	2-Methyl-1-butanol	0.12±0.01[b]	0.32±0.02[a]			
23	769	2.982	2-Penten-1-ol			0.12±0.01		

24	995	4.659	6-Methyl-heptanol	1.85±0.0[8a]				0.26±0.01[b]
25	858	4.934	2-Hexen-1-ol	0.05±0.00[d]	0.17±0.01[cd]	0.51±0.03[b]	1.19±0.07[a]	0.26±0.01[c]
26	860	5.020	Hexyl alcohol	0.51±0.03[b]	0.67±0.03[b]	2.08±0.12[b]	3.77±0.20[a]	2.07±0.98[b]
27	960	7.780	n-Heptanol			0.10±0.00		
28	969	8.092	1-Octen-3-ol	0.07±0.00[c]	0.06±0.00[c]	0.35±0.02[b]	0.38±0.02[b]	0.94±0.05[a]
29	969	8.835	Citronellol				1.49±0.06	
30	971	9.401	3-Ethyl-4-methyl-1-pentanol			0.12±0.01		
31	1042	9.535	4-Isopropyltoluene			0.08±0.00[b]	0.31±0.02[a]	
32	1055	9.664	2-Ethylhexanol	1.84±0.11[c]	1.16±0.01[c]	11.2±0.52[a]	9.17±0.43[b]	10.61±0.59[ab]
33	1059	9.795	Eucalyptol	0.07±0.00[bc]	0.10±0.00[b]	0.39±0.02[a]	0.10±0.01[d]	0.06±0.00[c]
34	1060	11.095	1-Octanol	0.13±0.01[b]	0.09±0.00[b]	0.92±0.05[a]	0.87±0.03[ab]	0.33±0.02[ab]
35	1063	11.157	Dihydromyrcenol				1.88±0.10	
36	1082	12.093	Linalool	0.05±0.00[c]	0.03±0.00[c]	0.88±0.04[b]	0.93±0.04[b]	2.68±0.14[a]
37	1138	12.753	Fenchyl alcohol	0.10±0.01[b]		0.53±0.03[a]	0.18±0.01[b]	
38	1153	14.249	Menthol			0.22±0.02		
39	1158	14.598	Borneol			0.96±0.05		
40	1159	14.622	1-Nonanol	0.08±0.00[c]	0.05±0.00[c]	0.44±0.02[b]	0.75±0.03[a]	
41	1164	14.817	DL-Menthol	0.68±0.03[b]	0.85±0.04[a]	0.65±0.03[b]	0.47±0.02[c]	
42	1187	14.914	4-Terpineol	0.15±0.01[c]	0.12±0.01[c]	0.80±0.03[a]	0.27±0.01[b]	
43	1198	15.422	(-)-α-Terpineol	0.07±0.00[c]	0.05±0.00[c]	0.51±0.02[a]	0.38±0.02[b]	0.06±0.00[c]
44	1228	17.356	Geraniol		0.99±0.05[b]	13.28±0.57[a]		
45	1258	18.133	1-Decanol	0.06±0.00[b]		1.50±0.07[a]		
46	1200	18.536	cis-Anethol	0.06±0.00[b]	0.04±0.00[b]	0.17±0.02[a]		
47	1262	18.978	Thymol			0.10±0.00		
48	1457	24.504	1-Dodecanol				0.37±0.15	
49	1543	28.659	Cedrol			0.07±0.00		
			Aldehydes and ketones					
50	508	1.589	Propionaldehyde			2.93±0.14[b]	2.78±0.12[b]	3.66±0.20[a]
51	543	1.725	Isobutyraldehyde		0.16±0.01[b]			0.53±0.02[a]
52	555	1.815	2-Butanone					0.92±0.04

