

# Comparison of the Volatile Constituents from Rabbiteye Blueberries (*Vaccinium ashei*) During Ripening

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## ABSTRACT

GLC-MS analyses of steam distillates prepared from Delite, Tifblue, and Woodard cultivars of rabbiteye blueberries at three stages of maturity showed qualitative and quantitative differences in their organic constituents. Fifty-one compounds, consisting of aldehydes, ketones, alcohols, aromatic hydrocarbons, terpenes, esters, and three methyl substituted indanones (the latter tentative) were identified and compared. Most compounds with molecular weights of 100 - 200 amu appeared to increase with maturity and indicate potential use as indices of quality. Terpenes, C<sub>6</sub> unsaturated aldehydes, and unsaturated alcohols were the predominant types of compounds identified in the distillates. Synthetic mixtures of linalool, *trans*-2-hexenal, *trans*-2-hexenol, *cis*-3-hexen-1-ol and geraniol possessed typical fruity aromas reminiscent of fresh blueberry odor-flavor as determined by informal sensory evaluations.

## INTRODUCTION

RABBITEYE BLUEBERRIES (*Vaccinium ashei* Reade) have received recent attention in the southeastern United States because of their potential as a cash crop to developing rural areas. Six cultivars have been recently introduced by the Coastal Plain Experiment Station, Tifton, GA (Brightwell, 1969), that are highly prolific, relatively resistant to insects and diseases, and offer high potential for increasing total acreage in production. Increase in production and economic importance of this crop has necessitated definitive analysis of flavor quality, especially characteristic of variety and stages of development.

Previous investigators have identified some volatiles of low-bush blueberries (*Vaccinium angustifolium*) (Hall et al., 1970). Parliment and Kolor (1975) identified 21 compounds in high bush blueberries (*V. corymbosum*) including low molecular weight esters, alcohols, aldehydes, and terpenes. However, only minor attempts were made to relate three of the identified compounds to blueberry aroma. Hirvi and Honkanen (1983) reported identification of 19 compounds. Horvat et al. (1983) described the identification of 42 compounds in Rabbiteye blueberries (*V. ashei*).

This investigation is a continuation of our earlier research on the identification of volatile compounds in Rabbiteye blueberries to establish definitive chemical indices whereby the quality of blueberries may be determined objectively.

## MATERIALS & METHODS

SAMPLES of Rabbiteye blueberries (cvs. Delite, Tifblue, and Woodard) at three stages of maturity were obtained from the University of Georgia Horticultural Farm, Athens, GA. The berries were separated into three stages of maturity using the following criteria: trace of anthocyanin pigmentation (pinkish); deep blue coloration but not separating easily from the bush, and deep blue coloration with berries readily released from the bush. Two hundred gram samples of the berries were selected and placed in a Waring Blendor with

200 mL distilled water; the container was sealed and the berries macerated for 3 min at medium speed. The resulting slurry was placed in a 3-L round-bottom flask containing 400 mL distilled water. A Likens-Nickerson steam distillation, continuous extraction head was attached (Likens and Nickerson, 1964) to the flask and 120 mL of glass distilled pentane was placed in the solvent flask. The isolation was carried out by boiling the pentane and blueberry macerate for 6 hr. After cooling the apparatus, the flask containing the pentane was removed, cooled to ca. 40°C and maintained at this temperature. The extract was then concentrated to ca. 0.5 mL by blowing a gentle stream of high purity nitrogen on its surface and further concentrated to 100 µL by allowing the solvent to evaporate at ambient temperature. Further concentration to 50 µL was later required to identify the trace constituents in these extracts.

## GLC-MS analyses

Analyses were performed with a Perkin Elmer Model 900 gas-liquid chromatograph equipped with a flame ionization detector on samples ranging from 0.2 - 1.0 µL in volume. The chromatograph was connected by means of an effluent splitter to a DuPont 21-490B mass spectrometer equipped with differential pumping on the analyzer section. Separations were made on a 50m X 0.05 cm glass open-tubular column coated with OV-1. GLC conditions were: carrier gas inlet pressure, 0.6 kg/cm<sup>2</sup>; injector and manifold temperature, 250°C; and column programmed from 55°C to 215°C at 1.5°C/min.

