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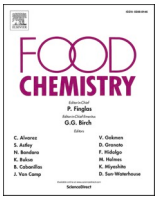

Analytical Methods

Optimization and validation of a SPME-GC/MS method for the determination of volatile compounds, including enantiomeric analysis, in northern highbush blueberries (*Vaccinium corymbosum* L.)

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ABSTRACT

Blueberry aroma is one of the most important quality traits that influences consumer purchasing decisions. This study aimed to optimize and validate a solid-phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS) method for the quantification of 73 volatile compounds in northern highbush blueberries. A SPME extraction of blueberries with water and specific proportions of sodium chloride, citric acid, and ascorbic acid, for 60 min at 50 °C using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber was optimal. The method was validated for sensitivity, reproducibility, linearity, and accuracy, and used to quantify volatile compounds through matrix-matched calibration curves in six blueberry cultivars ('Duke', 'Draper', 'Bluecrop', 'Calypso', 'Elliott', and 'Last Call'). Terpenes represented the most abundant volatile fraction, followed by aldehydes and alcohols. Linalool and 2-(E)-hexenal were key compounds that differentiated blueberry cultivars via Principal Component Analysis (PCA). Enantiomeric analyses revealed an excess of (−)-limonene, (−)-α-pinene, and (+)-linalool for all cultivars with potential impacts on the blueberry aroma.

1. Introduction

The total supply of fresh blueberries has increased five-fold over the past ten years (Statistics Canada, 2020; USDA, 2020). United States of America and Canada are the two world's largest blueberry producers, contributing 37 and 24 % of global blueberry production in 2019, respectively (FAOSTAT, 2020). In fact, blueberry was the fruit with highest farm gate value (*i.e.*, $270,692,000) in Canada in 2019, representing a 19 % of total volume of marketed fruit (AAFC, 2020). Additionally, although blueberries are native to North America, blueberry cultivars are now grown in a wider array of climatic regions across South America, Europe, Asia, and Australia (Gilbert et al., 2014). Consequently, blueberries are a fruit crop of importance in the global fruit market.

Traditionally, most breeding efforts have been focused on improving blueberry traits such as yield, fruit size, colour, shelf life (Cheng, Peng, & Yuan, 2020), and the balance of fruit sugar and acidity (Gilbert, Schwieterman, Colquhoun, Clark, & Olmstead, 2013). However, in a survey conducted by Gilbert et al. (2014) to determine the fruit traits that drive the likelihood of blueberry purchases, increased likelihood was strongly associated with more intense blueberry flavor. Therefore, blueberry cultivars with intense and pleasant flavours could be more successful in the marketplace, as satisfied consumers are more likely to repeat the purchase of those specific cultivars. The volatile profile and content of highbush blueberries (*Vaccinium corymbosum* L.) is known to be highly dependent on the genetic background (Cheng et al., 2020), but it also depends on the environmental conditions where blueberries are grown (Du et al., 2011), the interaction of genetics with environmental conditions (Gilbert et al., 2015), and the conditions applied during post-harvest storage (Yan, Yan, Pan, & Yuan, 2020). Thus, the volatile profile of highbush blueberries is characterized by aldehydes and alcohols, such as 2-(E)-hexenal, hexanal, and 3-(Z)-hexen-1-ol, which are associated to 'fresh green' and 'grassy' aromatic notes. Additionally, terpenes such as linalool, α-terpineol, limonene, and, geraniol are also present in high concentrations (Du et al., 2011; Du & Rouseff, 2014; Farneti et al., 2017; Gilbert et al., 2013). Of note, aldehydes and alcohols coming from lipids oxidation could be artificially generated during sample treatment because of the action of lipoxygenases (LOXs) released during berry grinding (Du & Rouseff, 2014). Likewise, volatiles could also be degraded via oxidations catalyzed by polyphenol oxidases (PPOs) (Du &

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Rouseff, 2014). Specifically, hexanal and 2-(E)-hexenal increase due to the action of LOXs (Pico, Bernal, & Gómez, 2015) while α-terpineol and guaiacol decrease due to the action of PPOs (Mate & Alcalde, 2016; Schroeder, Pöllinger-Zierler, AIchernig, Siegmund, & Guebitz, 2008). Although NaCl, citric acid, ascorbic acid, or NaF have been already employed for mitigating the alteration of the natural volatile profile of blueberries during sample analysis (Beaulieu et al., 2014; Du & Rouseff, 2014; Eichholz et al., 2011; Farneti et al., 2017), a thorough optimization of the proportion of these chemicals added to the blueberry samples has not been reported so far. The proper control for oxidation and artificially high presence of LOX-derived aldehydes and alcohols is crucial to determine the chemical groups that truly dominate the blueberry volatile profile.

While there is documentation of the importance of volatile compound analysis in blueberries (Beaulieu et al., 2016; Cheng et al., 2020; Du et al., 2011; Du & Rouseff, 2014; Eichholz et al., 2011; Farneti et al., 2017; Gilbert et al., 2013; Rohloff et al., 2099; Senter, 1985; Xinyu et al., 2020), a complete validation of analytical methodologies for volatiles quantification is still lacking, even though validation of analytical methods is essential to obtain reliable results (Betz, Brown, & Roman, 2011). One of the most important validation parameters considered for quantification is the "matrix effect" that takes place in the mass spectrometric detector, which leads to the increase (ion enhancement) or decrease (ion suppression) of the analyte signal in blueberry samples compared to the pure solvent of external calibration curves. Consequently, if present, the matrix effect results in an underestimation or overestimation of the analyte concentration in the blueberry sample. Despite its importance, the matrix effect has been commonly disregarded in the blueberry literature. As far as we know, only Du et al. (2011) performed matrix-matched calibration curves to correct for the matrix effect in the volatile quantification in southern highbush blueberries. Meanwhile, Cheng et al. (2020) quantified the volatile compounds of southern highbush, northern highbush, and rabbiteye blueberries using external calibration curves built with standards in saturated saltwater. Although this method corrects for the effects of the salt addition, it disregards the effect of the blueberry matrix on the volatile signal in the mass spectrometric detector. Other common practice to report blueberry volatiles results have been the use of "relative peak areas" (Eichholz et al., 2011; Polashock, Saftner, & Kramer, 2007; Saftner, Polashock, Ehlenfeldt, & Vinyard, 2008; Xinyu et al., 2020) and the "semi-quantification using an internal standard" (Farneti et al., 2017). Nevertheless, in both cases, it is only possible to compare the same compound among samples with similar matrices – as the signal of the compound would be affected by a similar "matrix effect" – while different compounds within the same sample could be affected by different matrix effects and would not be comparable. Therefore, a validated method that allows an accurate quantification of volatile compounds is essential to assessing the effects of the genetic background, environmental conditions, season, and post-harvest storage techniques on northern highbush blueberry volatile profiles and contents.

Finally, considering that different enantiomers from the same volatile compound often have different aroma characteristics and sensory thresholds (Ruiz del Castillo & Dobson, 2002; Werkhoff, Bretschneider, Güntert, Hopp, & Surburg, 1991), the evaluation of enantiomeric volatile profiles would provide critical knowledge about the determinants of blueberry aroma. However, enantiomeric analyses have been commonly neglected in the blueberry aroma literature.

The aim of the current study was to optimize and validate a solid-phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS) methodology for the quantification of a wide range of volatile compounds (including terpenes, alcohols, aldehydes, esters, ketones, and acids) in a range of commercially relevant northern highbush blueberry cultivars. Specifically, the limits of detection (LODs), limits of quantification (LOQs), intra- and inter-day repeatability, linearity, matrix effect, and accuracy (in terms of % relative error in quantification) were assessed using the optimized SPME-GC/MS method. Additionally, the enantiomeric profiles of linalool, limonene, α-terpineol, and α-pinene were examined in the six northern highbush blueberry cultivars. The methodology was applied to the quantification, through matrix-matched calibration curves, of 73 volatile compounds in 'Duke', 'Draper', 'Bluecrop', 'Calypso', 'Elliott' and 'Last Call' blueberry cultivars sampled at the time of commercial harvest. The quantification of volatiles through matrix-matched calibration curves would ensure the correction of the matrix effects. Moreover, this is the first report of the full volatile profiles for 'Calypso' and 'Last Call' in specific.

2. Materials and methods

2.1. Materials, reagents, and standards

Neat standards (≥98 % purity) of 1,4-cineole, eucalyptol, citronellol, nerol, myrcene, α-phellandrene, α-ionone, (+)-α-pinene, (−)-α-pinene, and citral were purchased from Extrasynthese (Genay, France) while the other 63 volatile compounds listed in Table 1 (including the enantiomeric species), methanol (HPLC grade), and ethyl alcohol (ACS grade) were obtained from Sigma Aldrich (Oakville, ON, Canada). The internal standard, ethyl butyrate-d3, was acquired from CDN Isotopes (Pointe-Claire, QC, Canada) and the mixture of C7–C30 *n*-alkanes from Supelco (Sigma Aldrich, Oakville, ON, Canada). Sodium chloride (NaCl), citric acid, and ascorbic acid were purchased from Fisher Scientific (Burnaby, BC, Canada).

2.2. Preparation of standard solutions

Stock solutions of α-phellandrene, β-phellandrene, ocimene, α-pinene, β-pinene, β-caryophyllene, limonene, α-terpinene, terpinolene, and γ-terpinene were prepared in ethanol due to their lower polarity while the rest of the volatile compounds from Table 1 were prepared in methanol. Even though low polarity volatiles would dissolve better in non-polar solvents, methanol and ethanol were selected to be able to spike the blueberry samples (with a high percentage of water) for the validation of the SPME-GC/MS methodology. The stock solution containing the 73 volatile compounds was prepared mixing the individual standards at equal concentrations (*i.e.,* 6.5 mg L^{-1} for volatiles injected in splitless and 65 mg L^{-1} for those injected in split 1:10), making up to the volumetric flask volume with methanol (for a final percentage of 91 % methanol and 9 % ethanol in the mixture). Dilutions of the stock solutions were done with methanol when necessary (*e.g.,* for matrix-matched calibration curves or validation assays).