53	643	2.008	2-Methylbutyraldehyde				1.57±0.04^{a}	1.22±0.07^{b}
54	644	2.279	1-Penten-3-one	0.04±0.00^{b}		0.65±0.03^{a}		
55	654	2.384	3-Pentanone		0.17±0.01^{b}			0.84±0.05^{a}
56	715	2.982	2-Pentenal			0.08±0.00		
57	791	3.613	4-Methyl-3-pentene-1-one		0.07±0.00^{c}	22.98±1.13^{a}	21.9±0.99^{a}	8.49±0.37^{b}
58	806	3.654	Hexanal	0.88±0.04^{b}		16.59±0.96^{a}	17.3±0.87^{a}	
59	831	4.225	Furfural	0.14±0.01^{b}	0.12±0.01^{b}	0.12±0.01^{b}	4.13±0.23^{a}	
60	853	5.500	2-Heptanone	0.01±0.00^{c}		0.22±0.01^{b}	1.68±0.07^{a}	
61	841	5.816	4-Methylhexanal	0.02±0.00^{b}		0.17±0.01^{b}	2.07±0.11^{a}	
62	913	7.358	2-Heptenal			0.04±0.00		
63	982	7.509	Benzaldehyde	0.07±0.00^{d}	0.04±0.00^{d}	0.65±0.03^{b}	0.92±0.06^{a}	0.21±0.01^{c}
64	1005	8.823	Octanal	0.03±0.00^{d}		0.17±0.01^{b}	0.21±0.01^{a}	0.13±0.00^{c}
65	1013	10.619	2-Octenal			0.04±0.00		
66	1052	11.762	2-Nonanone	0.03±0.00^{b}	0.03±0.00^{b}			0.22±0.01^{a}
67	1104	12.251	Nonanal	1.38±0.06^{c}	1.39±0.07^{c}	7.01±0.42^{b}	9.22±0.51^{a}	2.18±0.14^{c}
68	1112	14.194	(2E)-Nonenal				0.18±0.02	
69	1151	15.300	2-Decanone	0.02±0.00				
70	1204	15.807	Decanal	1.84±0.01^{a}	0.50±0.03^{bc}	0.58±0.02^{b}	0.47±0.03^{c}	
71	1208	16.063	2,4-Dimethylbenzaldehyde	0.16±0.01^{c}	0.10±0.01^{c}	0.57±0.03^{a}	0.45±0.02^{b}	
72	1263	16.358	5-Hydroxymethylfurfural				4.46±0.29	
73	1402	22.595	Dodecyl aldehyde	0.01±0.00^{b}			0.14±0.01^{a}	
74	1420	23.826	(Z)-Geranyl acetone	0.36±0.02^{d}	0.58±0.03^{c}	0.74±0.04^{b}	1.19±0.06^{a}	
			Others					
75	877	4.514	3,7-Dimethyl-1-octene	0.02±0.00^{b}		0.61±0.03^{a}		
76	883	5.574	Phenylethylene	0.05±0.00^{c}	0.04±0.00^{c}	0.18±0.01^{b}	0.27±0.01^{a}	
77	1029	10.851	Acetophenone	0.04±0.00^{b}	0.03±0.00^{b}	0.14±0.01^{b}	3.37±0.20^{a}	
78	1231	14.992	Naphthalene	0.48±0.02^{c}	0.46±0.02^{c}	1.63±0.08^{b}	2.35±0.13^{a}	0.45±0.02^{c}
79	1407	22.876	Cedarene				0.84±0.05	
80	1668	25.545	Butylated hydroxytoluene	0.77±0.04^{c}	1.17±0.06^{b}	2.53±0.11^{a}		

3.2. Identification of the volatile compounds in five blueberry varieties

As shown in Table 3, the volatile compounds in the five blueberry varieties were identified. A total of 80 volatiles were identified, including 19 esters, 30 alcohols, 18 aldehydes, 7 ketones and 6 other compounds. The number of identified volatile compounds in each blueberry variety ranged from 30 to 53. WH-M and CB-G had the highest (53) and the second highest number (47) of volatile compounds, respectively, while CB-Y had the smallest number (30) of volatile compounds.

