Received: 11 October 2019 | Revised: 19 March 2020 | Accepted: 24 March 2020
DOI: 10.1002/ffj.3583

RESEARCH ARTICLE

Volatile composition of eight blueberry cultivars and their relationship with sensory attributes

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Funding information
National Key Research and Development Plan of China, Grant/Award Number: 2017YFD0400101; National Natural Science Foundation of China, Grant/Award Number: 31701561; Fundamental Research Funds for the Central Universities, Grant/Award Number: 2662017JC014

Abstract

The volatile compositions of eight blueberry cultivars ('Premier', 'Gardenblue', 'Legacy', 'Brigitta', 'Misty', 'O'Neal', 'Bluerain' and 'Northland') grown in the middle region of China were investigated. Volatiles were extracted by headspace solid-phase microextraction (HS-SPME) and analysed by gas chromatography—quadrupole time of flight—mass spectrometry (SPME-GC-QTOF-MS). A total of 28 volatiles were identified and quantified, including 5 esters, 11 terpenoids, 3 aldehydes, 6 alcohols and 3 volatile phenols. Different blueberry cultivars had distinct varietal volatile profiles. Rabbiteye cultivars, 'Premier' and 'Gardenblue', were characterized by a large amount of esters, especially for ethyl acetate. 'Misty' had the highest terpenoid content. Principal component analysis (PCA) and partial least squares regression (PLS) were selected to correlate the chemical data with sensory perceptions. PCA showed that esters were dominant in rabbiteye blueberries, especially for ethyl acetate. No distinct pattern of volatile profile was found for the highbush and half-highbush blueberry cultivars. PLS showed that the grassy descriptor was positively correlated with linalool and hexanal. The minty descriptor was positively correlated with eucalyptol.

KEYWORDS
blueberry volatiles, half-highbush, northern highbush, rabbiteye, southern highbush

1 | INTRODUCTION

Flavour is one of the most important aspects to determine the fruit quality and its market value. Although taste and aroma are well integrated in their contributions to the overall flavour, aroma is often considered playing a dominant role in the fruit flavour quality.[1,2] For many years, most efforts have been primarily devoted to improve and maintain the external quality of blueberries, such as yield, fruit size, colour and shelf life.[3,4] As consumers' demand for flavour is growing, more recent studies are focusing on sensory attributes such as colour, texture and taste,[5] but still with little attention to the aroma. In the blueberry industry, fruit selection for a subjective goal such as aroma is challenging since aromas are not easy to quantify.[6] The most commonly used method is sensory evaluation.[6,7] However, this method is expensive and time-consuming. Great effort is required to minimize the errors in measurement and errors in conclusions and decisions. Instrumental analysis of the volatile compounds is another choice of aroma evaluation, which is fast, reliable and relatively lower cost. However, because of missing compounds or distorted quantitative values, it is often difficult to reconstruct high-quality facsimile flavours from these data.[8] To get desired aroma attributes through chemical analysis, it is necessary to find certain chemical compound(s) that correspond to the sensory data, which is not easy due to the complexity of human odour perception and diversity of the volatile compounds.[9]

The aroma of fresh blueberries is dependent on many factors. The large genetic variability in the nature of blueberry aroma results in differences in flavour among cultivars.[10,11] It is reported that linalool and E-2-hexenal were common major aroma impact volatiles, but dominant aroma-active volatiles were different for each cultivar.[10,12] Farneti et al analysed the volatile composition of eleven different blueberry cultivars and found that for the most cultivar, aldehydes, alcohols, terpenoids and esters can be used as putative biomarkers to evaluate the blueberry aroma variations.[4] Other reports also showed that terpenes such as linalool and α-terpineol were important varietal compounds in blueberries which could affect the aroma of the corresponding products such as blueberry wines.[13,14] Although some research papers have been published on sensory characteristics of blueberries,[15,16] very little information is available regarding the relationship between sensory attributes and their volatile composition. It is reported that total aromatic volatile concentrations were not correlated with sensory scores for flavour, overall eating quality or to any other sensory characteristic of blueberries,[17] indicating large number of compounds detected by instrument is not really contribute to the sensory quality of blueberries.

China is Asia's fastest growing blueberry market, and China's own blueberry production significantly increased in recent years.[18] However, little data are available regarding the flavour quality and cultivar differences of blueberries in this region. Based on the above considerations, the specific objective of this study was to investigate the volatile composition of eight blueberry cultivars: two rabbiteye blueberry (*Vaccinium ashei*) 'Premier' and 'Gardenblue', two northern highbush blueberry (*Vaccinium corymbosum L.*) 'Legacy' and 'Briggita', three southern highbush blueberry (interspecific hybrids of *Vaccinium virgatum*, *V corymbosum*, and *Vaccinium darrowii*) 'Misty', 'O'Neal', 'Bluerain' and one half-highbush blueberry (interspecific hybrids of *V corymbosum* and *Vaccinium angustifolium*) 'Northland', grown in middle Yangtze region in China. Multivariate statistical techniques have been used to elucidate the relationships between sensory and instrumental data for blueberries. More knowledge of the blueberry volatile and sensory profile can help growers estimate the blueberry aroma quality at harvest, as well as to maintain a sustainable production of high-quality blueberries.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

All chemicals were of analytical reagent grade unless otherwise stated, and water was obtained from a Milli-Q purification system. Folin-Ciocalteu reagent, sodium carbonate, sodium acetate, potassium chloride, sodium chloride, methanol (HPLC grade) and gallic acid were purchased from SCR ® (Shanghai, China). Ethyl acetate (HPLC grade), hexanal (98%), E-2-hexanal (≥95%), eucalyptol (99%), linalool (≥95%), linalool oxide (mixture of isomers, ≥97.0%), (-)-myrtenol (95%), carveol (97%, mixture of isomers), borneol (≥99.0%, sum of enantiomers, GC), β-citronellol (95%), ethyl-2-methylbutyrate (99%), Z-3-hexenol (98%), E-2-hexenol (96%), benzyl alcohol (≥99%), phenylethyl alcohol (≥99%), methyl butanoate (99%), methyl salicylate (≥99%), benzaldehyde (≥99%), E-asarone (98%), Z-asarone (70%), eugenol (99%), methyl isoeugenol (≥98%), isoeugenol (98%, mixture of cis and trans) were obtained from Sigma-Aldrich (St. Louis, MO). All volatile standards were prepared by dilution with HPLC grade methanol.

