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Review

A Review of the Fruit Volatiles Found in Blueberry and Other *Vaccinium* Species

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ABSTRACT: Variations in volatile organic compound (VOC) type and content can result in noticeable differences in fruit aroma. The genus *Vaccinium* encompasses over 500 distinct species of berry-producing plants, several of which are important to commercial horticulture. Understanding which VOCs are produced by different members of this genus could greatly aid efforts to improve the aroma of multiple *Vaccinium* crops by breeding for desirable fruit volatiles. This review summarizes the published research available on this topic, examining prior work done to characterize the volatile profiles of blueberries, cranberries, bilberries, lingonberries, whortleberries, and other *Vaccinium* berries. In addition, analytical methodologies used to obtain *Vaccinium* berry volatile profiles are discussed. Possible future directions for *Vaccinium* berry volatile research are also examined.

KEYWORDS: *Vaccinium, VOCs, volatile collection, breeding, aroma, sensory*

■ INTRODUCTION

Flavor is considered a fundamental component of fruit quality. The experience of flavor as interpreted by the human brain is the result of a combination between the senses of taste and retronasal olfaction.[1,2] While perception of sweet, salty, sour, bitter, fat, and umami by the tongue is the outcome of the sense of taste, the sensation of aroma is the direct result of particular volatile organic compounds (VOCs) being detected by the olfactory bulb of the nasal cavity.[2,3] When combined with sensory data from the tongue, the olfactory perception of different types of VOCs in different ratios enables humans to experience an almost infinite range of distinct flavors.

A common tendency in plant breeding has been to generally overlook flavor when developing new commercial fruit cultivars in favor of traits such as yield, fruit size, disease resistance, firmness, and shelf life. Nevertheless, consumers have made it very clear through surveys and purchasing patterns that they prefer fruits with both high-quality aroma and superior flavor.[4] The premium that consumers are willing to pay for more flavorful varieties would therefore justify the development of new fruit cultivars specifically bred for flavor-related traits like aroma.[5] This was the logic that led to the production of the flavor-enhanced "Tasti-Lee" tomato cultivar,[6,7] now sold at a premium price in grocery stores across the U.S.

The historical difficulty of accurately determining VOC content has resulted in fruit breeders ignoring aroma volatile levels when breeding for better flavor and instead focusing on more easily measured compounds such as sugars and acids. However, it has been demonstrated that simply modifying levels of taste compounds such as these is insufficient for generating higher-quality fruit flavor; aroma volatiles are a necessary component of this goal.[8,9] Acknowledging the contribution of VOCs to flavor has resulted in the selection and development of commercially successful fruit cultivars like Cotton Candy grapes and the aforementioned Tasti-Lee tomato. Replicating such successes with fruit crops from the genus *Vaccinium* will require a thorough understanding of the nature of the VOCs found in berries of those species.

The economic significance of more flavorful *Vaccinium* berries should not be underestimated; after all, the market for blueberries alone has become one of the fastest growing in the world.[10,11] Global blueberry production in 2018 was estimated at 914 000 t and is projected to keep rising according to the International Blueberry Organization.[12] The introduction of low-chill southern highbush blueberry cultivars in the latter half of the 20th century has enabled countries and regions with warmer climates to become important players in global blueberry production.[12] To illustrate, states in the Southeastern U.S. such as Georgia, Florida, and Texas now produce over 21% of all fresh-market highbush blueberries sold per year, in places where historically only lower quality rabbiteye blueberries with inferior production values could be grown. Cranberries, while previously the most valuable and widely consumed *Vaccinium* berry in the U.S.,[13] still maintain commercial importance both domestically and internationally particularly during the holiday season.[14] Additionally, bilberries, lingonberries, and whortleberries are popular fruits in much of Europe.[15] These *Vaccinium* berry types each have distinctive flavors and are reported to be beneficial to human health with their low sugar content and high levels of antioxidants, fiber, and other health-promoting compounds.[16−21] Consumer demand for *Vaccinium* fruits is thus

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a motivating force to develop cultivars with improved berry aroma and flavor.

ROLE OF AROMA VOLATILES IN FRUIT FLAVOR

Volatile content has been identified as an important fruit trait due to the effects of numerous VOCs on consumer perception of flavor.[22,23] For example, specific VOCs that have been tied to increased perceived sweetness include geranial, 6-methyl-5-hepten-2-one, and β-ionone in tomato[24] along with linalool, ethyl butanoate, methyl butanoate, and furaneol in strawberry.[23,24] Conversely, these studies also found other VOCs in both of these fruits which increased perceived sourness. The sensory effects many VOCs are known to exhibit illustrate the impact these compounds can have on human perception of overall fruit flavor.

By definition, aroma volatiles are those volatile compounds which alone or in combination have the capacity to generate an olfactory sensation (an aroma). Whether these compounds actually produce such a sensation in a particular instance depends heavily on the concentration of the compound(s) within the fruit matrix: different aroma volatiles have different threshold levels below which an olfactory sensation is not produced in humans.[25,26] Thus, not all of the VOCs present in a fruit may play a role in forming that fruit's aroma. As such, when analyzing the effect that fruit VOCs have on flavor, it is crucial to understand not just the aroma description of each compound but also the threshold amount necessary for the average human olfactory system to perceive it. Most studies profiling fruit volatiles do not compare their measurements of VOCs to odor activity or olfactory thresholds for each compound: this is evident at least in research concerning *Vaccinium* aroma volatiles with a few exceptions such as the studies of Du in 2011 and 2014 and a handful of other studies mostly using gas chromatography-olfactometry.[27−30] This remains one of the main challenges for researchers studying the contribution of fruit aroma to fruit flavor: how to objectively determine the levels at which particular VOCs begin to exert an effect on overall flavor. Developments have been more promising in the chemical analysis aspect of fruit aroma research. While early studies on fruit VOCs were often only able to detect between 10 and 30 compounds,[31,32] modern identification and quantitation techniques have enabled researchers to identify greater numbers of distinct VOCs from given fruits than previously reported.[33]