Esters are considered to be contributors to fruity and floral notes (WANG *et al.*, 2009). A total of 21 ester compounds were detected in the five blueberry varieties. Ethyl acetate is a common compound that has a strong fruity aroma. Among the 5 groups, the sum of the esters was higher in WS-M and WH-M blueberries than in the other cultivated groups. Esters were abundant in wild blueberries, contributing 87.66-90.44% of the total volatiles (Table 4). Although 13 esters in total were found in wild blueberries in this study, ethyl acetate, ethyl formate, butyl acetate and amyl acetate accounted for more than 80% of the total esters in WH-M and WS-M. The unique esters of WH-M were methyl butyrate, prenylacetate, ethyl 3-methyl-2-butenoate, ethyl 3,3-dimethylacrylate, and ethyl 2-hydroxy-3-methylbutanoate, with the latter two in agreement with previous results (BEAULIEU *et al.*, 2014). L-Bornyl acetate was only detected in CB-G. 2-Methylpropyl benzoate was detected in all varieties except CB-Y. Esters were not considered to be as important as aldehydes to the aroma in highbush blueberries, while they have been identified as important volatiles in some rabbiteye blueberries, which is consistent with previous results (Du and ROUSEFF, 2014).

The total content of alcohols accounted for 4.7-35.98% of the total volatiles (Table 3). The content of alcohols was significantly higher in cultivated blueberries than in wild blueberries. Of the 30 alcohols identified in this study, 8 were identified in all five varieties: 2-hexen-1-ol, hexyl alcohol, 1-octene-3-ol, eucalyptol, 1-octanol, linalool, 2-ethylhexanol, and (-)-α-terpineol. Among them, 2-ethylhexanol was dominant, with relative contents ranging from 1.16% to 11.20% (Table 4). 2-Methyl-1-butanol was detected in WS-M and WH-M. 2-Penten-1-ol, 3-ethyl-4-methyl-1-pentanol, borneol and menthol were only detected in CB-G. The unique alcohols in CP-G and CB-Y were citronellol and cyclopentanol, respectively.

A total of 25 different aldehydes and ketones in blueberry juice were identified, accounting for 3.16%-68.67% of the total volatiles (Table 4). The sum of the aldehydes and ketones in CP-G was significantly higher than that in other varieties, and it was also significantly higher in cultivated blueberries than in wild blueberries. Nonanal and benzaldehyde were the predominant aldehydes found in the five blueberry varieties. WH-M had a significantly higher decanal content than that of the other aldehydes. In all cultivated groups, 4-methyl-3-pentene-1-one was the major component, accounting for more than 20% of the total aldehydes in CB-G and CP-G. 2-Pentenal, 2-heptenal, and 2-octenal were only detected in CB-G. Additionally, (2E)-nonenal, 5-hydroxymethylfurfural and dodecyl aldehyde could be used to distinguish CP-G from the other varieties. 2-Butanone was only detected in CB-Y.

Table 4. The aroma-active compounds (ROAV > 1) in different blueberries*.