2.2 | Blueberry samples

Blueberries 'O'Neal', 'Misty' and 'Brigitta' were hand-harvested from a local blueberry farm in Huangpi, Hubei, China (N31°06′, E114°28′). Harvest date was 3 June 2017. Blueberries 'Premier', 'Legacy', 'Northland', 'Bluerain', 'Gardenblue' were hand-harvested from the same field but the harvest date was 2 June 2017. All fruits were harvested at commercial maturity, as determined by complete blue skin colour. After harvest, the fruits were cooled in an air-conditioned room (25°C) to remove the field heat and transported to the laboratory. Fruits were sorted for the absence of surface defects and uniform blue coloration before further analysis.

2.3 | Basic parameter measurements of blueberries

Berry weight and water content were measured on the harvest date. Berry weight was determined using the average weight of 100 random berries. Water content of blueberry sample was measured using an oven drying method at 100°C for 24 hours. Approximately 100 g of berries were randomly selected and placed in a zip-lock bag. The berries were pressed manually to collect the juice. Total soluble solid (TSS) was measured at room temperature using a PAL-1 pocket refractometer (Atago USA, Inc, Bellevue, WA). The pH of the juice was measured using a pH meter. The rest of the blueberry samples were kept at −20°C before the following analysis.

2.4 | Analysis of total monomeric anthocyanins and total phenolics

Approximately 30 g of frozen blueberry sample was blended with liquid nitrogen, and 0.5 g of powder was taken into a 10 mL centrifuge tube, and 9 mL of ethanol (contain 0.1% HCL, v/v) was added. The centrifuge tubes were sonicated for 1 hour and centrifuged (12 000 rpm, 30 minutes, 4°C). The supernate was collected for total monomeric anthocyanin (TMA) and total phenolic content (TPC) analysis. Each sample was extracted in triplicates. TPC was determined using the Folin-Ciocalteu colorimetric method.[19] The spectrophotometric method based upon pH-induced changes in absorbance was used to assay TMA.[20]

2.5 | Analysis of volatile compounds

Approximately 30 g of frozen berries was blended with liquid nitrogen. Ten g of blended sample was weighed into a 30 mL centrifuge tube and 18 g of NaCl was added. The mixture was shaken at 4°C for 24 hours and centrifuged (12,000 rpm, 30 minutes, 4°C), and the clear juice was used for volatile analysis. Two mL of juice was diluted with 8 mL of saturated saltwater in a 20 mL vial with a small magnetic stir bar. An aliquot of 10 μL internal standard (50 mg/L 4-octanol in methanol) was added. Sample vials were equilibrated at 50°C in a water bath for 15 minutes with stirring. After equilibration, headspace volatiles were collected on a 2 cm SPME fibre coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm film thickness, Supelco, Bellefonte, PA) for 45 minutes at 50°C. After extraction, volatile desorption was performed by introducing the SPME fibre into a GC injection port for 5 minutes; injection split ratio was 1:10. The extraction and desorption were conducted manually.

A 7200 accurate-mass GC-QTOF-MS (Agilent Technologies, Santa Clara, USA) was used for volatile analysis in this study. The GC separation was performed using a fused silica HP-5MS (5% phenyl methyl siloxane, 30 m × 250 μm × 0.25 μm) column. The GC oven temperature was programmed starting at 40°C for 5 minutes, and increased to 180°C at 3°C/min and held for 1 minute, then increased to 250°C at 20°C/min and held for 2 minutes. Ultra-pure grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The interface and ion source temperatures were set to 300 and 250°C, respectively. Electron ionization mass spectrometric data from *m/z* 25 to 300 were collected, with an ionization voltage of 70 eV. Mass calibration was performed daily. The GC-QTOF-MS data processing was performed with MassHunter B.06.00 software (Agilent Technologies). Compound identifications were made by comparing mass spectral data samples with the Wiley 275.L (G1035) database and confirmed by authentic pure standards and standard retention indices (RIs). Figure S1 showed a representative chromatograph and peak identification.

Seven-point calibration plots were constructed using peak areas obtained by adding known amounts of standards to 10 mL of saturated saltwater. Ten microlitres of internal standard were also added to each calibration mixture at the same final concentrations as in the sample. After mixing and equilibration, the volatiles were extracted with SPME and analysed with GC-QTOF-MS under the same conditions as for sample analysis. Calibration plots for each volatile were constructed and were used to calculate the concentrations of volatiles in the samples (Table S1). Triplicate analyses were performed for each sample and standards.

2.6 | OAV

The specific contribution of each odorant to the overall wine aroma was determined by calculating the odour activity value (OAV) as the ratio of the concentration of each compound to its detection threshold concentration.

2.7 | Sensory evaluation

Sensory evaluation was conducted on the frozen blueberry samples. To eliminate the appearance and texture differences, the blueberries were blended into a puree, divided into 30 mL-cups, and bring to room temperature before serving. The descriptive terminology and the sensory profile of blueberry were developed by using Quantitative descriptive analysis (QDA). A six-member trained sensory panel (4 females and 2 males), aged between 21 and 31 years, recruited from the Huazhong Agricultural University was involved. The panellists were asked to take the sample by a spoon and evaluate the aroma of the sample through retronasal perception. The panellists were trained to recognize different intensities of five sensory attributes (grassy, fruity, floral, minty and spicy) obtained from the bibliography, and the use of the 0 to 10 line-scale (very weak to very strong). References (blended puree of multiple cultivars) are used in training to calibrate panellist perceptions. The experiment consisted of 3 repetitions. Each panellist evaluated a total of 24 samples. All the samples were completely randomized and coded. Panellists were asked to evaluate 6 samples at each session and to have a 1 minute rest between each sample. The evaluation was performed in individual booths. Before sensory evaluation, all participants signed an informed consent form.