2.3. Plant material

Six northern highbush blueberry cultivars, 'Duke', 'Draper', 'Bluecrop', 'Calypso', 'Elliott', and 'Last Call', were hand-harvested at commercial maturity from three blueberry farms located in the Fraser Valley, British Columbia, Canada between July and September of 2020 (see Supplementary Material 1). After harvest, blueberries were sorted for the absence of surface defects, uniformity of blue coloration, and similarity of size. Blueberries were stored under commercial storage conditions (0.5 °C) for 12 h prior to being frozen at – 80 °C. Frozen blueberries were ground under liquid nitrogen to maximize preservation of volatile compounds and the resulting blueberry powder was kept at – 80 °C prior to SPME-GC/MS analyses.

A control sample was utilized for the development and validation of the SPME-GC/MS method, which consisted of a mixture of 12 berries from each of six cultivars and three growing locations (216 berries in total). These berries were crushed, ground, and stored, as described above, prior to analysis.

Table 1
Assessment of the optimized SPME-GC/MS analytical method in terms of intra- and inter-day repeatability (RSD %), % matrix effect, limits of detection (LOD), limits of quantification (LOQ), linearity (including the linear range, coefficient of determination R^2, *t*-test of the slope), and accuracy (as the % relative error in the quantification with matrix-matched calibration curves) for six northern highbush blueberry cultivars. Compounds are arranged in increasing order of retention time (showed in Supplementary Material 3).

Volatile Compound	Intra-day Repeatability (% RSD)	Inter-day Repeatability (% RSD)	% Matrix effect	LOD (μg/Kg)	LOQ (μg/Kg)	Linear range (μg/Kg)	R^2	p-value (t slope test)	% Relative Error
Methyl 2-methyl butanoate [E]	10.27	10.86	22.13	0.001	0.004	2.400–152.9	0.9953	0.0000	3.485
α-Pinene [T]	1.832	9.176	−62.96	0.206	0.681	2.400–152.9	0.9973	0.0000	3.907
Ethyl butanoate [E]	1.454	13.52	24.54	0.043	0.143	4.800–159.9	0.9953	0.0000	2.965
1-Propanol [ALC]	10.28	10.46	23.83	0.056	0.186	2.400–152.9	0.9940	0.0002	6.903
Ethyl 2-methyl butanoate [E]	5.572	13.63	26.68	0.006	0.021	2.400–152.9	0.9997	0.0000	0.682
Ethyl isovalerate [E]	0.2702	4.012	22.88	0.003	0.010	2.400–152.9	1.0000	0.0000	1.431
Hexanal [ALD]	4.885	6.704	−47.41	0.3188	1.052	264.7–4235	0.9981	0.0000	2.092
β-Pinene [T]	Nd	Nd	−62.75	0.062	0.206	2.400–152.9	0.9968	0.0000	5.970
2-Methyl-1-propanol [ALC]	5.592	8.540	−81.44	5.841	19.275	264.7–4235	0.9987	0.0000	6.686
α-Phellandrene [T]	2.709	12.80	−49.68	0.002	0.005	2.400–152.9	0.9989	0.0000	4.365
1-Butanol [ALC]	Nd	Nd	26.09	0.164	0.541	19.10–152.9	0.9963	0.0019	8.097
Myrcene [T]	6.480	6.365	−76.29	0.008	0.026	264.7–4235	0.9957	0.0001	5.342
1,4-Cineole [T]	5.465	10.78	20.87	0.006	0.019	2.400–152.9	0.9987	0.0000	4.182
Heptanal [ALD]	2.762	2.024	19.61	0.022	0.073	2.400–152.9	0.9900	0.0050	11.11
2-Heptanone [K]	7.883	4.993	−26.20	0.008	0.025	2.400–152.9	0.9978	0.0011	6.362
Limonene [T]	11.15	9.738	−86.55	0.774	2.555	264.7–4235	0.9972	0.0000	3.596
β-Phellandrene [T]	12.41	10.68	−28.44	0.008	0.026	2.400–152.9	0.9996	0.0000	2.404
Eucalyptol [T]	3.468	9.652	−38.67	0.104	0.342	2.400–152.9	0.9979	0.0000	2.868
2-(E)-Hexenal [ALD]	6.099	6.642	−63.21	0.425	1.403	264.7–4235	0.9981	0.0000	2.790
3-Methyl-1-butanol [ALC]	Nd	Nd	−41.66	6.984	23.047	264.7–4235	0.9987	0.0006	11.77
2-Hexanol [ALC]	1.510	16.53	−21.00	0.115	0.380	264.7–4235	0.9902	0.0045	14.09
γ-Terpinene [T]	9.658	7.555	−67.01	0.001	0.005	2.400–152.9	0.9984	0.0000	1.651
β-Ocimene [T]	10.97	9.825	−69.49	0.001	0.004	9.600–152.9	0.9999	0.0051	4.236
Cymene [T]	8.796	6.820	−75.80	0.0004	0.001	2.400–152.9	0.9980	0.0000	1.598
Terpinolene [T]	10.52	7.751	−87.30	0.122	0.401	264.7–4235	0.9931	0.0035	9.735
Hexyl acetate [E]	11.06	8.769	−12.54	0.001	0.002	2.400–152.9	0.9974	0.0000	1.62
Octanal [ALD]	8.559	7.895	−52.25	0.016	0.054	2.400–152.9	0.9946	0.0465	13.83
6-Methyl-5-hepten-2-one [K]	2.240	8.576	−89.12	0.018	0.058	2.400–152.9	0.9923	0.0000	6.796
2-Heptanol [ALC]	2.073	13.93	17.24	0.005	0.015	2.400–152.9	0.9944	0.0000	6.655
Rose Oxide (isomer 1) [T]	3.439	9.736	−50.12	0.001	0.003	2.400–152.9	0.9957	0.0000	4.975
Rose Oxide (isomer 2) [T]	Nd	Nd	35.54	0.006	0.019	2.400–152.9	0.9918	0.0000	4.699
1-Hexanol [ALC]	2.178	7.018	−83.43	0.561	1.852	264.7–4235	0.9981	0.0000	3.682
3-(E)-Hexen-1-ol [ALC]	2.214	12.30	−13.35	0.163	0.537	2.400–152.9	0.9941	0.0000	7.349
2,4-(E,E)-Hexadienal [ALD]	13.71	12.41	−43.75	0.088	0.291	38.20–152.9	0.9946	0.0465	7.386
2-Nonanone [K]	0.020	4.731	–32.62	0.003	0.011	2.400–152.9	0.9963	0.0000	10.66
3-(Z)-Hexen-1-ol [ALC]	1.814	7.693	−77.97	5.023	16.58	264.7–4235	0.9987	0.0000	5.750
Nonanal [ALD]	10.23	14.13	−47.15	1.095	3.612	2.400–152.9	0.9972	0.0014	9.803
2-(E)-Hexen-1-ol [ALC]	1.459	10.91	2.925	22.68	74.85	2.400–152.9	0.9935	0.0000	6.863
2-(Z)-Hexen-1-ol [ALC]	0.164	15.77	21.77	0.028	0.092	264.7–4235	1.0000	0.0000	2.950
Linalool Oxide (isomer 1) [T]	5.484	7.263	−31.14	0.178	0.586	264.7–4235	0.9991	0.0191	7.493
Furfural [ALD]	0.076	7.985	−43.29	0.409	1.349	2.400–152.9	0.9983	0.0009	1.869
1-Octen-3-ol [ALC]	4.242	4.183	−16.08	0.100	0.331	2.400–152.9	0.9923	0.0000	6.544
1-Heptanol [ALC]	1.575	7.480	−15.33	0.248	0.817	2.400–152.9	0.9984	0.0000	1.489
Linalool Oxide (isomer 2) [T]	6.075	7.431	−29.34	0.523	1.725	264.7–4235	0.9910	0.0045	7.781
Benzaldehyde [ALD]	7.279	5.541	10.45	0.133	0.441	19.10–152.9	0.9947	0.0000	5.740
Decanal [ALD]	Nd	Nd	−74.49	0.059	0.195	2.400–152.9	0.9909	0.0047	11.50
2-(E)-Nonenal [ALD]	14.16	11.20	−53.64	0.006	0.021	2.400–152.9	0.9978	0.0000	4.378
Linalool [T]	3.584	5.489	−28.31	0.161	0.533	264.7–42,350	0.9991	0.0118	6.812
Caryophyllene [T]	8.790	5.822	−80.89	0.012	0.041	9.600–152.9	0.9900	0.0004	8.667
2,6-(E,Z)-Nonadienal [ALD]	0.777	4.096	20.27	0.083	0.272	2.400–152.9	0.9998	0.0000	4.046
1-Octanol [ALC]	5.286	5.070	−52.38	0.126	0.416	2.400–152.9	0.9999	0.0000	2.994
2-Undecanone [K]	2.288	4.088	−82.87	0.003	0.010	2.400–152.9	0.9993	0.0000	2.543
Phenylacetaldehyde [A]	Nd	Nd	−69.56	0.001	0.003	2.400–152.9	0.9995	0.0000	6.161
Geranial [T]	0.277	14.04	−85.58	0.167	0.550	2.400–152.9	0.9950	0.0000	4.053
1-Nonanol [ALC]	7.163	11.17	−71.04	0.213	0.703	2.400–152.9	0.9954	0.0000	4.805
2,4-(E,E)-Nonadienal [ALD]	13.05	11.20	−63.18	0.012	0.039	2.400–152.9	0.9993	0.0000	2.016
α-Terpineol [T]	1.708	4.494	−71.53	0.412	1.360	264.7–4235	0.9909	0.0469	8.859
γ-Terpineol [T]	3.986	8.722	−1.84	0.083	0.273	264.7–4235	0.9892	0.0464	11.23
Neral [T]	0.832	6.465	−96.01	0.012	0.040	2.400–152.9	0.9975	0.0000	3.808
(+)-Citronellol [T]	2.700	5.466	85.63	0.074	0.243	2.400–152.9	0.9959	0.0000	4.008
β-Damascenone [T]	4.796	9.815	−82.73	0.024	0.079	2.400–152.9	0.9975	0.0000	4.121
Nerol [T]	0.111	8.091	−53.01	0.139	0.458	264.7–4235	0.9902	0.0049	6.332
α-Ionone [T]	11.31	9.479	−89.06	0.009	0.029	2.400–152.9	0.9985	0.0000	3.505
Guaiacol [ALC]	1.413	6.017	−91.97	0.015	0.050	2.400–152.9	0.9982	0.0000	2.784

(*continued on next page*)

Table 1 (*continued*)