No.	Volatile	Threshold (μg/L)	Sensory attributes	Aroma classification	ROAV				
					WH-M	WS-M	CB-G	CP-G	CB-Y
1	Ethyl formate	150	Fruity	1	0.54 ±0.03^{b}	0.44± 0.02^{b}			1.83 ±0.10^{a}
2	Ethyl acetate	5	Fruity	1	100.00±5.00^{a}	100.00±4.89^{a}	13.95±0.63^{b}	3.43±0.20^{b}	100.00 ±5.47^{a}
3	Butyl acetate	66	Sweet, banana,	1,3	1.22±0.06^{b}	1.06 ±0.05^{b}			2.22 ±0.12^{a}
4	1-Octene-3-ol	1	Mushroom	4	0.57 ±0.00^{c}	0.45 ±0.00^{c}	4.99±0.29^{b}	4.12 ±0.22^{b}	27.07±1.44^{a}
5	Linalool	6	Sweet lemon	1	0.07 ±0.00^{c}	0.04 ±0.00^{c}	2.09 ±0.10^{b}	1.68 ±0.07^{b}	12.86 ±0.67^{a}
6	Geraniol	40	Rose	2		0.19 ±0.01^{b}	4.74 ±0.20^{a}		
7	2-Methylbutyraldehyde	1	Stimulating, coffee, sweet	1,3,6				17.03 ±0.43^{b}	35.14±2.02^{a}
8	Hexanal	5	Fragrant, grassy	5	1.44 ±0.07^{c}		47.33 ±2.74^{a}	37.53±1.89^{b}	
9	4-Methylhexanal	3	Fruity, rose	1,2	0.05 ±0.00^{b}		0.81±0.05^{b}	7.48±0.40^{a}	
10	Octanal	0.7	Rose, orange	1,2,3	0.35 ±0.00^{c}		3.46 ±0.20^{b}	3.25 ±0.15^{b}	5.35 ±0.00^{a}
11	Nonanal	1	Floral, citrus, slightly spicy	1,2,6	11.32 ±0.49^{c}	10.50 ±0.53^{c}	100.00±5.99^{a}	100.00±5.53^{a}	62.79 ±4.03^{b}
12	Decanal	3	Fruity	1	5.03 ±0.03^{b}	1.26±0.08^{d}	2.76±0.10^{a}	1.70±0.11^{c}	

*Intensity: 1-fruity, 2-floral, 3-sweet, 4-fatty, 5-fragrant, 6-stimulating

3.3. Determination of the aroma active compounds in different blueberries

Considering that volatile compounds have different thresholds and people have different sensitivities to them, the relative content cannot reflect the true contribution that every volatile compound makes to the whole aroma profile. Therefore, we used ROAVs to detect the contribution of volatile compounds to the whole aroma profile (YI *et al*., 2016). Fourteen aroma active compounds were selected from five blueberry varieties, which are shown in Table 4.

There were four aroma active compounds (ethyl acetate, 1-octene-3-ol, linalool, nonanal) with higher ROAVs in five varieties. Ethyl acetate and nonanal possessed the highest ROAVs in wild blueberries and cultivated blueberries, respectively. CB-Y had the highest ROAV summations, which was significantly higher than the other four varieties.

Aldehydes were the most abundant chemical group, with aromatic activity found in five blueberry varieties. 2-Methylbutyraldehyde, hexanal, 4-methylhexanal, 1-octanal, nonanal and decanal contributed to stimulating, fragrant, fruity, rose, floral and fruity aroma notes, respectively. 2-Methylbutyraldehyde was observed only in CP-G. 2-Methylbutyraldehyde has stimulating, coffee, and sweet aroma notes, with a very low threshold (1 μg/L) in CP-G. Aldehydes made a major contribution to blueberry aromas, which is in agreement with previous results (Du and ROUSEFF, 2014; HORVAT and SENTER, 1985).

Alcohols were the next most abundant group, including 1-octene-3-ol, linalool, and geraniol, contributing mushroom, lemon and rose aroma notes. 1-Octene-3-ol and linalool were identified in the five blueberry varieties.

Three esters, including methyl acetate, ethyl acetate and butyl acetate, were aroma active. Ethyl formate had a high threshold value (150 μg/L) and a high relative content. However, its ROAVs were low (0.32-1.53). Ethyl acetate contributed a fruity aroma to the five varieties and possessed the highest ROAV in wild blueberries. Butyl acetate contributed sweet and banana aroma notes. However, it has not been previously reported as contributing to blueberry aroma.

Although wild blueberries had higher contents of volatile compounds, their characteristic aroma notes were less than those of cultivated blueberries. The reason may be that the aroma of fruit is not completely dependent on the concentration of the volatile compound but it is closely related to its threshold. The threshold of volatile compounds found differed greatly among the varieties studied. For example, the relative contents of ethyl formate in the two wild blueberries were higher than those in cultivated blueberries, but the ROAVs were lower because the threshold value of methyl acetate was high (150 μg/L).