2.8 | Statistical analysis

The compound concentration differences between cultivars were determined using one-way ANOVA, using SPSS 22.0 software. Means were separated using Tukey's Honest Significant Difference (HSD) test for multiple comparisons, with $\alpha = 0.05$. For sensory evaluation, two-way ANOVA was conducted with cultivar and panellist as variables. Mean scores of the sensory evaluation for each attribute were also separated using Tukey's HSD test. A principal component analysis (PCA) was performed using an online software MetaboAnalyst (https://www.metaboanalyst.ca/home.xhtml).[21] Partial Least Squares (PLS) analysis was performed using the statistical package Unscrambler v. 9.7 (CAMO Software, Oslo, Norway). In the PLS analysis, the X variables included the mean concentration of chemical data and were the indicator variables; and our Y variables were the descriptive panel aroma attributes. The predictive ability of the model for individual sensory attributes and the overall sensory profile was assessed with PLS 1 and PLS 2 models.

3 | RESULTS AND DISCUSSION

The eight blueberry cultivars that belong to four different types showed distinct characteristics in their appearances and chemical compositions. Table 1 shows the basic berry parameters such as berry weight, water content, pH, total soluble solids (TSS), total monomeric anthocyanins (TMA) and total phenolic contents (TPC). The TMA content of different blueberry cultivars ranged from 453 ~ 2690 mg/kg (as cyanidin-3-glucoside equivalent). The TPC ranged from 1617 ~ 4710 mg/kg (as gallic acid equivalent). The rabbiteye blueberry cultivars 'Premier' and 'Gardenblue' have relatively lower water content, higher TSS, and higher TMA and TPC compare to other cultivars. The northern highbush blueberry 'Brigitta' is relatively large (highest berry weight) but has the lowest pH value, and lowest TMA and TPC, since berry size accounted for a significant proportion of variation in TPC and TMA, as the surface area/volume

TABLE 1 Basic parameters, total monomeric anthocyanins and phenolics in eight blueberry cultivars

Cultivar	Type	Berry weight (g/berry)	Water content (%)	pH	Total soluble solids (Brix)	Total monomeric anthocyanin (mg/kg cyanidin-3-glucoside equivalent)	Total phenolics (mg/kg gallic acid equivalent)
Premier	Rabbiteye	1.68ab	82.7b	3.20a	12.8a	2073ab	4027ab
Gardenblue	Rabbiteye	1.20b	82.3b	3.00b	12.6a	2690a	4710a
Legacy	Northern highbush	1.53ab	85.2a	3.06b	11.0b	1473bc	3368bc
Brigitta	Northern highbush	2.14a	86.2a	2.76c	11.6b	453d	1617d
Misty	Southern highbush	1.30b	86.8a	3.25a	11.2b	970cd	3520abc
O'Neal	Southern highbush	1.40ab	86.2a	3.33a	11.4b	933cd	2453cd
Bluerain	Southern highbush	0.68c	86.6a	3.27a	11.4b	1623bc	3938ab
Northland	Half-highbush	0.78c	86.5a	2.97bc	9.9c	1593bc	3466abc

Note: Means (n = 3) followed by the different letter within a column differ at 95% confidence (Tukey's HSD test).

ratio decreases with increasing size.[22] The southern highbush blueberry 'Bluerain' and half-highbush blueberry 'Northland' are very small berries. 'Northland' also has the lowest TSS. Overall, rabbiteye blueberry has a significantly higher TMA than the highbush and half-highbush cultivars, which was consistent with previous reports.[23]

3.1 | Volatile composition of eight blueberry cultivars

Blueberry cultivars vary both quantitatively and qualitatively in the volatiles they produce. A total of 28 volatiles was identified and quantified via GC-QTOF-MS using authentic internal and external standards. The quantified volatiles were grouped into six chemical groups (Figure 1), including 5 esters, 11 terpenoids, 3 aldehydes, 6 alcohols, and 3 volatile phenols (Table 2). In general, the amount and presence/absence of aldehydes, esters, and terpenoids were the main differentiating volatile factors between cultivars (Figure 1).[10] Table 2 lists odour thresholds of each compound from the literature as an indication of possible contribution of the compound to the blueberry aroma.[24]

Concentrations of total esters in the eight blueberry cultivars ranged from 104 to 2992 μg/kg, which accounted for 3.0%~66.2% of total volatiles. Esters were most predominant in rabbiteye blueberry cultivars 'Premier' and 'Gardenblue' (Figure 1). The total ester concentrations in 'Premier' and 'Gardenblue' were much higher compared with other cultivars mainly due to the high content of ethyl acetate. This was consistent with a previous report that ethyl acetate was one of the major volatile components in rabbiteye blueberries.[25,26] Ethyl acetate, ethyl 2-methylbutanoate and methyl isovalerate were at concentrations well above their reported thresholds in all or some of the cultivars, indicating their fruity aroma contribution to these blueberries. In contrast, methyl butanoate and methyl salicylate were at concentrations below their reported