Volatile Compound	Intra-day Repeatability (% RSD)	Inter-day Repeatability (% RSD)	% Matrix effect	LOD (μg/Kg)	LOQ (μg/Kg)	Linear range (μg/Kg)	R^2	p-value (t slope test)	% Relative Error
Hexanoic acid ^{AC}	10.84	13.37	−38.61	6.847	22.59	264.7–4235	0.9909	0.0004	6.992
Geraniol ^{T}	2.902	5.778	−91.05	0.140	0.461	264.7–4235	0.9980	0.0010	8.788
Benzyl alcohol ^{ALC}	14.71	10.86	−56.59	2.850	9.405	2.400–152.9	0.9983	0.0000	11.87
Phenylethyl alcohol ^{ALC}	14.14	11.64	−69.39	0.286	0.944	2.400–152.9	0.9983	0.0000	6.434
β-Ionone ^{T}	11.24	11.87	−87.59	0.017	0.056	2.400–152.9	0.9940	0.0000	6.379
Eugenol ^{T}	5.564	15.69	−59.61	0.078	0.258	2.400–152.9	0.9931	0.0000	7.863
Nonanoic acid ^{AC}	12.28	15.76	−90.64	0.986	3.254	19.10–152.9	0.9932	0.0034	8.678
Decanoic acid ^{AC}	5.941	5.687	−96.21	1.621	5.349	264.7–4235	0.9924	0.0003	10.34
Farnesol ^{T}	4.050	13.42	29.29	0.517	1.707	2.400–152.9	0.9924	0.0000	5.176

* Nd means “not detected” (*i.e.*, under the limits of detection, < LOD)
* Superscripts refer to the chemical class: AC = acid, ALC = alcohol, ALD = aldehyde, E = ester, K = ketone, T = terpenoid

2.4. Optimization of the solid phase microextraction (SPME) technique

The optimization of the SPME methodology (*i.e.*, sample quantity as well as extraction temperature and time) was performed using a Central Composite Design (CCD) 3^3 with triplicated central point (Supplementary Material 2), Principal Component Analysis (PCA), and Response Surface Method (RSM). The resultant optimized conditions involved mixing 4.25 g (±0.0050 g) of blueberry powder, 1.70 g (±0.0050 g) of NaCl, 0.085 g (±0.0005 g) of citric acid, 0.085 g (±0.0005 g) of ascorbic acid, and 4.25 mL of water in a 20 mL vial chilled with liquid nitrogen and sealed with a magnetic screw cap provided with polytetrafluoroethylene (PTFE)/silicone septa. 25 μL of ethyl butyrate-d3 (15 mg L^{-1} in methanol) was added to the vial as an internal standard. Blueberry mixtures were pre-incubated (without the fibre) in the oven of the SPME autosampler for 10 min at 50 °C with an agitation speed of 500 rpm. Subsequently, a divinylbenzene/carboxen/polydimethysiloxane (DVB/CAR/PDMS) fiber of 1 cm and 50/30 μm (Sigma Aldrich, Oakville, ON, Canada) was exposed to the volatile compounds for 60 min at 50 °C with the same agitation speed. Afterwards, the fibre was inserted into the GC injector port for a thermal desorption of 3 min at 250 °C.

2.5. GC/MS chromatographic conditions

2.5.1. Analysis of non-enantiomeric volatile compounds

GC/MS analyses of the non-enantiomeric volatile compounds were performed on a 7890A gas chromatograph coupled to a 5975C single quadrupole mass spectrometer detector equipped with a GC Sampler 80 (SPME autosampler) and Chemstation software (version E.02.02.1431), all obtained from Agilent Technologies (Santa Clara, CA, USA). The separation was achieved on a polar DB-Wax column (100 % polyethylene glycol, 30 m × 0.25 mm ID × 0.25 μm) from J&W Scientific (Agilent Technologies, CA, USA). The chromatographic conditions were specifically optimized considering the common volatile compounds found in the literature of highbush blueberries, which included the 73 volatile compounds displayed in Table 1. Thus, the GC was operated under programmed temperature conditions: from 35 °C (4.36 min) to 65 °C (3 min) at 1.5 °C/min, subsequently up to 138 °C (0 min) at 3 °C/min, and finally to 230 °C (2 min) at 60 °C/min, resulting in a total run time of 55 min. The carrier gas was helium at a flow rate of 0.9 mL/min. The injector temperature was 250 °C, working in split 1:10 for the most abundant volatiles (*i.e.,* hexanal, myrcene, limonene, 2-(E)-hexenal, linalool, α-terpineol, and geraniol) and in splitless mode for the other 66 compounds listed in Table 1. The interface, ion source, and quadrupole temperatures were 250, 230, and 150 °C, respectively. Samples were simultaneously injected in SCAN (included a mass range of 35–500 *m/z*) and selected ion monitoring (SIM) mode, operating in electron ionization mode with energy of 70 eV. All the 73 volatile compounds in Table 1 were identified by comparing their retention times and accurate mass spectra with standards as well as using their Kovats Index (see Supplementary Material 3) and the Mass Spectra Library (NIST MS Search 2.2 & MS Interpreter). Kovats Index were calculated through the injection of a C7–C30 *n*-alkane series injected under the same chromatographic conditions.

2.5.2. Analysis of enantiomeric volatile compounds

The determination of the four enantiomeric volatile compounds (*i.e.,* α-pinene, limonene, linalool, and α-terpineol) was performed on the 7890A gas chromatograph coupled to a 5975C single quadrupole mass spectrometer detector. The separation was achieved on a chiral Cyclosil-B column [30 % Heptakis (2,3-di-O-methyl-6-O-*t*-butyl dimethylsilyl)-ß-cyclodextrin in DB-1701, 30 m × 0.25 mm ID × 0.25 μm] from J&W Scientific (Agilent Technologies, CA, USA). The chromatographic conditions were optimized following a gradient of temperatures that started at 30 °C (90 min) and increased to 230 °C (2 min) at 10 °C/min, with a total run time of 112 min. The carrier gas was helium with a flow rate of 1 mL/min in splitless mode. The rest of chromatographic and mass spectrometric conditions were the same as described in sub-section 2.5.1. Identification of enantiomeric compounds was achieved using their retention times and accurate mass spectra with standards as well as their Kovats Index (see Table 2) and the Mass Spectra Library (NIST MS Search 2.2 & MS Interpreter).

2.6. Validation of the SPME-GC/MS method

The analytical parameters were evaluated following the Association of Official Analytical Chemists (AOAC) Guidelines (2011). A “control sample” (*i.e.,* mixture of berries from ‘Duke’, ‘Draper’, ‘Bluecrop’, ‘Calypso’, ‘Elliott’ and ‘Last Call’ harvested at three different locations, as described in sub-section 2.3) was used for the validation of the SPME-GC/MS method. Spiked samples explained hereafter refer as the “control sample” with water, NaCl, citric acid, and ascorbic acid to which 100 μL containing the 73 studied volatile compounds were added (being the concentration dependent of the parameter under validation, ranging from 0.05 mg L^{-1} to 65.0 mg L^{-1}) before SPME-GC/MS injection.

2.6.1. Sensitivity: Limits of detection (LODs) and quantification (LOQs)

These parameters were calculated comparing the peak area of the standard in the matrix of the control sample and the area of the background noise from a blank (the air of an empty vial) at the same retention time. The area of the standard in the control matrix was calculated as the peak area of the spiked sample minus the peak area of the non-spiked sample. Injections were made in triplicate (n = 3). LODs were calculated as three times the signal to noise ratio (S/N) while LOQs were calculated as ten times the S/N.

2.6.2. Precision: intra-day and inter-day repeatability

For intra-day repeatability, control samples were injected in triplicate and the percent relative standard deviation (RSD) of each analyte was calculated (n = 3). In terms of inter-day repeatability, blueberry samples were injected in triplicate on three alternate days and RSD (%)

Table 2
Enantiomeric percentages (based on the peak areas from the isomeric chromatograms) found in six northern highbush blueberry cultivars ('Duke', 'Draper', 'Bluecrop', 'Calypso', 'Elliott' and 'Last Call') for α-pinene, limonene, linalool, and α-terpineol. Enantiomeric % followed by the same letters indicate no significant differences ($p > 0.05$) for each compound. Kovats Index (KI) are also included in the table, both from the current experiments and from the literature. No enantiomeric report of KI was found, thus the theoretical values are provided based on the column DB-1701.

Volatile Compound	Enantiomers	KI Experimental	KI Literature	'Duke' %	'Draper' %	'Calypso'%	'Bluecrop'%	'Elliott'%	'Last Call'
α-Pinene	(−)-α-Pinene	935	945	79.46b	77.57b	80.12b	88.99 a	78.99b	79.10b
	(+)-α-Pinene	937		20.54b	22.43b	19.88b	11.01 a	21.01b	20.90b
Limonene	(−)-Limonene	1,055	1,056	81.58b	69.70 e	76.74c	74.83 d	63.01f	93.27 a
	(+)-Limonene	1,058		18.42b	30.30 e	23.26c	25.17 d	36.99f	6.73 a
Linalool	(−)-Linalool	1,230	1,202	1.91 ab	2.03b	3.16b	7.42c	1.10 a	1.81 ab
	(+)-Linalool	1,236		98.09 ab	97.97b	96.84b	92.58c	98.90 a	98.19 ab
α-Terpineol	(−)-α-Terpineol	1,353	1,300	61.25 cd	77.29 d	60.11 cd	25.71 a	55.94 bc	37.75 ab
	(+)-α-Terpineol	1,355		38.75 cd	22.71 d	39.89 cd	74.29 a	44.06 bc	62.25 ab

$$*\%\ enantiomer = \frac{area\ enantiomer}{area\ enantiomer(+) + area\ enantiomer(-)} \times 100$$

was calculated (n = 9). As SPME samples cannot be re-injected, each injection required a fresh sample. A maximum RSD of 15 % was deemed acceptable for repeatability.