Six descriptors (fruity, floral, sweet, fatty, fragrant, and stimulating) were used to provide an assessment of the five blueberries. To reflect the difference in aroma among different blueberry varieties, the ROAV of each blueberry aroma component was taken as the logarithm base 10, and the aromatic series of the five blueberry juices on the spider web diagram are shown in Fig. 4.

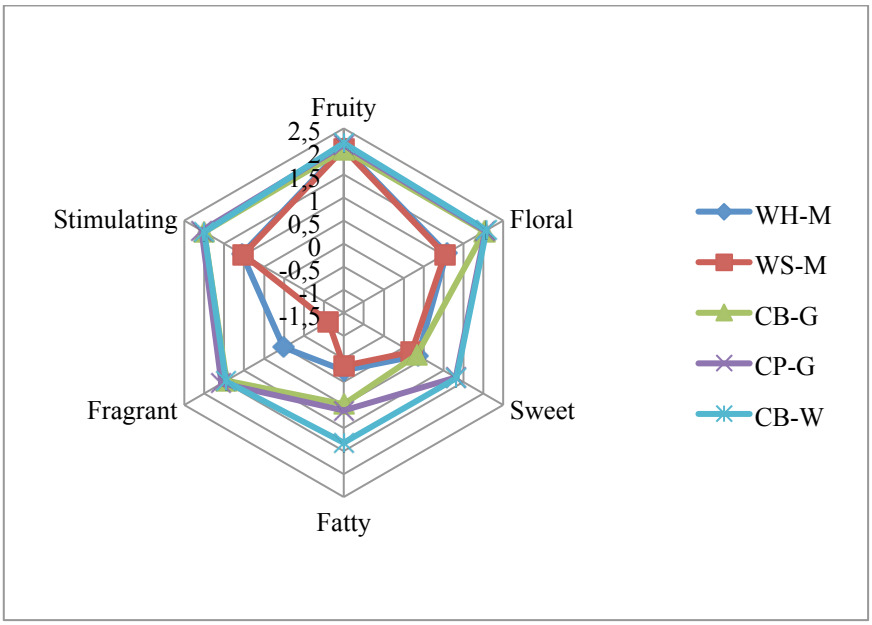

Figure 4. Aromatic series in blueberries based on aroma activity values.

The analysis showed that WH-M and WS-M could mostly be described as having fruity and floral notes due to the higher ROAVs of ethyl acetate and nonanal in the samples. CB-G and CP-G had higher values for the attributes fragrant and floral due to their large quantities of hexanal and nonanal. The difference between CB-G and CP-G lies in the fact that CB-G exhibited a greater sweet component. The ROAV of 1-octene-3-ol was higher in CB-Y; thus, CB-Y was perceived to have a fatty aroma. Considering the volatile composition of these blueberries, samples had higher values for the attributes fruity and fragrant due to their large quantities of aldehydes and alcohols.

4. CONCLUSIONS AND FUTURE WORK

The P&T extraction method coupled to GC-MS analysis was a quick and efficient method for the evaluation of blueberry volatiles, and the results demonstrated that 3 mL of sample volume purged at 25°C for 11 min were the best extraction conditions. A total of 80 volatiles were identified in five blueberry varieties using the P&T-GC-MS technique. The volatiles of blueberries were composed of mainly aldehydes, alcohols, esters, and terpenes. Among the identified compounds, 12 compounds (ROAV>1), including ethyl formate, ethyl acetate, butyl acetate, 1-octene-3-ol, linalool, geraniol, 2-methylbutyraldehyde, hexanal, 4-methylhexanal, octanal, nonanal and decanal, were considered aroma active. The spider web diagram showed that the sensory characterization of the five varieties was distinct due to the different quantities of volatile compounds.

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