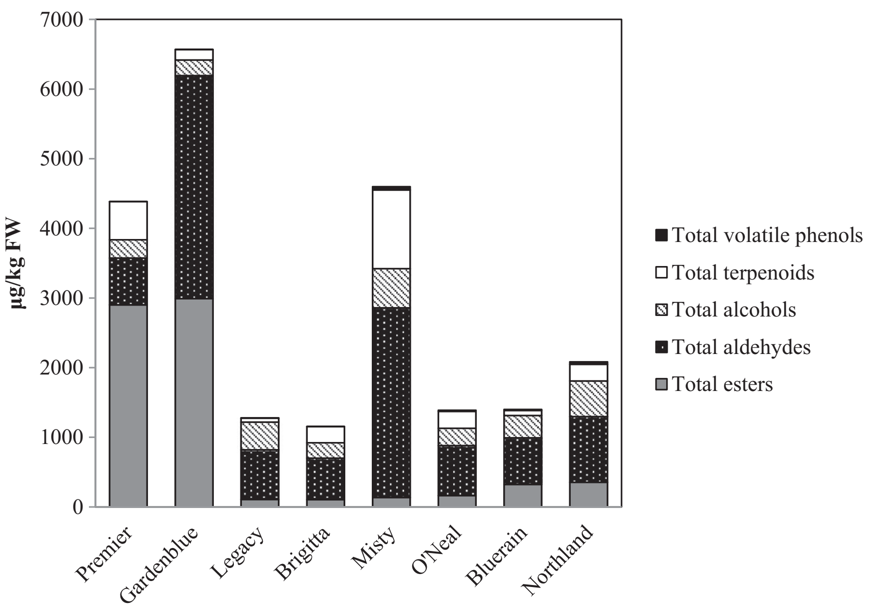


FIGURE 1 Volatile content of eight blueberry cultivars

TABLE 2 Concentration (μg/kg FW) of volatile compounds found in eight blueberry cultivars

RT	RI_{NIST}	RI_{cal}	Compound	Premier	Gardenblue	Legacy	Brigitta	Misty	O'Neal	Bluerain	Northland	Threshold[a]
			Esters									
2.18	628	742	ethyl acetate	2885a	2966a	75.0b	83.9b	113b	60.2b	289b	326b	5
3.58	724	768	methyl butanoate	2.62e	6.66d	15.5a	8.57cd	8.30cd	11.1bc	8.40cd	13.7ab	59
4.99	765	795	methyl isovalerate	5.57b	2.11b	1.26b	2.34b	1.94b	77.0a	8.59b	0.88b	4.4
7.89	849	851	ethyl 2-methylbutanoate	9.89c	16.5a	15.7a	9.37c	11.0bc	13.7ab	11.3bc	14.9a	0.1
24.89	1198	1189	methyl salicylate	0.37c	0.89bc	1.39bc	0.29c	2.30b	1.06bc	6.67a	-[b]	40
			Total esters	2903	2992	109	104	137	163	324	355	
			Aldehydes									
5.76	801	807	hexanal	130c	1160b	451c	300c	1650a	510c	533c	410c	4.5
7.90	854	852	E-2-hexenal	540c	2040a	255c	296c	1040b	207c	135c	530c	17
13.10	960	953	benzaldehyde	0.84b	1.73b	6.59b	0.32b	33.5a	0.65b	0.80b	1.46b	350
			Total aldehydes	671	3202	713	596	2724	718	669	941	
			Alcohols									
8.12	858	856	Z-3-hexenol	6.63d	21.6cd	54.4b	1.58d	123a	54.0b	32.5bcd	48.9bc	70
8.66	853	865	E-2-hexenol	0.50c	33.1a	14.3bc	12.7bc	37.1a	4.68c	11.4bc	24.3ab	1000
8.75	851	868	hexanol[c]	7.46d	97.0a	20.0d	12.0d	68.4b	24.3d	45.3c	82.4ab	500
16.84	1032	1025	2-ethyl-1-hexanol[c]	10.7c	7.25c	36.0a	4.04c	33.1a	19.0b	23.1b	23.0b	1280
16.98	1039	1029	benzyl alcohol	107abc	39.0d	158ab	120ab	130ab	43.0cd	170a	94.5bcd	2546
20.98	1111	1111	phenylethyl alcohol	131bc	27.1f	116c	71.6de	171b	105cd	39.9ef	241a	564
			Total alcohols	263	225	399	222	563	250	322	514	
			Terpenoids									
16.84	1030	1024	eucalyptol	4.55c	1.64c	5.30c	23.7a	14.0b	2.41c	6.01c	1.30c	1.3
19.81	1070	1068	Z-linalool oxide	65.1a	3.43c	16.2bc	3.32c	6.64c	25.0b	1.62c	17.0bc	100
18.98	1172	1072	E-linalool oxide	56.7a	0.30c	6.33c	3.33c	5.34c	16.4b	3.63c	17.3b	190
20.41	1096	1099	linalool	209b	96.3c	16.1c	106bc	614a	121bc	43.2c	104bc	6
26.70	1233	1125	β-citronellol	0.55b	-	-	-	0.98a	-	-	-	62
23.52	1162	1160	borneol	0.25	-	0.39	-	-	-	-	0.20	-
24.76	1186	1187	α-terpineol	211b	47.8cd	10.9d	94.4c	463a	54.5cd	16.5d	93.7c	330
25.02	1194	1191	myrtenol	0.27bcd	0.14d	0.21cd	0.14d	1.22a	0.67b	0.44bcd	0.60bc	7
26.19	1217	1216	carveol	0.38b	0.20b	0.13b	0.64b	25.8a	1.75b	0.17b	4.35b	250
43.20	1561	1556	β-asarone	-	-	-	-	-	19.6	-	-	-
45.27	1646	1649	α-asarone	-	-	-	-	-	0.28	-	-	-

(Continues)

TABLE 2 (Continued)

RT	RI_{NIST}	RI_{cal}	Compound	Premier	Gardenblue	Legacy	Brigitta	Misty	O'Neal	Bluerain	Northland	Threshold[a]
			Total terpenoids	548	150	55.6	232	1131	242	71.6	239	
			Volatile phenols									
38.34	1492	1495	methyl isoeugenol	0.14	-	-	0.08	0.08	0.40	0.07	-	1600
36.25	1438	1438	isoeugenol	0.62cd	0.25d	0.62cd	1.80b	1.32b	3.04a	0.38d	1.24bc	100
32.40	1355	1354	eugenol	0.43d	0.60d	4.82d	1.65d	45.7a	13.8c	14.4c	34.0b	6
			Total volatile phenols	1.2	0.9	5.4	3.5	47.1	17.2	14.8	35.2	

Note: RI_{cal}, retention indices calculated from retention time; RI_{NIST}, retention indices from NIST library; RT, retention time (min).