VACCINIUM BERRY VOLATILE RESEARCH

The genus *Vaccinium* includes hundreds of species native to various regions ranging from the tropics to the Arctic, many of which have berries consumed by humans. *Vaccinium* species included in this review include blueberries, cranberries, bilberries, lingonberries, and whortleberries as well as other lesser known relatives (Table 1) and cover the vast majority of *Vaccinium* taxa commercially important to humans. Many researchers have quantified the volatile profiles of different *Vaccinium* species over the past six decades using an assortment of different techniques and have found there to be much variation between species. Yet while most consumers can tell that there are noticeable differences in flavor between different *Vaccinium* species, accurately determining which VOCs contribute most to characteristic flavors and to what extent is considerably more difficult. Attempts to answer this question must begin with an accounting of those volatile compounds that have been previously found in ripe berries of plants from the genus *Vaccinium*. An extensive list of such compounds along with information on which species each was found in has been compiled over the course of this work (Supplemental Table 1).

Table 1. *Vaccinium* Species with at Least One Published Ripe Fruit VOC Aroma Profile Organized by Taxonomic Section with Common Name Given

full species name	taxonomic section	common name
Vaccinium angustifolium Ait.	*Cyanococcus*	lowbush blueberry
Vaccinium corymbosum L.	*Cyanococcus*	highbush blueberry
Vaccinium virgatum Ait.	*Cyanococcus*	rabbiteye blueberry
Vaccinium hirsutum Buckley	*Cyanococcus*	hairy blueberry
Vaccinium macrocarpon Ait.	*Oxycoccus*	American cranberry
Vaccinium oxycoccos L.	*Oxycoccus*	European cranberry
Vaccinium myrtillus L.	*Myrtillus*	bilberry
Vaccinium vitis-idaea L.	*Vitis-idaea*	lingonberry
Vaccinium padifolium Sm.	*Hemimyrtillus*	Madeira whortleberry
Vaccinium cylindraceum Sm.	*Hemimyrtillus*	Azores whortleberry
Vaccinium uliginosum L.	*Uliginosum*	bog bilberry
Vaccinium stamineum L.	*Polycodium*	deerberry
Vaccinium acrobracteatum K. Schum	*Nesococcus*	N/A
Vaccinium gaultheriifolium (Griff.) Hook.	*Calcicolus*	N/A

Volatiles of Blueberries (*Vaccinium* sect. *Cyanococcus*). In common parlance, the word blueberry refers to fruit produced by any species that is a member of *Vaccinium* taxonomic section *Cyanococcus*. Commercially important *Vaccinium* species in this section include the highbush blueberry *Vaccinium corymbosum* L., the lowbush blueberry *Vaccinium angustifolium* Ait., and the rabbiteye blueberry *Vaccinium virgatum* Ait. (*V. ashei* Reade). All are native to various parts of North America and are commercially produced inside and outside of their native range. Recent work has tended to focus on the volatiles of *V. corymbosum* due to its growing importance in global blueberry production (Table 2).[27,34]

The earliest study of blueberry volatiles was performed in 1970 and reported only four VOCs in fruit of the lowbush blueberry *V. angustifolium* (Table 2).[32] Nearly 20 years later, another study reported 23 distinct volatile compounds in ripe fruits of this species.[35] Most recently, a study published in 2012 listed 20 compounds that were found in *V. angustifolium* berries.[36] The two latter studies reported a roughly similar number of compounds but differed greatly on which group of volatiles was declared to be most abundant: Lugemwa et al. found that esters were the most plentiful while Forney et al. found that terpenoids predominated.[35,37]

The earliest volatile analysis of the highbush blueberry, *V. corymbosum*, reported 21 VOCs using commercially available blueberry fruit in 1975.[38] In 1983 another study of *V. corymbosum* greatly expanded the number of volatiles reported, listing 48 distinct compounds (though 21 were only detected at trace levels).[39] Interest in the volatiles of highbush blueberries greatly intensified in the last decade. Beginning in 2011, a number of high-quality studies utilizing state of the art

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Table 2. List of the Literature on Ripe *Vaccinium* Berry Volatiles Indicating the Year, *Vaccinium* Species, Volatile Analysis Method, and Number of VOCs Identified[a]