2.6.3. Linearity and accuracy

To verify whether matrix-matched calibration curves were required, the matrix effect of each volatile compound from Table 1 was calculated using spiked control samples, non-spiked control samples, and the standard in the same solution used for the preparation of the blueberry samples (*i.e.*, 4.25 mL of water with 1.7 g of NaCl, 0.085 g of citric acid, and 0.085 g of ascorbic acid). Due to the nature of SPME, the matrix effect (*i.e.,* changes of the mass spectrometric signal due to the presence of matrix compounds) and the extraction efficiency (*i.e.*, percentage of the analyte that is recovered from the matrix) cannot be separated. Thus, the combined matrix effect was calculated following the Equation (1):

$$\%\ Matrix\ effect = \left(\frac{area\ spiked\ sample - area\ non\text{-}spiked\ sample}{area\ standard\ in\ solution} - 1\right)x100 \quad (1)$$

Considering the existence of a matrix effect higher than 15 %, matrix-matched calibration curves were prepared. Thus, eight aliquots of the control sample were spiked with increasing concentrations of the standard mixture, which contained the 73 volatile compounds, within the concentration range shown in Table 1. The coefficients of determination (R^2) were calculated and a *t*-test of the slope for verifying the linearity was also performed ($t_{experimental} > t_{critical}$). The relative error (% RE) of quantification was also calculated to determine accuracy, and maximum RE of 15 % was deemed acceptable. The % RE for each volatile compound was calculated as the average of "low, medium and high % RE inside the linear range", following the Equation (2).

$$\%RE = \frac{Ct - Ce}{Ct}x100 \quad (2)$$

where Ct means "theoretical concentration "and Ce "experimental concentration".

2.7. Quantification of volatile compounds in six northern highbush blueberry varieties

The 73 volatile compounds validated using the control sample (see Table 1) were analyzed in the six commercial northern highbush blueberry samples harvested at Location 1, including 'Duke', 'Draper', 'Bluecrop', 'Calypso', 'Elliott', and 'Last Call' (see Supplementary Material 1). As the control sample was considered as representative of the six blueberry cultivars, the matrix-matched calibration curve described in sub-section 2.6.3 was employed for volatiles quantification of each cultivar.

2.8. Calculation of the volatile enantiomeric percentage in six northern highbush blueberry varieties

The ratio of the (+)- or (−)-enantiomers (% enantiomer) was calculated based on the sum of the peak areas of the corresponding enantiomers, following the Equation (3). Standards in methanol (except α-pinene that was in ethanol) were injected to confirm the (+)- and (−)-enantiomers. The % enantiomer was calculated in the six northern highbush commercial blueberry cultivars from Location 1.

$$\%enantiomer = \frac{area\ enantiomer}{area\ enantiomer(+) + area\ enantiomer(-)} \times 100 \quad (3)$$

2.9. Statistical analyses

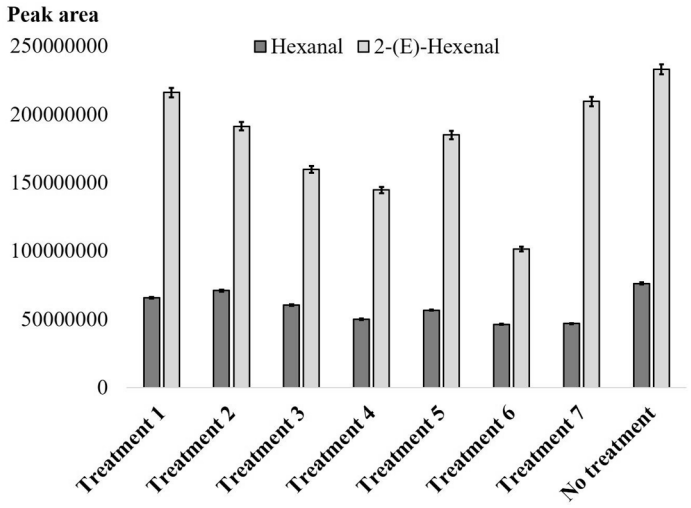
The optimization of the SPME conditions using a CCD 3^3 with 3 central points as well as the extraction of the Principal Components (PC) for the RSM and the RSM of the volatiles data were computed using the software Statgraphics Centurion version XVII (Statpoint Technologies, Warrenton, VA, USA). The one-way ANOVA for identifying significant differences of the volatile compounds among the six cultivars was also performed using Statgraphics Centurion version XVII. Meanwhile, the PCA graphic that describes similarities and differences among cultivars based on their volatile profiles was performed with the JMP® software v15.1.0 (SAS Institute, Cary, NC, USA), with data standardized prior to the analysis.

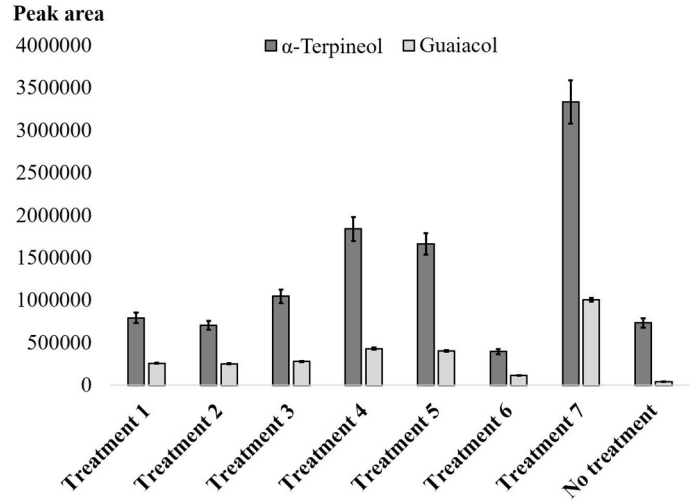
3. Results and discussion

3.1. Optimization of the SPME conditions

3.1.1. Pre-test for the selection of SPME conditions

To prevent changes in the natural blueberry volatile profile (*i.e.,* endogenous volatiles) due to the LOX and PPO action during SPME-GC/MS analyses, a thorough optimization of the chemicals added for this purpose was performed. Thus, seven experimental proportions (*i.e.,* treatments) of NaCl (0.2–0.8 g), citric acid (5–50 mg), and ascorbic acid (5–50 mg) were compared for the prevention of the artificial generation of volatiles (*i.e.,* exogenous volatiles) from lipids oxidation (Fig. 1A) and/or the degradation of endogenous volatiles (Fig. 1B). Hexanal and 2-(E)-hexenal were used as markers for volatile artificial generation due to LOX activity during the SPME-GC/MS extraction, while α-terpineol and guaiacol were used as markers of via PPO-mediated degradation of endogenous volatiles. For the seven experimental treatments, a mixture of 1 g of the control blueberry powder (section 2.1) with 1 mL of water was considered a fixed parameter to which different proportions of NaCl, citric acid, and ascorbic acid were added prior to SPME extraction at 40 °C for 30 min and 10 min of preincubation. Samples mixing just blueberry powder and water were also injected (used as a control for the seven experimental treatments).





Treatment 1: 0.4 g NaCl - 10 mg citric acid - 5 mg ascorbic acid
Treatment 2: 0.4 g NaCl - 5 mg citric acid - 10 mg ascorbic acid
Treatment 3: 0.4 g NaCl - 10 mg citric acid - 10 mg ascorbic acid
Treatment 4: 0.4 g NaCl - 20 mg citric acid - 20 mg ascorbic acid
Treatment 5: 0.4 g NaCl - 50 mg citric acid - 50 mg ascorbic acid
Treatment 6: 0.2 g NaCl - 20 mg citric acid - 20 mg ascorbic acid
Treatment 7: 0.8 g NaCl - 20 mg citric acid - 20 mg ascorbic acid
No treatment: 0 g NaCl – 0 mg citric acid – 0 mg ascorbic acid

Treatment 1: 0.4 g NaCl - 10 mg citric acid - 5 mg ascorbic acid
Treatment 2: 0.4 g NaCl - 5 mg citric acid - 10 mg ascorbic acid
Treatment 3: 0.4 g NaCl - 10 mg citric acid - 10 mg ascorbic acid
Treatment 4: 0.4 g NaCl - 20 mg citric acid - 20 mg ascorbic acid
Treatment 5: 0.4 g NaCl - 50 mg citric acid - 50 mg ascorbic acid
Treatment 6: 0.2 g NaCl - 20 mg citric acid - 20 mg ascorbic acid
Treatment 7: 0.8 g NaCl - 20 mg citric acid - 20 mg ascorbic acid
No treatment: 0 g NaCl – 0 mg citric acid – 0 mg ascorbic acid

Fig. 1. Volatile compounds (as peak areas) indicating the potential enzymatic modifications of the volatile profile during blueberry SPME-GC/MS analysis. These compounds include volatiles extra-generated by lipid oxidation (hexanal, dark grey bar; 2-(E)-hexenal, light grey bar) (1A) and volatiles degraded by polyphenol oxidases (α-terpineol, dark grey bar; guaiacol, light grey bar) (1B). The seven treatments (labelled on the horizontal axis) differed based on the proportions of NaCl, citric acid, and ascorbic acid added prior to SPME extractions. Volatile compounds from a control sample (only blueberry and water) are also included in the graph, labelled as "no treatment". The error bars represent the standard deviation.

As expected, the addition of NaCl with citric and ascorbic acids to the mixture of blueberry and water always resulted in a significant decrease of hexanal and 2-(E)-hexenal (Fig. 1A), despite the potential salting-out effect of NaCl. Likewise, significant increases of α-terpineol and guaiacol (Fig. 1B) was always observed with the addition of NaCl, citric acid, and ascorbic acid. Considering the treatments 1–5, that all had 0.4 g of NaCl and, therefore, similar salting-out effects, the best results were achieved with the use of 20 mg citric acid and 20 mg of ascorbic acid (treatment 4). Treatment 4 resulted in an inhibition of the endogenous generation of hexanal and 2-(E)-hexenal during SPME of 34 and 38 %, respectively, compared to the mixture of blueberry and water. Likewise, the degradation of α-terpineol and guaiacol during SPME was reduced by 96 and 90 %, respectively. The remaining volatile compounds that are not expected to be affected by enzymatic activity during SPME remained constant or slightly increased due to the salting-out effect in comparison to the mixture of blueberry and water (data not shown). Of note, the two treatments with 15 mg of antioxidants differed based on the relative proportions of citric and ascorbic acids. In particular, treatment 1 (*i.e.,* 10 mg citric acid and 5 mg ascorbic acid) and treatment 2 (*i.e.,* 5 mg citric acid and 10 mg ascorbic acid) resulted in a greater inhibition of the generation of hexanal and 2-(E)-hexanal during SPME-GC/MS, respectively. Consequently, the potential inhibition of LOX is dependent on the antioxidant species used. In contrast, the potential inhibition of PPO was independent of the antioxidant species, as evidenced by the lack of significant differences in α-terpineol and guaiacol levels between treatments 1 and 2. Finally, the proportion of NaCl was decreased from 0.4 g to 0.2 g and increased to 0.8 g (treatment 4, 6, and 7, respectively) while maintaining the levels of citric and ascorbic acid constant at 20 mg each. It was speculated that the increase of NaCl from 0.4 g to 0.8 g could have a salting-out effect, increasing the concentration of α-terpineol and guaiacol, but could also inhibit LOX activity, reducing the concentration of hexanal and 2-(E)-hexenal. The lowest NaCl level (0.2 g) was ruled out because it increased α-terpineol and guaiacol less than treatments 4 and 7 (Fig. 1B), mostly due to a reduced salting-out effect. The highest NaCl level (0.8 g) led to a considerably higher signal of 2-(E)-hexenal regarding to treatments 4 and 6 (Fig. 1A). Consequently, treatment 7 was discarded.