Means (n = 3) followed by the different letter within a line differ at 95% confidence (Tukey's HSD test). No letter means the difference was not significant between samples.

[a]Threshold values in water from ref. 24 are present as an indication of possible odour contribution.

[b]-, no peak detected.

[c]Concentration was estimated by 4-octanol.

thresholds, indicating that they provide little to no aroma in these cultivars.

Total aldehyde content ranged from 596 to 3202 μg/kg (Table 2) in eight cultivars, which accounted for 15.3%~59.2% of total volatiles. The dominant aldehydes in the blueberries were C6 aldehydes such as hexanal and E-2-hexanal, which were found at concentrations well above their reported thresholds, suggesting a major contribution to the blueberry flavour. Hexanal and E-2-hexenal have characteristic green/grassy odours.[12] These C6 aldehydes are generally considered as lipoxygenase (LOX)-derived compounds, which were generated from fatty acid oxidation during sample maceration, as well as formed in the mouth during chewing.[27]

Alcohols are normally found in fruits. The total alcohol concentration in blueberries ranged from 222 to 563 μg/kg among the eight cultivars, but none of the alcohols were above their reported thresholds (Table 2).

Terpenoids are important aroma compounds in blueberries that contribute to the floral characteristic.[12,28] Terpenoids were most abundant in 'Misty', followed by 'Premier' (Table 2). Eucalyptol, Z-linalool oxide, E-linalool oxide, linalool, α-terpineol, myrtenol and carveol were present in all cultivars, but β-citronellol was only detected in 'Premier' and 'Misty'. Borneol was only detected in 'Premier' and 'Legacy'. Among the 9 terpenoids detected in the blueberry samples, only eucalyptol and linalool were at concentrations above their sensory thresholds. Linalool has a pleasant floral aroma[29] and the aroma of eucalyptol is described as 'eucalyptus', 'fresh', 'cool', 'medicinal' and 'camphoraceous'.[30] α-Terpineol was only present in 'Misty' at concentration above its threshold. Other terpenes were all at concentrations below their thresholds in all cultivars. Interestingly, we found that 'O'Neal' blueberry contained two sesquiterpenes E-asarone and Z-asarone, which are firstly reported in blueberries. Our results were consistent with the previous finding that E- and Z-asarone were detected in O'Neal blueberry wines.[13,14] Although E- and Z-asarone are often reported in plants, especially in essential oils,[31] due to the low volatility, they may not contribute to the overall aroma of the blueberries.

There were 3 volatile phenols among the identified odorants in the eight blueberry cultivars (Table 2). However, only eugenol was at concentration above its reported threshold in 'Misty', 'O'Neal', 'Bluerain' and 'Northland', which could contribute to the clove-like aroma in blueberry.[28]

3.2 | Sensory evaluation of blueberries

Although blueberry aroma is subtle compared with many other fruits, the sensory panels still perceived a lot of different aromas in the blueberry samples. To simplify the results, we carefully combined these aromas into five descriptors: 'grassy' refers to the aroma of green, grassy and leafy like aromas; 'fruity' refers to the aroma of apple, berry, red fruit and dark fruits; 'floral' refers to the aroma of flower-like, citrus and perfume-like aroma; 'minty' refers to the minty and cooling sensations; and 'spicy' refers to the clove-like, balsamic and other spices-like aromas. Regarding the sensory evaluation results, a two-way ANOVA has been carried out considering factors such as the cultivar and the panellist (Figure 2). The panellist factor had no significant effect on the 'grassy' and 'floral' intensity ($P > .05$). However, there was a significant difference between panellists for 'fruity', 'minty' and 'spicy' attributes, indicating the panellists had different individual perceptions for these descriptors. Nevertheless, significant differences were still detected between the eight cultivars. Overall, the 'fruity' and 'floral' were rated higher for all samples, which were not surprising, but cultivar differences were also very obvious. 'Misty' was rated with the highest 'grassy' scores, and also relatively high in 'fruity' and 'floral'. 'O'Neal' had the highest 'fruity' notes, 'Brigitta' had the highest 'minty' notes, 'Premier' had the highest 'floral' notes and 'Northland' had the highest 'spicy' notes.

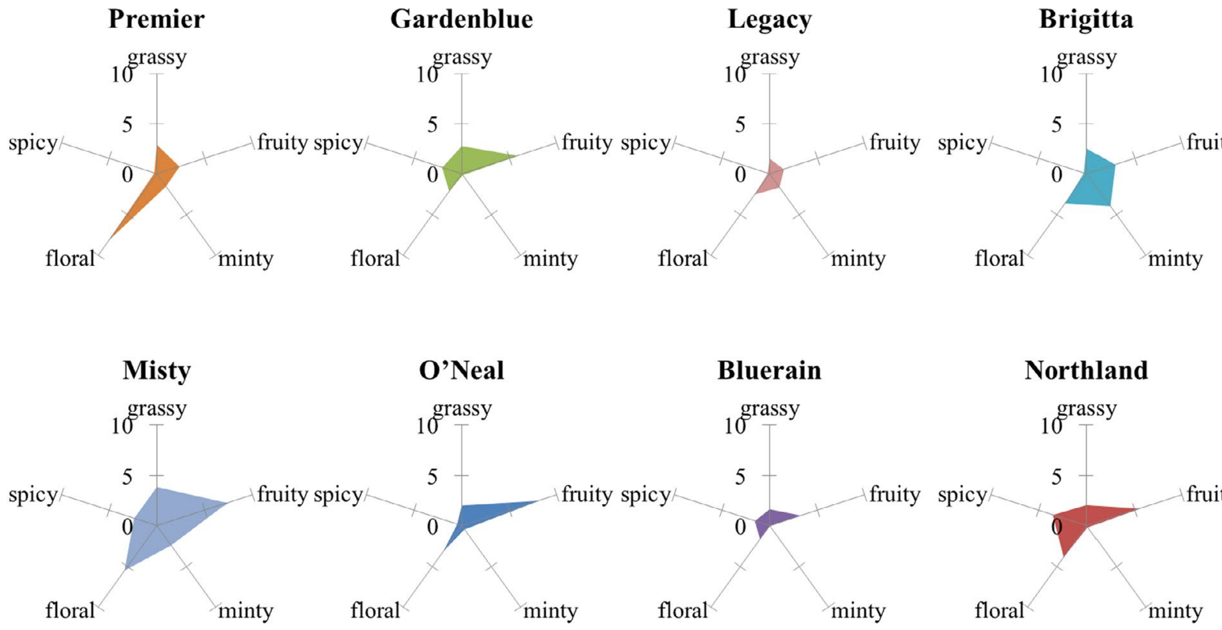
3.3 | Principal component analysis (PCA) of sensory descriptors and volatile compounds