year published	species	method of quantification	authors	VOCs per species
1967	*V. vitis-idaea*	vacuum distillation	(Anjou, K. and von Sydow, E., 1967)[59]	85
1967	*V. macrocarpon*	vacuum distillation	(Anjou, K. and von Sydow, E., 1967)[52]	89
1968	*V. macrocarpon*	vacuum distillation	(Croteau and Fagerson, 1968)[53]	42
1969	*V. vitis-idaea*	vacuum distillation	(Anjou, K. and von Sydow, E., 1969)[60]	45
1969	*V. myrtillus*	vacuum distillation	(Anjou, K. and von Sydow, E., 1969)[56]	109
1970	*V. angustifolium*	static headspace[b]	(Hall et al., 1970)[32]	4
1970	*V. myrtillus*	vacuum distillation	(von Sydowet al., 1970)[57]	24
1975	*V. corymbosum*	dynamic headspace	(Parliment and Kolor, 1975)[31]	18
1981	*V. macrocarpon*	solvent extraction	(Hirvi et al., 1981)[54]	40
1981	*V. oxycoccos*	solvent extraction	(Hirvi et al., 1981)[54]	69
1983	*V. corymbosum*	solvent extraction	(Hirvi and Honkanen, 1983)[66]	48
1983	*V. myrtillus*	solvent extraction	(Hirvi and Honkanen, 1983)[66]	53
1983	*V. uliginosum*	solvent extraction	(Hirvi and Honkanen, 1983)[66]	28
1983	*V. virgatum*[c]	steam distillation	(Horvat et al., 1983)[45]	41
1985	*V. virgatum*[c]	steam distillation	(Horvat and Senter, 1985)[46]	20
1989	*V. angustifolium*	dynamic headspace	(Lugemwa et al., 1989)[35]	23
1995	*V. boreale*	dynamic headspace	(Baloga et al., 1995)[50]	NA
1995	*V. corymbosum*	dynamic headspace	(Baloga et al., 1995)[50]	NA
1995	*V. darrowii*	dynamic headspace	(Baloga et al., 1995)[50]	NA
1995	*V. elliottii*	dynamic headspace	(Baloga et al., 1995)[50]	NA
1995	*V. myrtilloides*	dynamic headspace	(Baloga et al., 1995)[50]	NA
1995	*V. pallidum*	dynamic headspace	(Baloga et al., 1995)[50]	NA
1995	*V. tenellum*	dynamic headspace	(Baloga et al., 1995)[50]	NA
1996	*V. stamineum*	vacuum distillation	(Horvat et al., 1996)[47]	9
1996	*V. virgatum*	vacuum distillation	(Horvat et al., 1996)[47]	6
1999	*V. corymbosum*	dynamic headspace	(Di Cesare et al., 1999)[78]	26
2008	*V. corymbosum*	SPME	(Saftner et al., 2008)[49]	20
2008	*V. virgatum*[b]	SPME	(Saftner et al., 2008)[49]	20
2009	*V. myrtillus*	SPME	(Rohloff et al., 2009)[58]	97
2009	*V. corymbosum*	SPME	(Zhang et al., 2009)[79]	48
2010	*V. corymbosum*	SPME	(Zhang et al., 2010)[80]	77
2011	*V. corymbosum*	SPME	(Du et al., 2011)[27]	42
2011	*V. corymbosum*	SPME	(Eichholz et al., 2011)[43]	12
2012	*V. corymbosum*	SPME	(Du et al., 2012)[41]	32
2012	*V. corymbosum*	FGC-SAW	(Du et al., 2012)[41]	11
2012	*V. macrocarpon*	SPME	(Ruse et al. 2012)[55]	21
2012	*V. angustifolium*	SPME	(Forney et al., 2012)[37]	20
2012	*V. corymbosum*	SPME	(Forney et al., 2012)[37]	20
2012	*V. hirsutum*	SPME	(Forney et al., 2012)[37]	20
2012	*V. acrobracteatum*	SPME	(Forney et al., 2012)[37]	15
2012	*V. gaultheriifolium*	SPME	(Forney et al., 2012)[37]	16
2013	*V. corymbosum*	dynamic headspace	(Gilbert et al., 2013)[44]	44
2014	*V. virgatum*	SPME	(Beaulieu et al., 2014)[48]	53[d]
2014	*V. corymbosum*	SPME	(Du and Rouseff, 2014)[28]	38
2014	*V. vitis-idaea*	SPME	(Viljanen et al., 2014)[61]	38
2015	*V. corymbosum*	dynamic headspace	(Gilbert et al., 2015)[34]	52
2016	*V. macrocarpon*	SPME	(Zhu et al., 2016)[29]	62
2017	*V. virgatum*	SPME	(Beaulieu et al., 2017)[77]	19
2017	*V. corymbosum*	PTR-TOF-MS, SPME[e]	(Farneti et al., 2017)[33]	36
2017	*V. cylindraceum*	PTR-TOF-MS, SPME[e]	(Farneti et al., 2017)[33]	54
2017	*V. myrtillus*	PTR-TOF-MS, SPME[e]	(Farneti et al., 2017)[33]	21
2017	*V. virgatum*	PTR-TOF-MS, SPME[e]	(Farneti et al., 2017)[33]	31
2017	*V. padifolium*	SPME	(Carvalho et al., 2017)[65]	72
2018	*V. padifolium*	SPME	(Porto-Figueira et al., 2018)[67]	57
2020	*V. corymbosum*	solvent extraction	(Ferrão et al., 2020)[42]	17

[a]Studies that reported several close isomers as each being a separate compound are considered throughout this review to report only a single compound if another study reports the mixture of the same isomers as an individual volatile. This convention allows the number of unique volatiles to be compared across studies that have different ways of reporting isomers. Compounds indicated by authors as likely deriving from solvent have been omitted from the total VOC count. [b]Formerly known as (*V. ashei* Reade). [c]Static headspace extraction prior to modern SPME method. [d]Includes VOC of immature and overripe berries. [e]VOCs quantified were obtained from PTR-TOF-MS as opposed to SPME GC-MS.

analytical techniques published the volatile profiles of numerous *V. corymbosum* varieties.[28,33,34,40−42] Unlike earlier analyses, these studies all examined intraspecific variation in the volatile profile composition throughout different *V. corymbosum* cultivars. Great intraspecific variation of VOC content was observed between cultivars even while using identical analytical methods: this is particularly evident in the 2014 work of Du et al.[28] As with earlier analyses, "green leaf" volatiles were prominently represented in all of the studies. Two other recent investigations identified smaller numbers of