On this basis, 1 g blueberry powder, 1 mL of water, 0.4 g of NaCl, 20 mg of citric acid, and 20 mg of ascorbic acid (treatment 4) was selected as the initial proportions for the optimization of the SPME parameters. Endogenous aldehydes and alcohols from LOX-mediated lipid oxidation have been commonly reported in the blueberry literature (Du, & Rouseff, 2014; Eichholz et al., 2011; Farneti et al., 2017; Xinyu et al., 2020). Thus, the control of lipids oxidation, succesfully achieved in the present study, is of utmost importance for avoiding articially LOX-derived compounds in the volatile profile of blueberries.

3.1.2. Blueberry sample amount, extraction time, and extraction temperature

A Central Composite Design (CCD) 3^3 with 3 central points was employed for the optimization of the blueberry sample quantity as well as the SPME extraction temperature and time. It consisted of 27

experimental treatments with randomized combinations of low, medium, and high levels for the optimized parameters, plus a treatment with each parameter at its medium value performed in triplicate, for a total of 30 experimental treatments (Supplementary Material 2). The maximum blueberry sample quantity of 4.25 g was selected considering vial volume (20 mL), while and the maximum time of 60 min was chosen because longer times did not increase the signal (data not shown). The maximum temperature value (50 °C) was selected to avoid changes in the original blueberry volatile profile, as temperatures of 60 °C were reported to promote the formation of ß-damascenone from its acetylenic diol precursor during SPME extraction of blackberry purees (Meret et al., 2011).

As there were 73 responses (*i.e.,* peak areas of volatile compounds) for each experiment of the CCD 3^3, a PCA was employed to reduce the dimensionality of the original data (Ribeiro et al., 2010). The application of RSM to the values of the four principal components (PC1-4, which explained 81.3 % of the variance) from the 30 experimental treatments resulted in the multiple response surface shown in Supplementary Material 4. With an overall desirability function of 77 %, 4.25 g of blueberry sample quantity, and 50 °C and 60 min of SPME were selected as optimum. The volatile profile of the control sample employed for the optimization (see sub-section 2.3) revealed hexanal, limonene, 2-(E)-hexenal, linalool, α-terpineol, and geraniol as the peaks with the largest areas (see Supplementary Material 5), which agreed with previous reports for northern highbush blueberries (Eichholz et al., 2011).

Our optimization of the SPME conditions, including the amount of the preservatives (*i.e.*, NaCl, citric acid, and ascorbic acid), guarantees the maximum signal and preserves the natural blueberry volatile profile, minimizing the formation of analytical artifacts.

3.2. Validation of SPME-GC/MS methodology

3.2.1. Limits of detection (LODs) and quantification (LOQs)

The LODs and LOQs of hexanal, myrcene, limonene, 2-(E)-hexenal, linalool, α-terpineol, and geraniol were calculated in split mode (1:10) while the rest of compounds from Table 1 were calculated in splitless mode. On this basis, the LODs ranged from 0.0004 μg kg^{-1} (cymene) to 22.68 μg kg^{-1} (2-(E)-hexenol), with an average value of 0.84 μg kg^{-1} for the LOD and 2.78 μg kg^{-1} for the LOQ (Table 1). Consequently, the optimized SPME/GC–MS method has suitable sensitivity for the analysis of volatile compounds present in low concentration. LODs of low μg kg^{-1} were in accordance with the lowest values reported in the matrix-matched calibration curves for southern highbush blueberries (Du et al., 2011).

3.2.2. Precision: intra-day repeatability and inter-day repeatability

Intra-day repeatability values varied between 0.02 % (2-nonanone) and 14.71 % (benzyl alcohol), while inter-day repeatability values varied between 2.02 % (heptanal) and 16.53 % (2-hexanol) (Table 1). With average RSD of 5.72 and 9.05 % for the intra- and inter-day repeatability, respectively, values were lower than the 15 % recommended for methods validation (AOAC Guidelines, 2011).

However, as eugenol, nonanoic acid, 2-(Z)-hexen-1-ol, and 2-hexanol had RSD between 15.69 and 16.53 % for the inter-day repeatability, the use of an internal standard was necessary. Therefore, ethyl butyrate-d3 was employed for the quantification of the analyzed volatile compounds.

3.2.3. Linearity and accuracy

The % matrix effect was estimated to decide if matrix-matched calibration curves were needed to quantify the blueberry volatiles. Percent matrix effects of ion enhancement or suppression in the mass spectrometric detector (*i.e.,* increase or decrease in signal compared to the original) greater than 15 % must be corrected (AOAC Guidelines, 2011). Based on the results of Table 1, matrix-matched calibration curves were needed for quantification of the targeted blueberry volatiles.

The linearity of the volatile compounds, defined as ability of the detector to obtain results that are directly proportional to their concentration within a given range, was verified in the matrix-matched calibration curves. Both the split 1:10 (for hexanal, myrcene, limonene, 24-(E)-hexenal, linalool, α-terpineol, and geraniol) and splitless (for the remaining compounds in Table 1) calibration curves presented coefficients of determination (R^2) greater than 0.99 and a $p < 0.05$ for the *t*-test of the slope (Table 1). Consequently, the present SPME-GC/MS method is considered linear in the reported concentration ranges for each volatile compound shown in Table 1.

Additionally, the 73 volatile compounds had % RE between 1.49 % (1-heptanol) and 14.09 % (2-hexanol), with an average % RE of 6.11 %. These RE values fall below the maximum values accepted (15 %) for validation of accuracy (AOAC Guidelines, 2011).

3.3. Quantification of volatile compounds in six northern highbush blueberry cultivars

3.3.1. Volatile groups found and total amounts

In blueberry, the content of volatile chemical groups varies among cultivars. Southern highbush blueberries have been characterized by aldehydes as the most abundant fraction, followed by esters and/or terpenes (Du, & Rouseff, 2014; Farneti et al., 2017). Our study provides new knowledge on the volatile fractions of northern highbush blueberry cultivars.

Fig. 2 shows the total content of each volatile chemical group (*i.e.,* the sum of individual compound concentrations) quantified in the six cultivars. Considering the total volatile content, 'Draper' showed the highest (48,279 μg kg^{-1}) followed by 'Duke' (31,673 μg kg^{-1}), 'Calypso' (18,854 μg kg^{-1}), 'Bluecrop' (12,708 μg kg^{-1}), 'Elliott' (11,509 μg kg^{-1}), and 'Last Call' (4,502 μg kg^{-1}). Terpenes were the most abundant group for all the samples and ranged from 2,970 to 42,396 μg kg^{-1}, generally followed by aldehydes (from 527 to 4,837 μg kg^{-1}), alcohols (from 690 to 1,003 μg kg^{-1}), and acids (from 436 to 476 μg kg^{-1}). Traces of ketones (from 9 to 69 μg kg^{-1}) and esters (from 0.2 to 8 μg kg^{-1}) were also found. In 'Last Call', similar amounts of aldehydes and alcohols were found. The tendency of terpenes as the most abundant compounds followed by aldehydes is in fully agreement with the volatile compounds showed in 'Duke' by Polashock, Saftner, & Kramer (2007), who inhibited the artificial generation of aldehydes from lipid oxidation using $CaCl_2$. Studies that did perform semi-quantifications reported contrasting results. Eichholz et al. (2011) reported that aldehydes were the group with the highest relative peak areas (in relation to the internal standard), followed by terpenes and alcohols with a total relative peak area about six- and ten-times lower than aldehydes, respectively. Likewise, Xinyu et al. (2020) found that aldehydes are the group with the highest peak areas (28.9 % of relative content), followed by terpenes (20.0 % of relative content) and alcohols (16.6 % of the relative content). In these two studies, results are presented as peak areas, and no quantitative comparison to our study is possible. Moreover, in these studies, a potential low-efficiency in the control for oxidation could have led to the high presence of artificial hexanal and 2-(E)-hexenal, as both studies only used NaCl for preventing lipids oxidation. However, the genetic × environment interaction could also have contributed to the different profile compared to that of our study.

Other northern highbush blueberry cultivars not included in the current study, such as 'Legacy', 'Brigitta', and 'Northland', have aldehydes as the most abundant compounds, followed by alcohols, with lower concentrations of terpenes and esters (Cheng, Peng & Yuan, 2020). Cheng et al. (2020) found esters to be present in higher concentrations (ranging from 109 to 335 μg kg^{-1}) than in the six cultivars in this study while terpenes were in much lower concentrations (ranging from 56 to 239 μg kg^{-1}). These differences among studies can be attributed to differences in the blueberry cultivars considered – which is confirmed by the current study where large differences among cultivars