PCA was used to identify the specific volatile compounds and descriptors best discriminating among the eight blueberry cultivars (Figure 3). When all 28 of the volatile compounds detected by SPME-QTOF-GC-MS were included, the first principal component (PC1) explained most of the total variation (83.2%), and PC2 explained 11.9% of the total variation (Figure 3A). Figure 3B is the corresponding loading plot used to establish the relative importance of each volatile component in order to relate volatile compounds to one another and with samples. The two rabbiteye blueberries 'Premier' and 'Gardenblue' were separated from other cultivars on PC1, indicating they had different volatile profiles compared with other cultivars. 'Premier' and 'Gardenblue' mainly contained high relative correlations with ethyl acetate.

The results for the 5 odour descriptors used in the sensory analysis were analysed in a second PCA. Figure 4 shows the relationships between sensory aroma characters and the blueberry samples. The first two principal components, PC1 and PC2, accounted for 79.9% of the total variance (45.8% and 34.1% respectively). In this way, blueberry 'Premier' that clustered at negative PC1 and positive PC2 scores contained high relative correlations mainly of floral. 'O'Neal', 'Misty', 'Northland' and 'Gardenblue' that clustered at positive PC1 scores contained high relative correlations mainly of fruity, grassy and spicy nuances.

3.4 | Partial least squares (PLS) regression analysis between volatile components and sensory descriptors

The relationship between sensory variables and chemical analysis (volatile compounds, TSS, TMA, TPC, pH, water content and berry



	O'Neal	Northland	Gardenblue	Bluerain	Brigitta	Premier	Misty	Legacy	Panelist *P*
grassy	2.03d	2.08d	2.79bc	1.67e	2.57c	2.94b	3.89a	1.55e	ns
fruity	8.14a	5.56c	5.91c	3.33d	2.99d	2.38e	7.42b	1.51f	0.013
minty	0.44d	0.18de	0.15e	0.03e	3.98a	1.54c	2.41b	1.66d	<0.001
floral	3.13d	3.84c	2.14e	1.63f	3.61cd	8.24a	5.51b	2.53e	ns
spicy	0.49d	3.52a	2.07b	1.56c	0.26d	0.27d	2.39b	0.21d	<0.001

FIGURE 2 Spider chart showing the intensity scores of each attributes of eight blueberry cultivars. The table blow showing the results of two-way ANOVA, mean intensity scores followed by the different letter within a line differ at 95% confidence (Tukey's HSD test). Panellist *P* value showing the statistical difference between panellists

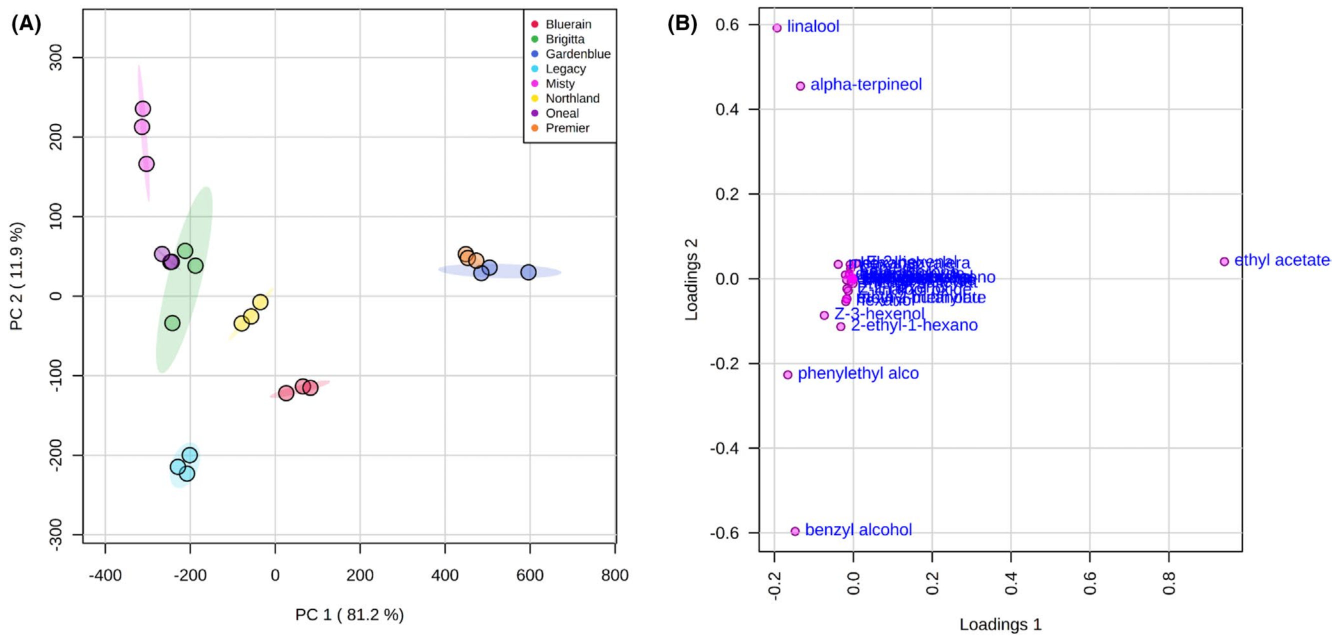


FIGURE 3 Two-dimensional PCA: scores plot for eight different blueberry cultivars (A) and loading plot for 28 volatile compounds detected by GC (B)