Table 1—Volatile compounds of rabbiteye blueberry identified by GLC-MS<sup>a</sup>

methanol	m/e 154; oxygenated terpene (8)
acetaldehyde*	isomer of butylbenzene* (9)
ethanol*	isomer of pentylbenzene
2-pentanone*	linalool* (12)
1-penten-3-ol*	terpinyl acetate <sup>a</sup>
toluene* (1)	isomer of dodecene
hexanal*	geraniol* (13)
2-furfural*	terpinene-4-ol*
<i>trans</i> -2-hexenal (2)	α-terpineol* (14)
<i>trans</i> -2-hexenol	<i>trans</i> caran- <i>cis</i> -3-ol <sup>a</sup> (15)
<i>cis</i> -3-hexen-1-ol	p-menth-8-ene-10-ol (16)
isomer of xylene	nerol* (17)
isomer of xylene	thymol* (18)
acetylfuran	isomer of octylbenzene <sup>a</sup> (19)
benzaldehyde	carvacrol <sup>b</sup>
pulegone	eugenol* (20)
i-propylbenzene	isomer of divinylbenzene <sup>a</sup> (21)
1,8-cineole	m/e 204; sesquiterpene (22)
myrcene (3)	isomer of pentamethyl-2,3-dihydroindene <sup>a</sup>
allocimene <sup>b</sup>	β-ionone* (23)
isomer of butylbenzene*	2-tridecanone* (24)
terpinolene (4)	m/e 204; sesquiterpene (25)
limonene* (5)	isomer of tetramethylindanone-1 <sup>a</sup> (26)
p-cymene* (6)	isomer of tetramethylindanone-1 <sup>a</sup> (27)
hexanol* (7)	geranyl formate* (28)
	isomer of pentamethyl-1-indanone <sup>a</sup> (29)
	m/e 204; sesquiterpene
	n-eicosane (31)
	octadecyl alcohol (32)

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\* Previously identified in Rabbiteye blueberry (*Vaccinium ashei*).

<sup>a</sup> Numbers in parentheses correspond to GLC peaks in chromatograms.

<sup>b</sup> Identified solely on the basis of a comparison of their mass spectra with standards in the literature.

## COMPARISON OF VOLATILE CONSTITUENTS...

Mass spectrometer conditions were: ion source temperature, 210°C; scan rate, 10 sec per decade; ionizing voltage, 70 eV; and ion source pressure,  $2 \times 10^{-5}$  Torr. Compounds were identified by comparison of their mass spectra and GLC retention times to those of known standards obtained from commercial sources or synthesized by established methods. All reference compounds were analyzed by GLC-MS for purity. Compounds identified on the basis of comparison of their mass spectra with published spectra are designated "tentatively" identified. Estimates of levels of components of interest, *trans*-2-hexenal, *trans*-2-hexenol, linalool and geraniol, were determined by normalization of peak areas. All blueberry isolates were run in duplicate. Precision of the GLC procedure was determined from the results of five repetitive analyses of a standard pentane solution containing these compounds in approximately the same concentration as the isolates.

### Odor evaluation of synthetic mixtures

Synthetic mixtures of select compounds identified in this investigation and those identified previously as "impact compounds" (Parliment and Kolar, 1975) were prepared in pentane in ratios similar to that observed in GLC profiles of the volatile fractions

of ripe blueberries. Aliquots of these solutions and the volatile fractions of ripe blueberries were applied to blotter paper, the solvent was evaporated and informal odor evaluations were made by four experienced odor-flavor researchers for their similarity of odor.

## RESULTS & DISCUSSION

EACH BLUEBERRY cv. examined showed an increase in medium to high molecular weight compounds with maturity. Fig. 1 shows the GLC chromatograms of the volatile fractions from green, midripe, and ripe Woodard berries. Note that most of the compounds represented by peaks 9 - 36 increased in levels with maturity, whereas peaks 1 through 7 decreased.

Similar results were obtained from analysis of the Delite and Tifblue berries. GLC analysis of the volatile fractions from ripe Woodard, Delite and Tifblue berries revealed qualitative and quantitative differences by cv. (Fig. 2). Highly concentrated samples (50  $\mu$ L) were required to reveal peaks 2, 8, 16\*, 19, 21, 23, 25, 29, 31 through 37 in the Woodard berries