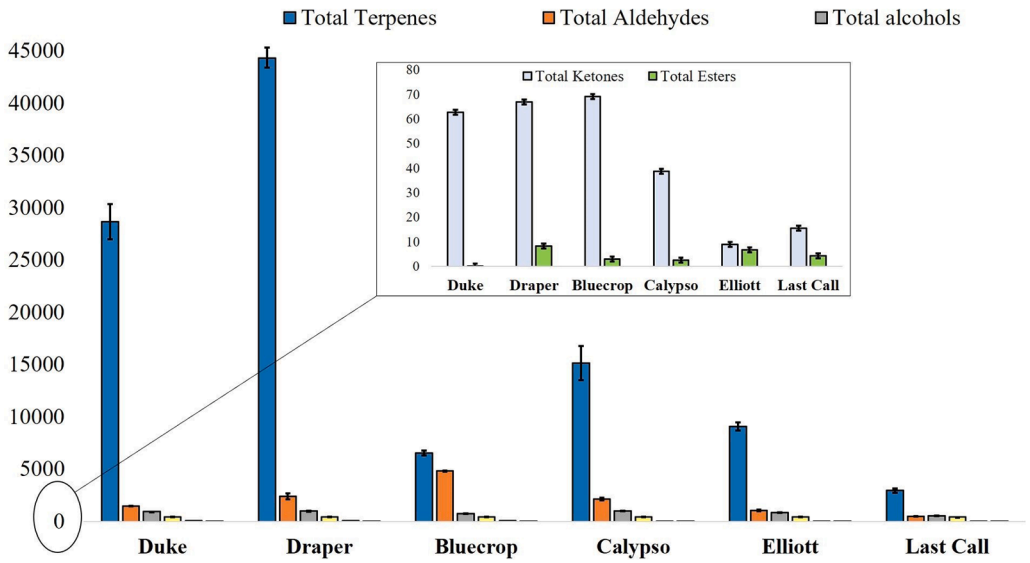


Fig. 2. Total content of volatile compounds, in μg kg^{-1}, for each group of volatiles targeted in six cultivars ('Duke', 'Draper', 'Bluecrop', 'Calypso', 'Elliott', 'Last Call'), including terpenes (dark blue bar), aldehydes (orange bar), alcohols (grey bar), acids (yellow bar), ketones (light blue bar), and esters (green bar). The error bars represent the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were observed between 'Draper' and 'Last Call' (Fig. 2). However, the technique used for quantification could also contribute to inconsistencies among studies, as Cheng et al. (2020) did not consider the potential matrix effect of the sample and performed the calibration curve in saturated saltwater rather than using a matrix-matched calibration curve. Esters showed a remarkable effect of ion enhancement in the current study, which could partially explain the higher concentration of esters in the study by Cheng et al. (2020) where there was no correction for the potential matrix effect of ion enhancement. Likewise, terpenes demonstrated a strong effect of ion suppression in the current study (Table 1), which could partially justify the lower terpenes concentration in Cheng et al. (2020). Our approach improves the accuracy of quantification for volatile compounds, which will allow to directly compare cultivars among experiments (Betz et al., 2011).

3.3.2. *Individual volatile compounds*

From the 73 targeted volatile compounds (Table 1), 71 volatiles were found above their LOD in all six cultivars, including 29 terpenes, 18 alcohols, 12 aldehydes, four ketones, five esters, and three acids (Table 3). Phenylacetaldehyde (an aldehyde) and β-pinene (a terpene) were below the LOD. A total of 15 volatile compounds were below their LOQ (Table 3) and will not be discussed hereafter.

Linalool was the most abundant volatile (Table 3), 'Draper' having the highest (34,201 ± 2,486 μg kg^{-1}) and 'Last Call' the lowest concentration (1,198 a ± 132 μg kg^{-1}). α-Terpineol and γ-terpineol were present in similar concentrations as the second and third most abundant compounds across all cultivars, except 'Draper' for which limonene was in similar concentration to these two terpineol isomers. The highest concentration of terpineol isomers was also found in 'Draper' (2,332 ± 97 μg kg^{-1} and 2,573 ± 89 μg kg^{-1} for α- and γ-, respectively) and the lowest in 'Last Call' (372 ± 46 μg kg^{-1} and 500 ± 48 μg kg^{-1}, respectively). Terpinolene was also abundant in all six cultivars. 'Duke' had the highest concentration of terpinolene (816 ± 62 μg kg^{-1}), closely followed by 'Draper' (719 ± 0.5 μg kg^{-1}), with 'Last Call' having the lowest concentration (444 ± 1 μg kg^{-1}). Geraniol was close in concentration to terpinolene in 'Draper' (688 ± 27 μg kg^{-1}) while for the other blueberry cultivars geraniol was found at approximately half the concentration of terpinolene. On this basis, it is suggested that 'Draper' produces more terpene precursors through the geranyl pyrophosphate (GPP) route than 'Last Call', as this pathway gives rise to intermediate cations that result in linalool (from geranyl and neryl cations) as well as the terpineol isomers, limonene and terpinolene (from terpinyl cation) (Sugiura et al., 2011). Substantial amounts of linalool, terpineol, terpinolene, geraniol, and, limonene have been reported in blueberries of both southern (Du et al., 2011; Farneti et al., 2017) and northern (Cheng et al., 2020; Eichholz et al., 2011; Xinyu et al., 2020) highbush cultivars. Linalool and geraniol, contributing to 'sweet', 'floral', 'fruity', 'citrus', and 'berry-like' notes, as well as α- terpineol, contributing to 'woody', 'herbaceous', and 'piney' notes, are recognized as aroma active compounds in highbush blueberries (Du & Rouseff, 2014). Linalool in particular is one of the terpenes with the lowest odour thresholds (OT) in water at 6 μg L^{-1} (Du et al., 2011).

Among aldehydes, 2-(E)-hexenal then hexanal were the most abundant across the six cultivars. The highest concentration was measured in 'Bluecrop' (2,497 ± 0.4 μg kg^{-1} and 1,748 ± 22 μg kg^{-1}, respectively) and the lowest concentration in 'Last Call' (260 ± 3 and 155 ± 3 μg kg^{-1}, respectively) (Table 3). The high abundance of hexanal and 2-(E)-hexenal agrees with reports in the range of 50–1,600 μg kg^{-1} and 1,500–3,000 μg kg^{-1}, respectively, for both southern (Du et al., 2011; Farneti et al., 2017) and northern (Cheng et al., 2020; Eichholz et al., 2011; Xinyu et al., 2020) cultivars. However, for southern highbush blueberries, aldehydes were considerably more abundant than terpenes for all the reported cultivars (Du et al., 2011), which could be also related to the lower efficiency in the control of LOX oxidation during the sample analysis. Both hexanal and 2-(E)-hexenal have been reported as aroma-impact compounds that contribute 'fresh green', 'grassy', and 'fruity' notes (Du & Rouseff, 2014) in highbush blueberries and have relatively low OTs in water (17 and 4.5 μg L^{-1}, respectively) (Du et al., 2011), which is expected to have a positive impact on the final aroma of blueberries. 2-(E)-Hexenal was one of the most potent aromas in four southern highbush blueberry cultivars analyzed by Du & Rouseff (2014) using GC-Olfactometry (GC-O).

2-(E)-Hexen-1-ol (351 ± 9 to 535 ± 9 μg kg^{-1}) and 1-hexanol (129 ± 12 to 258 ± 8 μg kg^{-1}) were the most abundant alcohols found in the six cultivars (Table 3); however, only 3-(Z)-hexenol and 2-heptanol have been reported as aroma active compounds in highbush blueberries (Du & Rouseff, 2014). As 2-(E)-hexen-1-ol and 1-hexanol are known to have high OTs in water (1,000 μg L^{-1} and 500 μg L^{-1}) compared to 3-(Z)-hexenol and 2-heptanol (70 μg L^{-1}) (Du et al., 2011), the alcoholic fraction is not expected to have a strong impact on the final aroma of the cultivars analyzed in the current study.

Hexanoic acid ('cheesy' and 'sour' notes) and 6-methyl-5-hepten-2-

Table 3
Concentration of volatile compounds, expressed as μg kg^{-1} of blueberries (fresh weight), found in six northern highbush blueberry cultivars ('Duke', 'Draper', 'Bluecrop', 'Calypso', 'Elliott' and 'Last Call'). Values in the table represent means ± standard errors, which are followed by the same letters for each compound when there are no significant differences ($p > 0.05$). Compounds are arranged in increasing order of retention time (showed in Supplementary Material 3).