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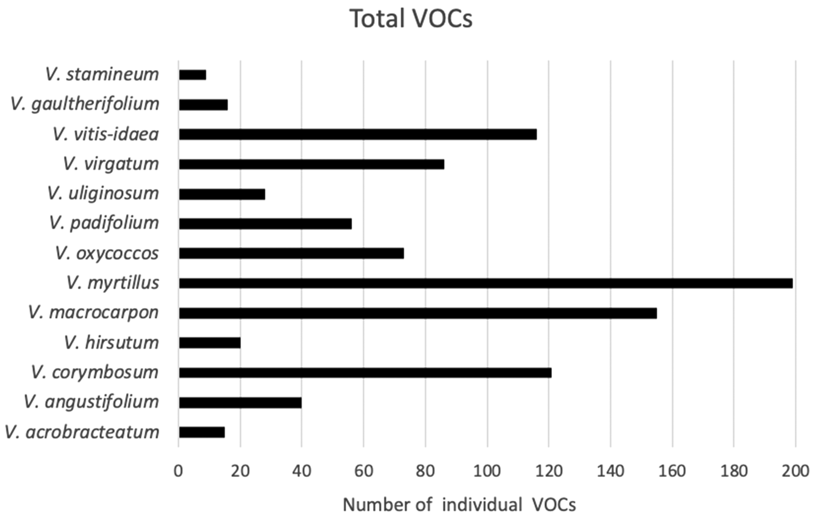

Figure 1. Total number of distinct VOCs by *Vaccinium* species found across studies in this review. VOCs considered were those found in studies where ripe berry volatiles were listed by species.[27,29,32,34,35,37−39,43,45−48,52−59,61,67,77]

volatiles in *V. corymbosum*. The first confirmed the presence of 12 previously reported compounds.[43] This was followed by a study published in 2012 which reported 20 compounds in *V. corymbosum*, some of them for the first time.[36] All 20 compounds reported by the aforementioned study were also detected by the same group in *V. angustifolium* fruits, though at different concentrations.

A 2013 study examined the levels of five blueberry VOCs during ripening which had been postulated to play significant roles in flavor quality:[38] 1-penten-3-ol, hexanal, (E)-2-hexenal, (E)-2-hexen-1-ol, and linalool.[44] Across five commercial *V. corymbosum* cultivars throughout the berry ripening process, average VOC levels increased, yet the concentration of the VOCs 1-penten-3-ol, hexanal, and (E)-2-hexenal were highest at the red stage (pink or immature) and decreased as the berries matured into the fully ripe blue stage.[44] This finding is consistent with another study that found the same pattern across three highbush blueberry cultivars for total aldehyde volatiles.[33] Additionally, the work of Gilbert et al. (2013) showed the monoterpene linalool increased in concentration during ripening in all but one cultivar, and in the 2017 Farneti et al. study this VOC was reported to increase during ripening and remained at high levels as the fruit matured.[33,44]

The earliest volatile analysis for the rabbiteye blueberry *V. virgatum* was published in 1983 and identified 42 compounds.[45] Several uncommon terpenoids were found by this study to be present in *V. virgatum* fruits. In a subsequent study by the same group, volatiles present in three rabbiteye cultivars were assessed during three ripening stages, with the result being that 20 VOCs were established as definitively present in ripe berries.[46] In a less comprehensive study in 1996, only the names of six of the most major compounds were given while several unknown VOCs were detected.[47] More recently, a study published in 2014 was able to identify 53 compounds from samples taken throughout ripening stages for five rabbiteye cultivars.[48] Consistent with studies conducted with highbush blueberries, this analysis also found great intraspecific variation among volatile profiles from different cultivars.

Highbush and rabbiteye cultivars have not generally been compared directly in the same study with the exception of the recent work of Farneti et al. in 2017.[33] In both rabbiteye[48] and highbush[27] blueberry cultivars, terpenoid abundance and diversity have been shown to vary considerably between certain cultivars and during ripening stages.[44,46,33] The same is true for green leaf aldehydes and related derivatives.[48,27] Rabbiteye and highbush blueberries have distinct sensory profiles which were hypothesized to be the result of their VOC differences.[49] The previously mentioned Farneti et al. study of mature *V. virgatum* and *V. corymbosum* fruits showed that the two species only shared 13 VOCs in common out of *V. virgatum*'s 31 VOCs and *V. corymbosum*'s 36 compounds.[33] These variations in VOCs could potentially be responsible for the differences in aroma between the two species noted by sensory panels.[28,34,49]

Volatile data for other species in section *Cyanococcus* remains limited with the exception of the wild blueberry species *V. hirsutum* Buckley where 20 VOCs have been reported. All 20 of these VOCs were simultaneously found in both highbush and lowbush blueberry fruits.[36] A study in 1995 did analyze berry volatiles from seven wild diploid blueberry species; however, only a single list of all compounds detected from all of the samples combined was published, thus yielding no species-specific volatile data.[50]

Many authors have converged on a consensus that the blueberry aroma profile is dominated in quantity by green leaf aldehydes such as hexanal and (E)-2-hexenal with additional relatively high levels of monoterpenes such as linalool.[28,38,51] Although relatively less abundant, esters have been proposed to be responsible for the fruity aroma characteristics noted in ripe blueberries at full maturity.[33] Most of the relevant analyses reported terpenes such as linalool to be present at higher levels in rabbiteye blueberries, and these compounds were found to be less abundant and diverse in lowbush blueberries. Nearly all of the studies showed highbush blueberries as displaying the greatest overall diversity of VOCs (Figure 1).