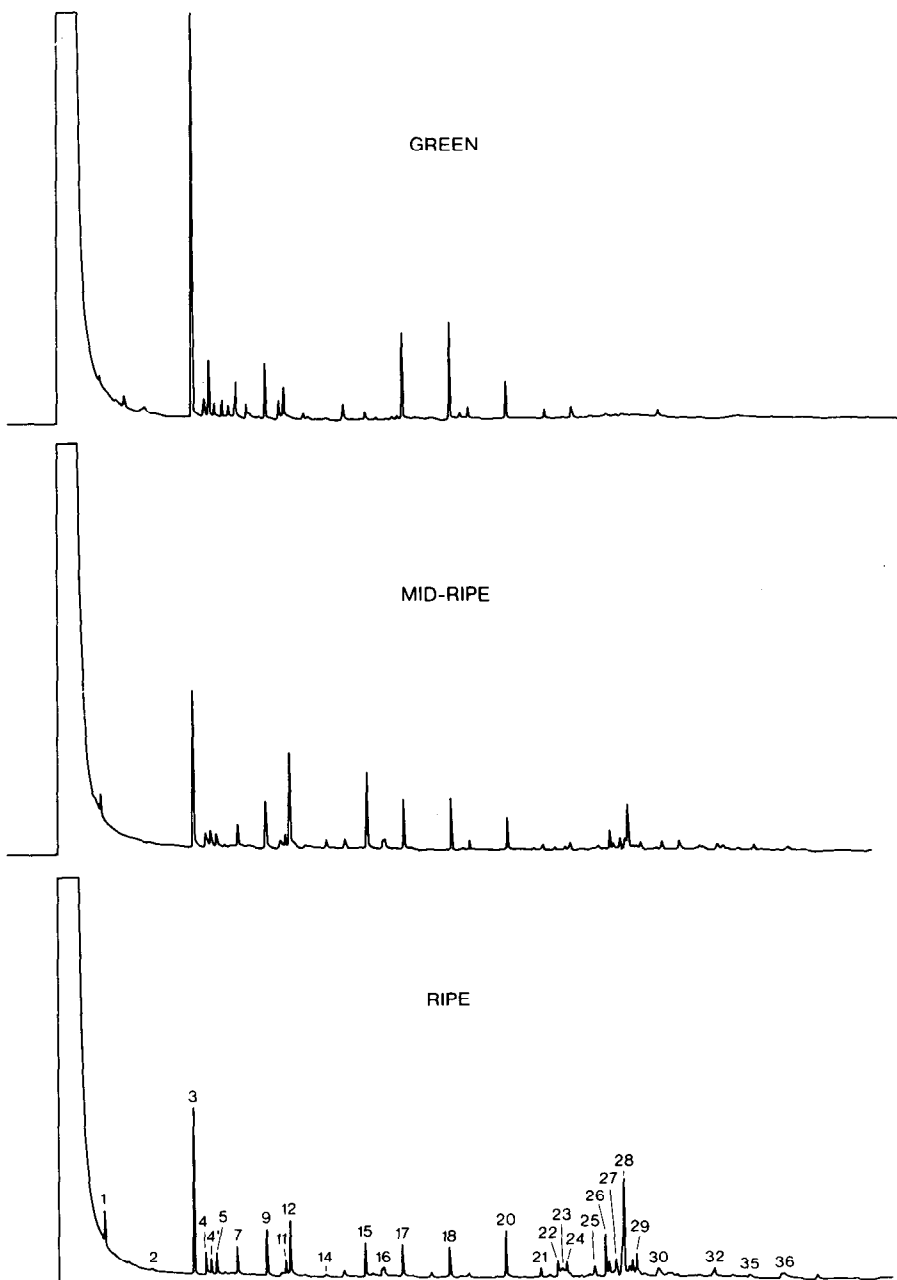


Fig. 1—GLC chromatograms of green (1), midripe (2), and ripe (3) Woodard blueberries.

which were absent in the more dilute concentrates. Analyses of highly concentrated distillates revealed the same range of compounds in all three cvs. Compounds identified are listed in Table 1 in order of elution and include several not identified in our prior research (Horvat et al., 1983). Of particular interest are the tentatively identified three methyl-substituted indanones and pentamethyl-2,3-dihydroindene that have not been previously reported in plant materials. The total number of compounds identified from blueberry volatiles (lowbush, highbush, and Rabbiteye) now totals 62. It appears that the range of compounds present in highbush and Rabbiteye blueberries are very similar.

The compounds selected for comparison as indices of maturity in the three ripening Rabbiteye blueberry cvs. were linalool, *trans*-2-hexenal, *trans*-2-hexenol, *cis*-3-hexen-1-ol and geraniol, the latter three having been previously identified as flavor impact compounds by Parliment and Kolor (1975). The importance of *cis*-3-hexen-1-ol and geraniol to blueberry aroma was established in the present

study by comparing pentane solutions of these two compounds and the three flavor impact compounds identified by Parliment and Kolor (1975) to ripe blueberry isolates by means of an informal odor panel. The consensus of the panel members was that these synthetic mixtures possessed the same aroma as the blueberry isolates. Estimates of the levels of these impact compounds was determined by GLC. Results of these analyses (Table 2) revealed that *trans*-2-hexenal and *trans*-2-hexenol decreased with ripening in all cvs., whereas linalool reached a maximum in the mid-ripe fruit of two of the cvs. In Tifblue, maximum levels of linalool occurred in the ripe berries (12.9%). However, geraniol reached a maximum concentration in the midripe fruit of all three cvs. Differences in levels of these four compounds in the mature fruit of the three cvs. studied appear to reflect subtle differences that were apparent in aroma and taste, although each cv. possessed a typical blueberry aroma.