Volatile compounds	'Duke'	'Draper'	'Bluecrop'	'Calypso'	'Elliott'	'Last Call'
Methyl 2-methyl butanoate [E]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
α-Pinene [T]	<LOQ	11.89b ± 1.011	0.1193 a ± 0.3044	<LOQ	<LOQ	<LOQ
Ethyl butanoate [E]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1-Propanol [ALC]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ethyl 2-methyl butanoate [E]	0.150 ± 0.045	ND	<LOQ	<LOQ	<LOQ	<LOQ
Ethyl isovalerate [E]	3.202c ± 0.093	<LOQ	2.195b ± 0.115	0.0803 a ± 0.0199	3.383c ± 0.275	2.246b ± 0.396
Hexanal [ALD]	416.0 a ± 23.72	621.1b ± 78.33	1748c ± 21.89	663.8b ± 66.76	345.3 a ± 14.92	154.7 a ± 3.110
2-Methyl-1-propanol [ALC]	94.92b ± 10.64	191.3c ± 25.10	23.20 a ± 0.590	159.3c ± 4.470	29.61 a ± 5.020	<LOQ
α-Phellandrene [T]	10.54b ± 1.340	11.98b ± 1.190	4.030 a ± 0.070	6.270 a ± 0.370	4.480 a ± 0.170	4.760 a ± 0.080
1-Butanol [ALC]	14.85 ± 0.9100	Nd	Nd	Nd	<LOQ	Nd
Myrcene [T]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1,4-Cineole [T]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Heptanal [ALD]	9.990b ± 1.110	0.950 a ± 0.0197	20.33c ± 1.240	8.690b ± 1.270	<LOQ	<LOQ
2-Heptanone [K]	<LOQ	Nd	3.28 ± 0.46	<LOQ	<LOQ	<LOQ
Limonene [T]	393.74b ± 29.07	2884c ± 155.5	<LOQ	179.8 a ± 18.16	<LOQ	<LOQ
β-Phellandrene [T]	28.69c ± 3.996	24.25c ± 1.650	1.886 a ± 0.075	16.13b ± 2.340	2.588 a ± 0.023	2.097 a ± 0.026
Eucalyptol [T]	42.92c ± 4.390	27.97 bc ± 1.490	10.09 a ± 1.490	25.78 ab ± 0.190	15.94 a ± 1.070	20.71 ab ± 1.270
2-(E)-Hexenal [ALD]	979.5 a ± 5.670	1,645b ± 217.9	2,497c ± 0.3714	1,407b ± 55.67	631.3 a ± 46.32	260.2 a ± 3.360
3-Methyl-1-butanol [ALC]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2-Hexanol [ALC]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
γ-Terpinene [T]	48.99b ± 4.530	77.31c ± 7.899	6.908 ab ± 0.577	24.37 ab ± 2.784	5.318 a ± 0.5741	14.01 ab ± 0.0245
β-Ocimene [T]	162.0c ± 14.00	268.7 d ± 24.29	38.81 a ± 0.4386	82.31b ± 10.61	38.63 a ± 1.470	69.58b ± 0.9612
Cymene [T]	16.46c ± 2.530	15.32c ± 1.640	<LOQ	10.08b ± 0.720	1.376 a ± 0.2362	4.290 a ± 0.3333
Terpinolene [T]	815.7 d ± 61.91	718.5c ± 0.495	474.1 a ± 5.612	607.2b ± 11.86	472.3 a ± 3.832	444.3 a ± 1.194
Hexyl acetate [E]	<LOQ	9.139 e ± 0.097	0.883 a ± 0.017	2.548c ± 0.128	3.368 d ± 0.186	2.070b ± 0.171
Octanal [ALD]	2.121 a ± 2.371	8.066b ± 0.044	19.09c ± 4.017	<LOQ	<LOQ	<LOQ
6-Methyl-5-hepten-2-one [K]	62.98c ± 5.97	58.88c ± 0.276	63.97c ± 1.422	31.23b ± 0.122	6.712 a ± 0.824	8.721 a ± 0.450
2-Heptanol [ALC]	<LOQ	Nd	<LOQ	Nd	<LOQ	< LOQ
Rose Oxide [T] (isomer 1)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Rose Oxide [T] (isomer 2)	Nd	Nd	Nd	<LOQ	ND	ND
1-Hexanol [ALC]	199.8c ± 0.931	205.7c ± 4.805	181.2b ± 3.813	257.5 e ± 8.513	230.6 d ± 4.4385	128.6 a ± 12.09
3-(E)-Hexen-1-ol [ALC]	24.30c ± 2.088	5.794 a ± 0.207	4.581 a ± 0.208	10.12b ± 0.927	11.84b ± 1.771	< LOQ
2,4-(E,E)-Hexadienal [ALD]	59.15c ± 2.363	61.60c ± 0.702	63.34c ± 3.042	49.85b ± 0.4458	42.97 a ± 2.668	42.90 a ± 2.617
2-Nonanone [K]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-(Z)-Hexen-1-ol [ALC]	71.58c ± 1.123	48.55 bc ± 4.637	34.54 abc ± 3.228	52.99c ± 2.801	13.56 a ± 0.4498	<LOQ
Nonanal [ALD]	16.03 a ± 1.898	17.67 a ± 1.110	465.5b ± 36.52	6.528 a ± 0.222	12.54 a ± 0.826	5.025 a ± 0.144
2-(E)-Hexen-1-ol [ALC]	486.19c ± 6.996	428.9b ± 13.04	496.7c ± 29.10	432.6b ± 10.56	534.9 d ± 9.034	351.0 a ± 8.902
2-(Z)-Hexen-1-ol [ALC]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Linalool Oxide (isomer 1) [T]	449.0 d ± 42.39	287.6b ± 17.79	110.5 a ± 1.016	348.2c ± 22.54	325.5 bc ± 4.799	95.11 a ± 2.089
Furfural [ALD]	20.99 a ± 0.074	28.24 ab ± 4.018	17.41 a ± 1.048	39.46b ± 2.533	31.61 ab ± 1.997	58.53c ± 14.27
1-Octen-3-ol [ALC]	<LOQ	<LOQ	5.56 ± 0.38	<LOQ	<LOQ	<LOQ
1-Heptanol [ALC]	<LOQ	0.438 ± 0.047	<LOQ	<LOQ	<LOQ	<LOQ
Linalool Oxide (isomer 2) [T]	82.06b ± 9.697	39.29 ab ± 1.330	<LOQ	47.25 a ± 6.490	45.23 a ± 0.868	<LOQ
Benzaldehyde [ALD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Decanal [ALD]	Nd	30.63 ± 0.041	Nd	Nd	Nd	Nd
2-(E)-Nonenal [ALD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Linalool [T]	22,706 d ± 1311	34,201 e ± 2486	4,573b ± 217.7	11,051c ± 1491	6,583b ± 333.6	1,198 a ± 132
Caryophyllene [T]	34.34c ± 3.630	14.93 a ± 0.020	16.87 a ± 0.140	23.94b ± 0.577	<LOQ	ND
2,6-(E,Z)-Nonadienal [ALD]	2.349b ± 0.441	10.97 d ± 1.297	4.423c ± 0.294	0.899 a ± 0.047	1.373 ab ± 0.155	1.669 ab ± 0.230
1-Octanol [ALC]	3.973c ± 0.902	20.77 e ± 1.196	8.650 d ± 0.456	1.632 a ± 0.064	2.614 ab ± 0.193	3.351 bc ± 0.265
2-Undecanone [K]	3.132 a ± 0.284	8.113b ± 0.246	2.092 a ± 0.109	7.538b ± 0.31	2.241 a ± 0.031	6.976 ab ± 0.680
Geranial [T]	62.97c ± 9.16	51.08b ± 2.74	0.89 a ± 0.02	5.06 a ± 1.27	<LOQ	ND
1-Nonanol [ALC]	0.75 a ± 0.07	3.21b ± 0.56	<LOQ	<LOQ	<LOQ	<LOQ
2,4-(E,E)-Nonadienal [ALD]	2.432 a ± 0.239	2.486 a ± 0.041	1.703 a ± 0.117	1.959 a ± 0.037	2.057 a ± 0.1522	2.740 a ± 0.124
α-Terpineol [T]	1,612 d ± 152.1	2,332 e ± 97.22	512.2 ab ± 9.192	1,017c ± 98.24	643.99b ± 16.37	371.9 a ± 46.02
γ-Terpineol [T]	1,810 d ± 145.6	2,573 e ± 89.48	643.6 ab ± 3.290	1,216c ± 1.150	782.5b ± 18.63	500.3 a ± 47.73
Neral [T]	15.73b ± 4.970	36.78c ± 5.130	1.767 a ± 0.128	2.774 a ± 0.604	<LOQ	0.437 a ± 0.083
(+)-Citronellol [T]	<LOQ	2.28 ± 0.43	<LOQ	<LOQ	<LOQ	<LOQ
β-Damascenone [T]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Nerol [T]	<LOQ	36.53 ± 1.170	<LOQ	<LOQ	<LOQ	<LOQ
α-Ionone [T]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Guaiacol [ALC]	47.63 bc ± 5.759	76.20c ± 3.236	1.617 a ± 0.030	72.02c ± 4.720	22.82 a ± 2.240	46.49 ab ± 4.550
Hexanoic acid [AC]	206.3 a ± 4.617	199.6 a ± 0.101	214.4 a ± 2.661	215.0 a ± 4.303	191.8 a ± 1.716	179.08 a ± 0.554
Geraniol [T]	373.1c ± 21.93	687.5 d ± 26.86	160.3 a ± 0.019	237.3b ± 17.51	181.7b ± 0.595	148.7 a ± 1.853
Benzyl alcohol [ALC]	10.74 ab ± 1.013	17.00c ± 1.371	6.701 a ± 0.061	14.02 bc ± 0.243	7.976 a ± 0.244	10.19 a ± 0.423
Phenylethyl alcohol [ALC]	3.941b ± 0.310	6.101c ± 0.435	2.938 a ± 0.032	2.939 a ± 0.032	3.237 a ± 0.024	< LOQ
β-Ionone [T]	2.119 a ± 0.013	2.149 a ± 0.013	2.165 a ± 0.074	2.149 a ± 0.014	2.063 a ± 0.007	2.081 a ± 0.015
Eugenol [T]	6.179c ± 0.397	3.883 ab ± 0.060	4.481b ± 0.085	3.770 ab ± 0.003	10.23 d ± 0.6589	3.650 a ± 0.010
Nonanoic acid [AC]	24.31 a ± 0.851	24.74 a ± 0.235	19.88 a ± 0.235	22.34 a ± 0.775	20.19 a ± 1.373	21.29 a ± 0.054
Decanoic acid [AC]	239.7 a ± 0.790	240.4 a ± 0.569	237.2 a ± 0.101	238.4 a ± 0.033	240.5 a ± 0.035	235.4 a ± 0.004
Farnesol [T]	7.416 a ± 0.435	<LOQ	<LOQ	249.25b ± 0.816	<LOQ	< LOQ

* Nd means "not detected" (*i.e.*, under the limits of detection, < LOD); <LOQ means "under the limits of quantification"
* Superscripts refer to the chemical classes: AC = acid, ALC = alcohol, ALD = aldehyde, E = ester, K = ketone, T = terpenoid

one ('citrus' notes) were the most abundant acids and ketones, respectively (Table 3). Due to the low concentrations of the acids and ketones in the cultivars analyzed and their reportedly high (3,000 μg L^{-1} for hexanoic acid) and medium (50 μg L^{-1} for 6-methyl-5-hepten-2-one) OTs in water (Du et al., 2011), they are not expected to have a strong impact on the blueberry aroma.

Esters, even at trace concentrations lower than 9 μg kg^{-1} (Table 3), can impact the final aroma of blueberries with pleasant 'fruity' notes due to their extremely low OTs. For example, OT values for ethyl 2-methylbutanoate, ethyl isovalerate, and hexyl acetate are 0.1, 4.4, and 2 μg L^{-1}, respectively (Leffingwell, 2021).

3.3.3. *Characterization of the blueberry cultivars based on their volatile profiles*

The proportions of the different volatile compounds greatly varied among cultivars (Table 3). To make it possible to both interpret this large dataset and minimize loss of information regarding differences in volatile profiles across cultivars, the PCA biplot (Fig. 3) was constructed with the peak areas of the 73 targeted volatile compounds. The first principal component (PC1), explaining 39.5 % of the variability of the original values, led to the separation of 'Bluecrop', 'Elliott', and 'Last Call' in the negative PC1, and 'Duke', 'Draper', and 'Calypso' in the positive PC1. This separation was mostly due to the higher content of terpenes for 'Duke', 'Draper', and 'Calypso', especially limonene in 'Duke' and linalool and γ- and α-terpineol in 'Draper'. Ethyl and methyl esters were the compounds that also distinguished 'Bluecrop', 'Last Call', and 'Elliott' in the negative PC1, and compounds like 2-(E)-hexen-1-ol caused the proximity between 'Last Call', and 'Elliott'. The second component (PC2), explaining 24.6 % of the variability of the original values, separated 'Draper' and 'Bluecrop' in the positive PC2 from the rest of cultivars, mainly attributed to the higher content of volatile compounds from the endogenous lipids oxidation, such as 2-(E)-nonenal, octanal, 2,6-(E,Z)-nonadienal, and 1-octanol found both in 'Draper' and 'Bluecrop'. Indeed, the remarkable high content in hexanal, nonanal, 1-octen-3-ol and, above all 2-(E)-hexenal (all compounds from endogenous lipids oxidation) determines the unique location of 'Bluecrop' in the negative PC1 and positive PC2. Likewise, 'Draper' is the only cultivar located in the positive PC1 and positive PC2 due to the high content of nerol and geraniol, that are both terpenoids.