Volatiles of Cranberries (*Vaccinium* sect. *Oxycoccus*). Volatile components of cranberries have been studied since the late 1960s. In 1967, the American cranberry, *Vaccinium macrocarpon* Ait., was the subject of a volatile analysis utilizing an extract of pressed cranberry fruit solids. This extract contained 89 identified VOCs.[52] A year later, another group published a separate analysis of *V. macrocarpon* volatiles. In contrast to the first study, this analysis used an organic solvent extract of only cranberry fruit juice. A total of 42 distinct VOCs

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J. Agric. Food Chem. 2020, 68, 5777−5786

were identified in *V. macrocarpon*, constituting an estimated 95% of all volatile compounds present.[53] The authors estimated that an additional 200 distinct VOCs (comprising the remaining 5% of juice volatiles) were not detectable in this analysis. Furthermore, they hypothesized that the unidentified volatiles were responsible for the "delicate aroma of natural cranberry extract" compared to an artificial preparation of cranberry aroma made from the 42 identified VOCs in their natural ratios.[53]

In a 1981 study, the VOCs of commercially cultivated American cranberries were compared to those from berries of a wild European relative (*Vaccinium oxycoccos* L.). This study found a total of 69 distinct compounds present in *V. oxycoccos*, while only 40 of those were found in *V. macrocarpon*.[54] The concentrations of volatiles found in the American cranberry were greatly reduced compared to its European counterpart. This suggests that generations of selection for other traits may have left the commercially cultivated American cranberry with a weaker aroma profile when compared to wild relatives, a phenomenon documented in other crops like tomato.[22]

In a study published in 2012, VOCs of commercially cultivated *V. macrocarpon* berries were again compared to those of wild European *V. oxycoccos* fruits. Five cultivars of *V. macrocarpon* were used, marking a departure from previous research where the volatile composition of only a single American cranberry cultivar was examined. This study only detected 21 volatile compounds across all cultivars and species used, with the five cultivars of *V. macrocarpon* showing notable differences in both VOC presence and concentration.[55] Furthermore, in contrast to the 1981 study, this particular analysis found that *V. oxycoccos* produced fewer distinct volatiles than *V. macrocarpon*. No evidence of higher *V. oxycoccos* VOC concentrations was found in this study.

In a similar study published in 2016, researchers sampled four cultivars of *V. macrocarpon* and analyzed their VOC profiles and sensory characteristics.[29] Across the four cultivars, 62 distinct volatiles were identified, 32 of which were present in at least one cultivar in high enough concentrations for detection via human olfaction.[29] Sensory analysis revealed that the aromas of the four cultivars were distinct enough for panelists to reliably differentiate between the cultivars when asked to describe the aroma notes of each.

There is some consensus across studies as to the most abundant VOCs in cranberry. All the studies examined agree that benzyl compounds, such as benzyl alcohol, benzoic acid, and benzaldehyde, make up a major proportion of total cranberry VOC content. Likewise, nearly all the studies agreed that α-terpineol was present in cranberries at relatively high levels. Apart from these compounds, however, there is disagreement among the studies as to exactly which other compounds are the most abundant in cranberry fruit. Whether this is due to differences in analytical methodologies or environmental and genetic effects remains an open question.

Volatiles of Bilberries (*Vaccinium* sect. *Myrtillus*). *Vaccinium myrtillus* L., the European bilberry, is a *Vaccinium* species with fruits very similar in appearance to wild blueberries but is from a taxonomically distinct section and has its own distinguishable aroma profile. Bilberry volatiles were first analyzed in a 1969 study where 109 distinct volatile compounds were found in ripe fruits.[56] However, a subsequent study in 1970 by the same researchers using pressed bilberry juice found only 24 VOCs, suggesting that greater VOC diversity exists within whole bilberry fruit tissue compared to freshly pressed bilberry juice.[57] A study published in 1983 compared bilberry VOCs to those of the highbush blueberry. Bilberry pressed juice was used to identify 53 volatile compounds, of which 33 were also found in highbush blueberries in the same study.[39] Comparison between the two species showed that γ-octalactone and γ-decalactone were found at relatively high concentrations in bilberry but were absent in blueberry.

The most thorough and most recent analysis of bilberry VOCs sampled 32 geographically separated *V. myrtillus* populations from Norway.[58] Researchers found 130 VOCs across all populations sampled, 97 of which were readily identifiable.[58] The geographically distinct samples used in this study gave researchers insight as to variations in VOC content among individual wild *V. myrtillus* populations, a first for this species.

Across all studies, it becomes clear that a great number of the volatile compounds reported in bilberries have also been reported in blueberries. As with blueberry, several of these studies also found that six carbon aldehydes such as hexenal and (E)-2-hexenal were also present in significant amounts. However, bilberry did present numerous compounds not detected by any of the studies in blueberry. Characterizing bilberry VOCs using sensory analysis or olfactometry would help elucidate which VOCs are responsible for the "distinctive flavor" often noted as belonging to *V. myrtillus*.

Volatiles of Lingonberries (*Vaccinium* sect. *Vitis-idaea*). The only species in its section, lingonberry (*Vaccinium vitis-idaea* L) VOCs were first analyzed in the 1960s in two studies performed by the same researchers. The first study used pressed lingonberry residue as the source of volatiles for analysis, while the second utilized freshly pressed lingonberry juice for sampling. These authors reported 85 identifiable fruit volatiles in the first study[59] and 45 in the second.[60] The extract from the press cake contained a greater number of nonpolar, hydrophobic VOCs, while the extract from lingonberry juice contained VOCs that were both more polar and more hydrophilic. A 2014 study comparing the impact of various bioprocessing methods on lingonberry aroma analyzed the volatiles of untreated lingonberries to serve as a control.[61] In the unaltered control sample, 38 distinct volatile aroma compounds were identified. All three studies reported that 2-methylbutanoic acid made up a large proportion of the total lingonberry volatiles. This compound has been reported to have an odor described as fermented, cheesy, and rancid.[62] The authors of the first two studies hypothesized that this molecule may be the key volatile primarily responsible for lingonberry's unique aroma.[59,60]