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Table 2—Concentration of flavor impact components from blueberry volatiles at three stages of maturity

	Delite			Tifblue			Woodard		
	Green	Midripe	Ripe	Green	Midripe	Ripe	Green	Midripe	Ripe
<i>trans</i> -2-hexenal	0.62 <sup>a</sup>	0.33 <sup>a</sup>	0.34 <sup>a</sup>	0.77 <sup>a</sup>	0.51 <sup>a</sup>	0.46 <sup>a</sup>	0.89 <sup>a</sup>	0.49 <sup>a</sup>	0.47 <sup>a</sup>
<i>trans</i> -2-hexenol	0.07	0.05	0.05	0.09	0.04	0.05	0.11	0.08	0.06
<i>cis</i> -3-hexen-1-ol	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0.07	0.05	Tr.
linalool	1.02	1.29	1.47	8.70	9.81	12.92	2.65	12.31	8.47
geraniol	2.92	3.40	2.24	0.13	0.28	0.24	0.57	4.08	2.33

<sup>a</sup> Numbers represent percentage of total area of GLC peaks in chromatograms. Relative standard deviation for *trans*-2-hexenal, *trans*-2-hexenol, and geraniol was 0.01 and linalool 0.25.

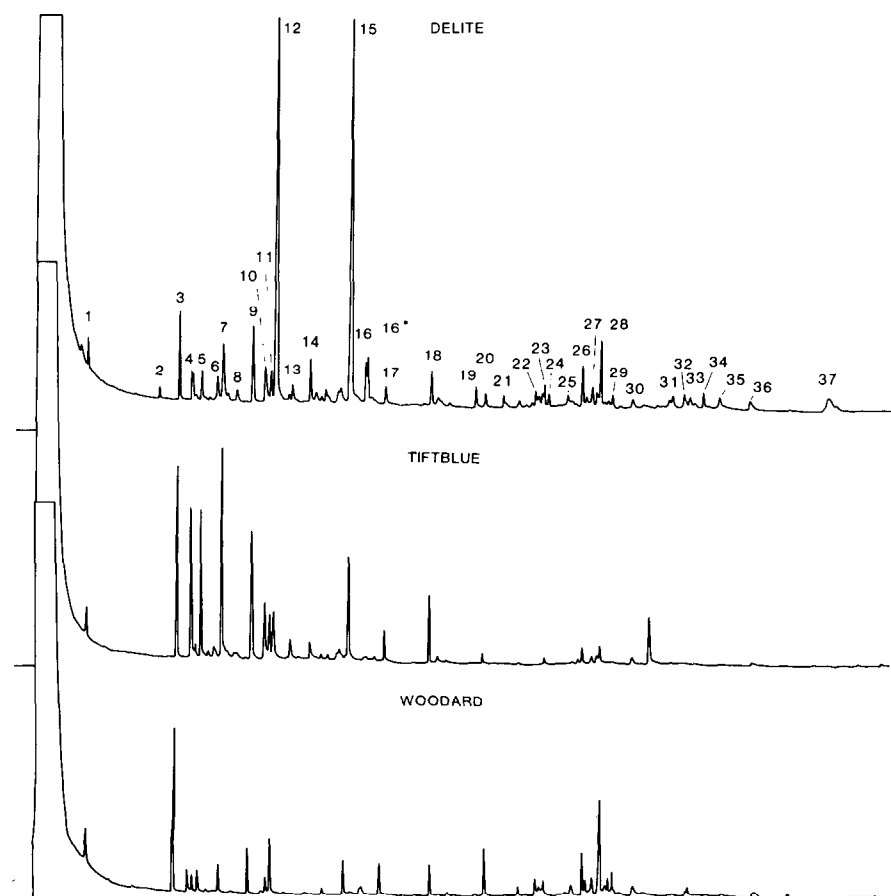


Fig. 2—GLC chromatograms of ripe Delite, Tifblue, and Woodard blueberries.