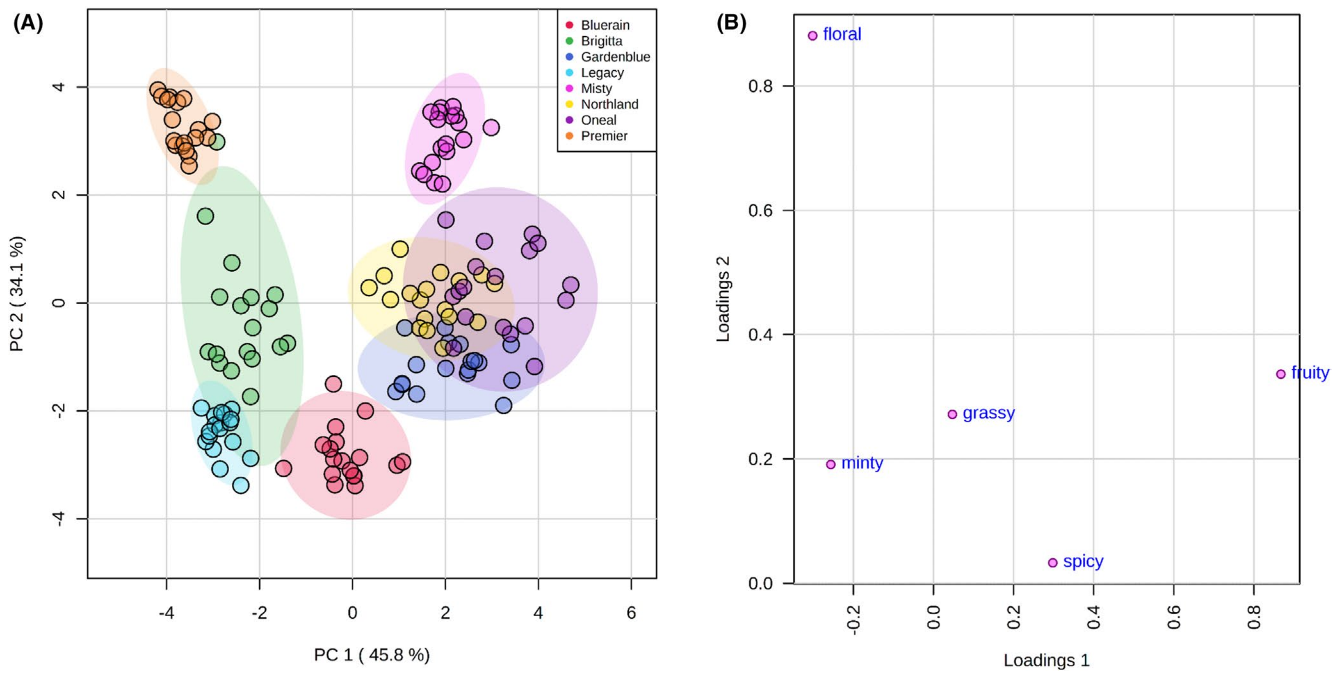


FIGURE 4 Two-dimensional PCA: score plot for eight different blueberry cultivars (A) and loading plot for the 5 odour descriptors (B)

weight) was established by PLS regression, a multivariate technique widely used to relate sensory and chemical data sets.[32-34] A PLS2 was initially used to correlate chemical data (volatiles with OAVs > 1, TSS, TMA, TPC, pH, water content and berry weight) and each matrix of sensory data. Then, PLS1 was used to model relationships between volatile compounds with OAVs > 1 and individual sensory attribute data. It also has to be mentioned that odour thresholds provide only a general indication and do not reflect the actual contribution of the compound to the aroma because it does not take into account interactions among volatiles and between volatiles and fruit matrix.[35] Nevertheless, pre-selection of the compounds according to the reported threshold could largely exclude those compounds with high concentration but no contribution to the overall aroma.

Partial least squares 2 modelling between the matrices of chemical data and aroma descriptors provided a two-factor model explaining 48% of the variance in X (chemical data) and 61% of that in Y (sensory descriptors) (Figure 5). The ensuring model was evaluated via the root mean square error for predictions (RMSEP), which was calculated to be lower than 10 for sensory descriptors. The central ellipsoid in Figure 4 indicates that all compounds inside the circle were poorly modelled and failed to explain variation in the sensory data. Unsurprisingly, TSS, TMA, pH, berry weight and water content