Volatiles of Whortleberries (*Vaccinium* sect. *Hemi-myrtillus*). The whortleberries are a group of taxonomically distinct *Vaccinium* species distributed across temperate mountainous regions of Europe and Asia.[63] Whortleberries have been proposed as a source of valuable new traits to introgress into commercial *Vaccinium* germplasm and have been found to cross easily with *Vaccinium* from section *Cyanococcus*.[64]

Two studies analyzed the VOCs present in ripe fruits from the Madeira whortleberry *Vaccinium padifolium* Smith, a rare member of the *Vaccinium* section *Hemimyrtillus* endemic to the Portuguese Madeira Islands. One study examined wild populations of *V. padifolium* from three distinct geographic areas, while the other analyzed berries of a named cultivar (Uveira). The first study identified a total of 72 distinct

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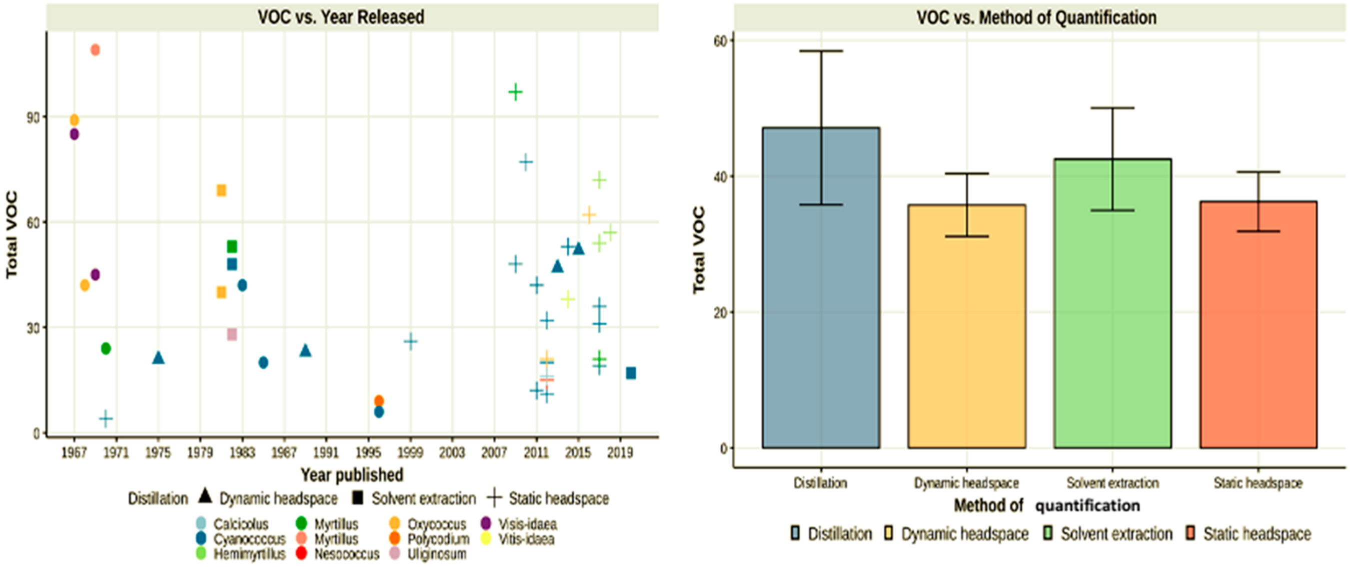

Figure 2. (A) Number of VOCs in studies of *Vaccinium* berry volatiles by year of publication, taxonomic section, and method of quantification. (B) Average number of *Vaccinium* VOCs identified by method with bars indicating standard error.

volatiles, with the same volatiles mostly present in fruits from each location but varying in concentration across geographic areas.[65] Fruits of *V. padifolium* were reported to produce large amounts of medium size straight chain alcohols; the authors hypothesized that this may be due to the greater exposure to UV wavelengths experienced by plants growing in the high-altitude Madeira Islands. Radiation has been shown to alter the berry volatile composition in other *Vaccinium*.[43,65] The second study also reported 72 volatiles as being present in fruits of *V. padifolium* (cultivar Uveira) across ripening stages; however, only 57 of these were detected in ripe berries.[65] Consistent with the first study, these authors observed a higher proportion of alcohols in the ripe stage.

Another species of whortleberry, the Azores Archipelago endemic *Vaccinium cylindraceum* Smith, was included in a 2017 study analyzing the VOC profiles of several *Vaccinium* species using proton transfer reaction-time-of-flight-mass spectrometry (PTR-TOF-MS).[33] Compared to the 10 other blueberry and bilberry accessions analyzed, *V. cylindraceum* had the most aromatic profile in terms of both VOC diversity and VOC concentrations; however, no sensory thresholds were tested in this study.[33]

Across studies dealing with whortleberry VOCs, it has been observed that the volatile profile of ripe berries largely resembles that of blueberries; one exception was that a greater quantity of medium chain alcohols was present in whortleberry species that grow at high elevations. Whether other *Vaccinium species* would display similar changes to their volatile profiles if grown at the same heights poses an open question. Apart from that observation, there is little else that can definitively be said about whortleberry aroma volatiles in general without more research.

Volatiles of Other *Vaccinium* Berries (Various Other Sections of the Genus *Vaccinium*). Berry volatile profile data exists for several other *Vaccinium* species from taxonomic sections other than those already discussed. Some of these species may find use as sources of traits for more conventional *Vaccinium* taxa: at least one has been shown to hybridize with the commercially important highbush blueberry.[66] This species, *Vaccinium uliginosum* L., belongs to the monotypic *Vaccinium* section *Uliginosum* and is commonly known as the "bog bilberry" despite not being from*Vaccinium* section *Myrtillus*. A 1983 study of the VOCs from several edible types of *Vaccinium* species included fruits of *V. uliginosum*. This study found 28 distinct VOCs in ripe *V. uliginosum* berries.[66] Bog bilberries had fewer compounds and less compound diversity when compared to blueberries and standard bilberries in the same study and were missing several VOCs prominent in other *Vaccinium* species.