Therefore, it can be concluded that the analyzed blueberry cultivars were mainly distinguished by their contents in terpenoids and volatiles generated by the endogenous lipid oxidation. Indeed, the ratio of linalool to 2-(E)-hexenal has been reported to play a role in imparting blueberry flavour to foodstuffs, with common ratios from 0.25 to 1 employed in food flavoring agents (US4041185A patent, 1975). Taking this into consideration, 'Bluecrop' presented the lowest ratio of linalool to 2-(E)-hexenal (1.8) and 'Duke' and 'Draper' the highest ratios (23.2 and 20.8, respectively). This could justify the fact that 'Bluecrop' is widely considered to have one of the best flavour profiles among commercial cultivars including 'Duke' and 'Draper' (OSU, 2014). However,

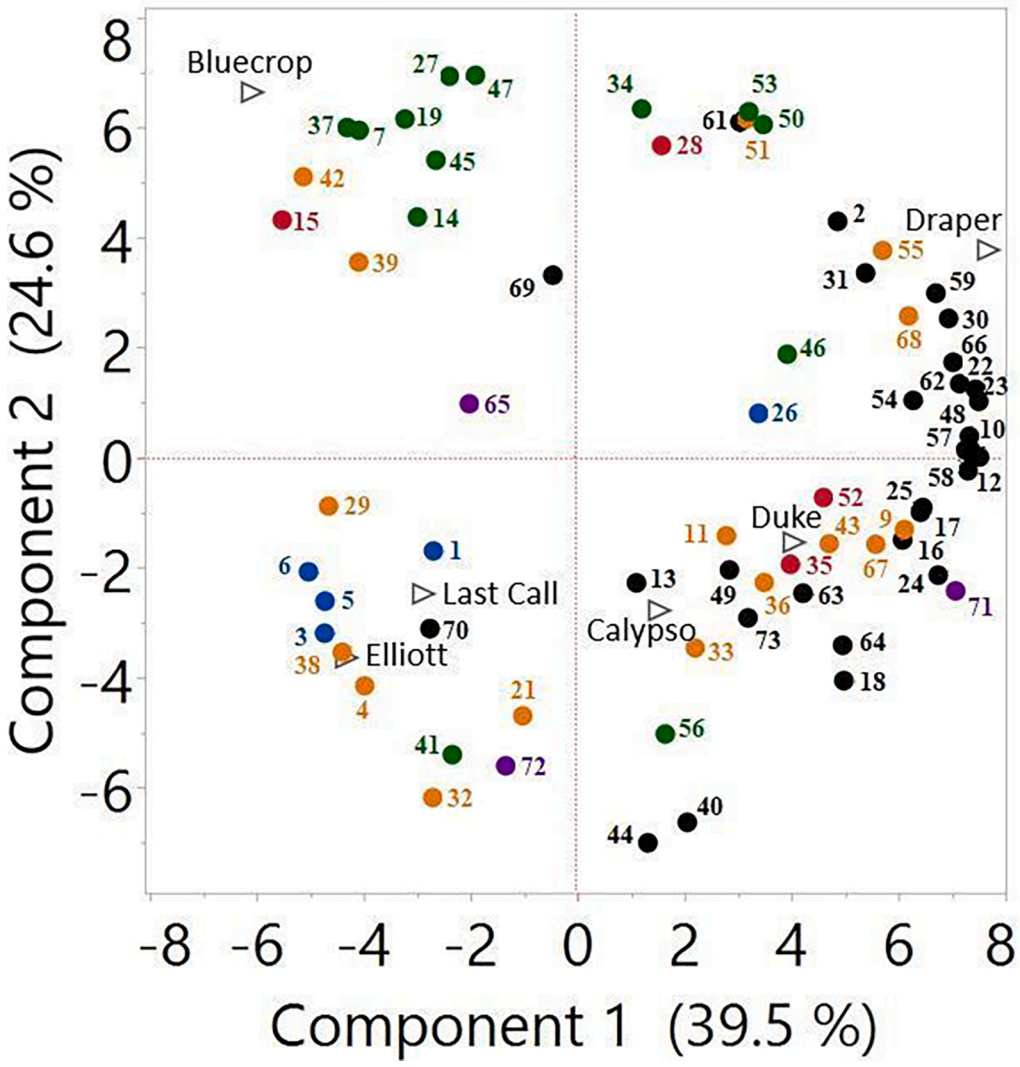


Fig. 3. PCA of volatile profiles analyzed in the six cultivars. The biplot represents the six blueberry cultivars (as white triangles) and the 73 volatile compounds analyzed. Terpenoids are depicted with black circles, aldehydes with green circles, alcohols with orange circles, acids with purple circles, ketones with red circles, and esters with blue circles. The numbers corresponding to each volatile compound are indicated in Supplementary Material 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

further sensorial analyses would be needed to confirm this explanation.

3.4. Enantiomeric analysis of terpenes

Understanding the enantiomeric composition provides additional insight into blueberry aroma since different enantiomers of the same volatile compound can have different aroma characteristics and sensory thresholds (Ruiz del Castillo & Dobson, 2002; Werkhoff, Bretschneider, Güntert, Hopp, & Surburg, 1991). Thus, the enantiomers of linalool, α-terpineol, limonene, and α-pinene were analyzed in the six cultivars of the present study (Table 2).

Limonene and α-pinene presented higher abundance of the (−)-enantiomer in all the cultivars analyzed (Table 2). The (−)-enantiomer for limonene varied from 63.01 % (in 'Elliott') to 93.27 % (in 'Last Call'). In the case of the (−)-enantiomer of α-pinene, only 'Bluecrop' (88.99 %) was significantly different ($p < 0.05$) from the other cultivars (around 79 %). The predominance of the (−)-terpenes has been already observed in raspberry (Bernreuther, Bank, Krammer, & Schreier, 1984). The (+)- and (−)- limonene have been described as contributing 'orange' and 'lemon' notes, respectively (Finefield, Sherman, Kreitman, & Williams, 2012), which indicates that the citrus-like notes can be slightly different in the six cultivars depending on the proportion (+)- to (−)-enantiomer. Both enantiomers of α-pinene present the same aroma notes (*i.e.*, 'pine') (Finefield et al., 2012). However, (+)-α-pinene is the only enantiomer with antimicrobial activity. Thus, all of the cultivars would present the same antimicrobial activities in terms of α-pinene content, except for 'Bluecrop', which would have more moderate antimicrobial activity due to the lower abundance of (+)-α-pinene.

In the case of linalool, the (+)-enantiomer was predominant, ranging from 92.58 % (in 'Bluecrop') to 98.90 % (in 'Elliott'). The predominance of (+)-linalool over the (−)-linalool agrees with previous enantiomeric studies of linalool in 'Duke', 'Draper', 'Bluecrop', and 'Elliott' (Karaarslan et al., 2014). As (+)-linalool has been described with 'sweet', and 'petitgrain' notes and the (−)-linalool with 'woody' and 'lavender' notes (Brenna, Fuganti, & Serra, 2003), linalool in 'Bluecrop' possibly contributes stronger 'citrus' aroma notes to the overall blueberry aroma than for the other cultivars.

The enantiomeric excess of α-terpineol varied among cultivars, with 'Bluecrop' (74.29 %) and 'Last Call' (62.25 %) presenting an excess of the (+)- over the (−)-enantiomer, and 'Draper' (22.71 %), 'Duke' (38.75 %), 'Calypso' (39.89 %) and 'Elliott' (44.06 %) presenting a deficit of the (+)-enantiomer. α-Terpineol has been reported to have similar amounts of the (+)- and (−)-enantiomers for some cultivars (Karaarslan et al., 2014), demonstrating the effect of the genetic background on the enantiomeric ratio. The (+)-α-terpineol has a lilac odor, while the (−)-enantiomer has a coniferous odor character (Martins de Sousa et al., 2020), which may result in more floral notes in 'Bluecrop' and 'Last Call' and more pine notes in the other cultivars. These differences may contribute to the determination of cultivar-specific aromas.

4. Conclusions

For the first time, a SPME-GC/MS method for the quantification of 73 volatile compounds in northern highbush blueberries was optimized (using a combination of CCD 3^3 with 3 central points experimental design, PCA, and RSM) and validated in terms of sensitivity, reproducibility, linearity, and accuracy. Considering that the proportion of the preservatives (*i.e.,* citric acid, ascorbic acid and NaCl) was optimized, it was guaranteed that the natural blueberry volatile profile was not modified during sample analysis. Additionally, the quantification of volatiles through matrix-matched calibration curves corrected the extraction efficiencies and matrix effects, ensuring that the differences in volatiles among cultivars were due to genetic and agronomical factors and not to analytical issues. This study demonstrates that it is highly recommendable to preserve the natural blueberry aroma profile and accurately quantify blueberry volatile compounds to get reliable conclusions.

The highest total volatile concentration was detected in 'Draper' (48,279 μg kg^{-1}), and the lowest in 'Last Call' (4,502 μg kg^{-1}). Generally, terpenes were the most abundant group of volatiles, followed by aldehydes and alcohols, while esters were found in trace amounts. The PCA of the volatiles from the six cultivars led to the conclusion that the volatile profile of 'Bluecrop' differed from the other cultivars mainly because of a higher 2-(E)-hexenal and hexanal content. A higher terpene content (particularly for linalool in 'Duke' and 'Draper)' characterized the other five cultivars. The enantiomeric profile revealed a common enantiomeric excess of the (−)-enantiomer for limonene and α-pinene, (+)-enantiomer for linalool, and a cultivar dependence for α-terpineol. As the ratio of linalool to 2-(E)-hexenal seems important for consumer acceptability, further sensorial analyses are needed to confirm how differences in this ratio observed among cultivar impact the aroma of the six cultivars considered.

CRediT authorship contribution statement

Joana Pico: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **Eric M. Gerbrandt:** Conceptualization, Funding acquisition, Writing - review & editing. **Simone D. Castellarin:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130812.

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