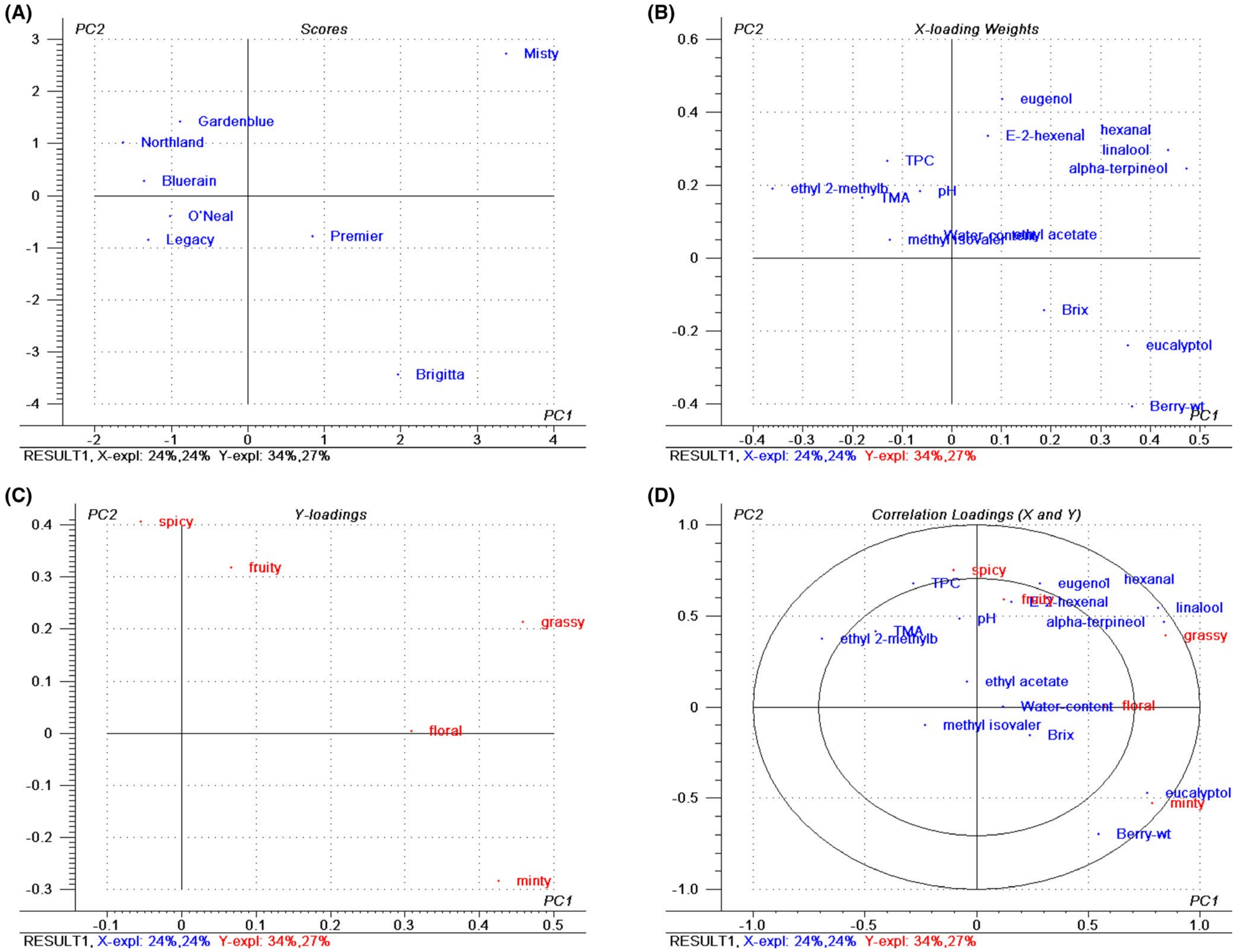


FIGURE 5 Two-dimensional PLS2: scores plot for eight different blueberry cultivars (A), loadings plots of X variables for the 9 volatile components with OAVs > 1 and other chemical data (B) and of Y variables for the 5 odour descriptors (C), together with correlations between the loadings of X and Y variables (D)

TABLE 3 One-dimensional PLS1: loading coefficients for X variables (volatile components with OAV > 1) used to estimate their weight into the Y variables (sensory descriptors)

	grassy	fruity	minty	floral	spicy
y-explained%	95	83	82	85	71
ethyl acetate	0.295	−0.089	−0.101	0.159	−0.011
methyl isovalerate	0.049	0.528	−0.158	−0.055	−0.127
ethyl 2-methylbutanoate	−0.192	0.182	−0.111	−0.234	0.138
Hexanal	0.148	0.175	0.038	0.033	0.177
Eucalyptol	0.146	−0.178	0.665	0.051	−0.118
α-terpineol	**0.324**	0.074	0.144	0.289	0.108
Linalool	**0.293**	0.168	0.093	**0.236**	0.122
Eugenol	−0.010	0.303	−0.335	0.051	0.302
E-2-hexenal	0.179	0.231	−0.012	−0.072	0.159

Note: Significant results are presents in bold.

had little relationship with the odour perception. But it was interesting that TPC located closely with spicy odour in the plot, indicating they had a positive correlation. Positive correlations (r > .700) of the grassy descriptor with linalool and hexanal were found. Similarly, the minty descriptor was positively correlated with eucalyptol. Negative correlations (r > .700) of ethyl 2-methylbutanoate with the grassy and minty descriptors were observed.

Additional loading coefficients for the volatiles with OAVs > 1 were estimated for odour descriptors of the blueberry by applying PLS1 to a single Y variable at time (Table 3). Results showed that floral were explained mainly by linalool. Grassy was mainly explained by positive contributions of linalool and α-terpineol. While other sensory attributes could not be well predicted by the model, which could be due to multiple reasons. Firstly, using linear relationships might lead to a problem that sensory function and chemical composition are sometimes nonlinear.[35] In other cases, volatile compounds may have different characteristics depending on the concentration found and the flavour characteristics are actually a combination of several volatile compounds.[36,37] Many volatiles can also interact with each other impacting flavour and aroma, even when individual concentrations are below their respective odour activity thresholds.[38]

4 | CONCLUSIONS

This study investigated the volatile composition of eight blueberry cultivars. Correlations between sets of sensory and chemical data as established with the aid of multivariate statistical procedures were used to improve our current understanding of the aroma of blueberries. The aroma of fresh blueberries is comprised of a complex mixture of volatile components. This study showed that esters were dominant in rabbiteye blueberries, especially for ethyl acetate. No distinct pattern of volatile profile was found for the highbush and half-highbush blueberry cultivars. Eucalyptol has significant impacts on perception of minty. Linalool and hexanal influence grassy aroma. However, volatile composition did not predict the associated sensory attribute intensities of the blueberries well, indicating that the sensory perception is complex and attributes are not perceived in isolation.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Plan of China (Grant number 2017YFD0400101), the National Natural Science Foundation of China (Grant number 31701561) and the Fundamental Research Funds for the Central Universities (Grant number 2662017JC014).

CONFLICT OF INTEREST

The authors claimed no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Cheng K, Peng B, Yuan F. Volatile composition of eight blueberry cultivars and their relationship with sensory attributes. *Flavour Fragr J*. 2020;00:1–11. https://doi.org/10.1002/ffj.3583