Vaccinium stamineum L. belongs to the section *Polycodium* and is known as the deerberry throughout the Southeastern United States. The berry VOC profile of this species was reported as part of a 1996 study where deerberry volatile content was analyzed at three stages in berry ripening. Deerberries showed a pattern of increasing linalool levels as ripening progressed.[47] In contrast, other deerberry terpenoids such as α-terpineol and the sesquiterpene β-caryophyllene decreased in concentration as berries ripened, consistent with similar findings in whortleberries.[47,67] Only nine major volatile compounds were measured in fully ripe deerberries, although the study listed several other minor volatiles as detected without specifying if they were present in the ripe stage or not.[47] This study is consistent with the previously discussed work in blueberries,[33,44] illustrating that as *Vaccinium* berries ripen the greatest abundance of VOCs can often be found at the stage right before full ripeness. Most of the VOCs found in the deerberry study have been readily detected in berries of other *Vaccinium* species, but one compound in particular stands out: 3-methyl-3-butenyl 3-methylbutanoate. This compound was not found in other *Vaccinium* fruits and was present in deerberry at high concentrations.[47]

A 2012 study of VOCs from several blueberry species also included analyses of the volatile profiles from ripe berries of two Southeast Asian *Vaccinium* species: *Vaccinium acrobracteatum* K. Schum. and *Vaccinium gaultheriifolium* (Griff.) Hook. f. Neither was reported to be cultivated by humans.[68,69] *Vaccinium acrobracteatum* is a Papua New Guinean species of *Vaccinium* found in the section *Nesococcus*. Fifteen VOCs were identified in fruits of *V. acrobracteatum*.[36] *Vaccinium gaultheriifolium* is a Vietnamese species of *Vaccinium* from section

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J. Agric. Food Chem. 2020, 68, 5777−5786

Calcicolus. The study identified 16 VOCs from ripe fruits of *V. gaultheriifolium*.[36] Both Southeast Asian *Vaccinium* species had fewer distinct VOCs than ripe fruits of the common blueberry species. Notably, a volatile profile for the highbush blueberry also reported by this study was found to contain all of the VOCs identified in both Southeast Asian species.[37] Furthermore, there was little difference between the VOC profiles of *V. acrobracteatum* and *V. gaultheriifolium*.

These studies of uncultivated *Vaccinium* species all report a common theme: berries from the more exotic species tend to have fewer VOCs than berries from more commercially important *Vaccinium* taxa. In contrast to expectations that these unusual *Vaccinium* species would have several rare and uncommon VOCs, it appears that existing studies report only a reduced range of compounds already identified in more mainstream *Vaccinium* species.

■ ANALYTICAL METHODS

The identification of VOCs begins with extraction of compounds from fruit followed by separation and quantification via gas chromatography (GC). The resulting fractions are then usually subject to mass spectrometry (MS) to discern the identity of each compound. A variety of different methods are employed to extract VOCs from fruit tissue. The major methods of extraction in the literature pertaining to fruit volatile analysis include solid-phase microextraction (SPME) from static headspace, dynamic headspace trapping, distillation extraction, and direct solvent extraction (Table 2).[70−73] Method selection during a particular analysis depends upon the type of sample used as well as the goal of the analysis.

Static headspace sampling using solid phase microextraction (SPME) is the most common modern method performed, as seen in Figure 2. The majority of *Vaccinium* fruit volatile studies performed in the past two decades employed SPME for VOC collection, and several have been able to quantify more than 50 distinct VOCs while using this method (Figure 2).[27,33,48,67]

Dynamic headspace sampling collects VOCs from a homogenized, sliced, or juiced sample of fruit by using an inert carrier gas to collect and transport volatile compounds into an absorbent fiber trap.[74] In the context of analyses of *Vaccinium* berry volatiles, it was found that similar numbers of distinct VOCs were reported by studies using SPME as were reported by studies which used dynamic headspace trapping to collect the volatile compounds (Figure 2). For many of the older studies this review examined, dynamic headspace was used as the method of choice to collect volatiles from *Vaccinium* fruit tissue. In contrast, *Vaccinium* volatile analyses utilizing SPME tend to be more recent; to illustrate, the first such analysis was only published in 2008.[49] This shift may be due to the fact that while SPME has been found to capture the same range of individual VOCs as dynamic headspace,[70] it is considered to be easier to perform and can also be automated.

A third method used to collect volatile compounds for analysis is solvent extraction of fruit juice, a method where organic solvents are used to partition VOCs from juice made from fruit tissue. The VOC-containing solvent extract is then subjected to spectrographic analysis.[75] While common in the oldest *Vaccinium* volatile studies, this method has largely been supplanted by dynamic headspace and SPME in studies from more recent decades (Figure 2).

Distillation techniques of VOC collection have also been used in older *Vaccinium* berry volatile analyses and include steam-distillation, vacuum distillation, and heating and sweeping. The effectiveness of distillation-based methods in capturing a wide range of *Vaccinium* volatiles appears to be high: a study which used such methods to capture VOCs from bilberry was able to qualitatively identify 109 compounds, comparable to the number of distinct VOCs detected by a 2009 bilberry volatile analysis conducted using SPME (Table 2).[58,60] However, distillation methods of fruit volatile collection are not frequently used anymore, largely because they are more labor intensive and difficult to perform than more recently developed methods such as SPME.[73]

Most contemporary research geared toward determining the volatile profiles of *Vaccinium* berries has utilized SPME, but a few studies have used dynamic headspace and liquid extraction (Figure 2 and Table 2).[34,42,44] The separation of compounds in these studies is most commonly accomplished by a gas chromatograph (GC) directly connected to a mass spectrometer (MS). Older GCs often use a flame ionized detector (FID) to quantify the relative abundance of each compound as it elutes off of the column. A few of the more recent studies have used other, faster analytical instruments and methodologies. An example of this would be the study of Farneti et al. (2017) which made use of proton-transfer-reaction time-of-flight mass spectrometry (PTR-TOF-MS): this methodology drastically reduced the time of separation and mass fragmentation of each sample as compared to SPME GC-MS.[33] Additionally, a study of blueberry volatiles using fast gas chromatography-surface acoustic wave (FGC-SAW) was used to conduct a quick scan of VOCs present in a sample's headspace.[41] However, despite the speed of this methodology, FGC-SAW was only able to quantify roughly one-third of the compounds detected by SPME GC-MS.[41]

■ DISCUSSION

After reviewing the body of published research, several trends become apparent. One which stood out prominently is that while there is overlap among VOCs found in major studies of any given *Vaccinium* species, there is no agreement on the exact concentrations of these compounds in ripe berry tissue. Multiple factors may be responsible for this discordance including differences in genotypes analyzed, quantification methodologies used, and environments where the plants were grown. All of these factors were reported to be associated with variation in the quantity of certain volatiles in individual studies of the same species.[27−29,48] It is thus not inconceivable that such differences alone or in combination may be responsible for the variable concentrations reported by different studies for the same volatile in the same *Vaccinium* species. Furthermore, while there was some general agreement among different studies on the presence or absence of many volatile compounds in fruits of any given species, this concordance did not apply to all of the compounds identified. Many analyses reported the presence of compounds that no other study of the same species was able to find (Supplemental Table 1).

However, despite the variable absolute concentrations of many VOCs reported by different authors working on the same species, there was general agreement in many cases on the relative quantities of particular VOCs and VOC classes as compared to others. To illustrate, in highbush blueberry many independent analyses reported high levels of lipid-derived volatile aldehydes and alcohols. Six-carbon volatile aldehydes in particular were almost universally found to make up the

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J. Agric. Food Chem. 2020, 68, 5777−5786

greatest proportion of VOCs as compared to other groups.[27,31,33,34] Likewise, in American cranberry aromatic (benzene ring structured) phenylalanine derivatives such as benzyl alcohol and benzaldehyde made up a large proportion of reported VOCs in nearly all analyses.[29,52,54,55] In lingonberry, separate studies reported that 2-methylbutanoic acid was present in large amounts when compared to other VOCs.[59−61] Despite different experimental methodologies, the presence and relative abundance of important VOCs and VOC classes in a given *Vaccinium* species can still be reliably established.

Another observation concerns the existence of intraspecific variation in VOC content among different cultivars or genotypes of the same species. While it is not surprising that VOC variation would occur among species within the same taxonomic section, the degree of intraspecific variation for particular compounds was very great in some instances. Noticeable genotype-based intraspecific VOC differences were reported for highbush blueberry[27,28,42] rabbiteye blueberry,[48] cranberry,[29,55] and bilberry.[58] Whether these differences in volatile content among the selected genotypes can translate to detectable genotype-specific differences in *Vaccinium* berry aroma needs to be more thoroughly investigated, though initial results from blueberry and cranberry indicate that this may indeed be the case.[28,29,34,42] Moreover, a recent genome-wide association study has shown that the variation of some VOCs in a highbush blueberry breeding population is controlled by a few major genomic regions, illustrating that VOCs are suitable traits for marker-assisted selection in plant breeding.[42,76]

The genus *Vaccinium* is diverse from both a geographic and evolutionary standpoint. Comparing volatile profiles from *Vaccinium* species of different taxonomic groupings allows researchers to see that this diversity is reflected in the realm of fruit volatiles (Figure 1). A complete list of which VOCs were found in which species is provided in Supplemental Table 1.

Future research efforts should focus on bringing together information from human sensory perception of fruit flavor and combining it with knowledge of which VOCs are present in *Vaccinium* fruits in order to ascertain which volatiles positively and negatively contribute to berry flavor. Breeders could use this information to develop *Vaccinium* berry varieties with improved flavor by breeding for desirable volatile components. Evidence from cranberry indicates that even compounds present in minuscule proportions can collectively have a large impact on the qualitative experience of berry aroma[53] and thus could ultimately affect berry flavor. Additionally, further research is needed to measure the postharvest effects of storage and refrigeration on the volatile content of *Vaccinium* berries. Studying postharvest changes in volatile quality and content in the commercially important *Vaccinium* such as blueberries can help develop methods to better store berries after picking in ways that do not diminish fruit aroma.

The past 60 years of research in this field have revealed many different *Vaccinium* berry volatile profiles and brought new, more robust methods of volatile quantitation to this field. As demand for *Vaccinium* berries grows, knowledge in this area will prove useful to areas such as molecular breeding for improved berry aroma and developing better methods of postharvest berry storage.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c01445.

List of unique VOCs found by *Vaccinium* species with their respective CAS no., including the total number of studies where a compound has been reported and the total number of species where a VOC compound has been reported (PDF)

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Notes

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■ ABBREVIATIONS USED

VOC, volatile organic compounds; GC, gas chromatograph; MS, mass spectrometer; GC-MS, gas chromatography-mass spectrometry; SPME, solid-phase microextraction; GC-O, gas chromatography olfactometry; PTR-TOF-MS, proton-transfer-reaction-time-of-flight-mass spectrometry; FGC-SAW, fast gas chromatography-surface acoustic wave

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