Sugars and Nonvolatile Acids of Blackberries

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The sugars and nonvolatile acid composition of blackberries were investigated with the purpose of acquiring base-line data which can be used to establish authenticity of blackberry products. Sugars and acids were isolated using ion-exchange techniques, and their Me$_3$Si derivatives were separated by GC. Fifteen samples were analyzed, with sample selection giving consideration to varietal, maturity, and processing effects. Glucose and fructose were the only sugars detected, the glucose/fructose ratio for all samples being 0.710. The acids (phosphoric, malic, lactoisocitric, citric–isocitric, and quinic) showed considerable variation, lactoisocitric being absent in six of the samples. Maturity had considerable effect on the composition, while processing into a concentrate induced little change.

Blackberries of commercial importance include a wide range of domestic varieties as well as certain wild forms. Increasing amounts of the fruit are being processed into concentrate for their subsequent manufacture into wine and beverages and for use as coloring and flavoring agents. Sugars and acids are the dominant chemical entities in fruit juices, and they are less affected by processing and storage than are constituents such as pigments and flavors.

Widdowson and McCance (1935) reported glucose and fructose to be the principal sugars of blackberry and, on the basis of the increase in reducing power after inversion, indicated that small amounts of sucrose were present. Lee et al. (1970) using paper chromatographic techniques reported that blackberries contained, in addition to those three sugars, trace amounts of maltose. Nelson (1925) discovered that isocitric was the dominant acid in blackberries. Whiting (1958) reported malic to be the predominant acid, with large amounts of isocitric and lactoisocitric; trace amounts of quinic and shikimic acids were also shown to be present. Fitelson (1969) detected citric and/or isocitric, and malic acids and an unidentified spot in blackberry juice by paper chromatography; he reported that seven of eight collaborators were able to detect that a sample of blackberry juice had been adulterated with citric acid.

The capability of analyzing for sugars and acids has been greatly extended through gas chromatographic analysis of trimethylsilyl (Me$_3$Si) derivatives. With the purpose of compiling base-line data in order to establish authenticity, the sugars and acids of blackberries were examined on a qualitative and quantitative basis. Varieties of commercial importance in both western and eastern United States, as well as in Europe, were selected; in addition, wild cultivars and hybrids such as Logan, Young, and Boysenberry, which have some blackberry parentage, were examined.

EXPERIMENTAL SECTION

Plant Materials. Fruits of Evergreen blackberry (*Rubus laciniatus*), Olallie (*Rubus spp hyb*), Cherokee (*Rubus spp hyb*), Dirksen Thornless (*Rubus spp hyb*), Logan (*Rubus ursinus* × *idaeus*), Young (*Rubus ursinus* × *idaeus*), and Boysenberry (*Rubus ursinus* × *idaeus*) were obtained from plantings at the Oregon State University Lewis Brown farm. Fruits of Western Mountain Trailing (*Rubus ursinus*) and Himalaya (*Rubus procerus*) were collected in the wild. Samples of Marion (*Rubus spp hyb*) and Evergreen blackberry pulp were obtained from local concentrate manufacturers. Santiam (*Rubus ursinus hyb*)

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fruit was obtained from a local preserve manufacturer. A freeze-dried sample of Bedford Giant, which is reputed to be a seedling of Veitchberry (*Rubus rusticanus* × *Rubus idaeus* 4×), was obtained from East Malling Research station in England. Samples were stored at -24 °C until analyzed.

Reagents. Chemical standards and solvents were of reagent grade, and deionized water was used in dilutions. "Tri-Sil" reagent was purchased from Pierce Chemical Co.

Cationic-exchange resin was prepared by washing the resin with deionized water two–three times; the anionic resin was prepared by washing twice with deionized water, followed by two washings with 0.1 N acetic acid.

Isolation of Sugars and Acids. An ion-exchange procedure (Akhavan et al., 1980) was used for isolation of sugars and acids. The fruit (50.0 g) was thawed and blended with 150 mL of 95% ethanol for 5 min, let stand for 1 h, and centrifuged (2000g for 10 min). The residue was washed twice with 25 mL of 80% ethanol, and the supernatants were combined. Concentrate and pulp samples were diluted to 10° brix, and a 50.0-mL sample was similarly extracted. The extract was percolated through a column containing 5 mL of cationic-exchange resin (Bio-Rad AG 50W-X4, 200–400 mesh in the hydrogen form) and then through a column containing 7 mL of anion-exchange resin (Bio-Rad AG 1-X8, 200–400 mesh, acetate form). The system was washed with deionized water until 2 L was eluted. A 100-mL aliquot of this sugar fraction was made up to 250 mL with distilled water; 1 mL was pipetted into a 3-mL vial which contained 100 μL of 0.2% (w/v) rhamnose in 85.5% ethanol as an internal standard. Samples were taken to dryness on a rotary evaporator (35 °C) and stored under vacuum over P$_2$O$_5$ for at least 24 h.

The acids were recovered from the anionic column by washing with 250 mL of 10 N formic acid, followed with deionized water until 1 L of eluant had been collected. A 100-mL aliquot of the acid fraction was taken to dryness on a rotary evaporator (35 °C), the residue being taken up in 10 mL of water. One milliliter of this solution was pipetted into a 3-mL vial containing 100 μL of 1% (w/v) tartaric acid in 85.5% ethanol as an internal standard. Samples were taken to dryness on a rotary evaporator and stored under vacuum over P$_2$O$_5$ for at least 24 h.

Preparation of Me$_3$Si Derivatives. Sugars in 3-mL vials were converted to their trimethylsilyl derivatives by adding 300 μL of "Tri-Sil" reagent, shaking vigorously in a Buchler Shaker for 5 min, heating at 70 °C for 20 min, shaking for an additional 15 min, and centrifuging. Two microliters of supernatant was injected into the gas chromatograph; samples were analyzed in triplicate.

Acids were derivatized by a similar procedure except that they were heated at 50 °C for 30 min and then centrifuged.

Gas–Liquid Chromatography (GLC) Equipment and Conditions. A Varian Aerograph Model 200 dual-column gas chromatograph equipped with hydrogen flame ionization detectors was used for analysis. Sugars were analyzed on a 3 m by 2 mm i.d. glass column packed with 5% SE-52 on 80/100 mesh Chromosorb W; the column was operated isothermally at 165 °C for 14 min, then programmed at 12 °C/min to 250 °C and held for the remainder of the run. Acids were analyzed on a column of the same dimensions packed with 3% SE-30 on 80/100 mesh Chromosorb W; the column was programmed at 6 °C/min from 100 to 250 °C and held. Nitrogen carrier gas flow rates were 25 mL/min; injector temperature was 190 °C, and the detector was maintained at 250 °C. Retention times and peak areas were determined with a Hewlett Packard Model 3380 A recording integrator.

Quantitative Determination of Sugars and Acids. Quantities of individual organic acids were calculated by the following formula:

$$\text{mequiv of acid/100 g of fruit} = \frac{A_a}{A_{is}} \frac{W_{is}}{K} \frac{XF}{R} \frac{2}{\text{equiv wt}}$$

A_a/A_{is} is the ratio of the peak area of the acid to the tartaric internal standard; W_{is} is the milligrams of internal standard added to the vial; and K is the detector response factor for a given acid. K values for phosphoric, malic, citric, and quinic acids had previously been determined in this laboratory (Akhavan et al., 1980). Detector response values for isocitric and lactoisocitric were determined from peak areas of standard acid derivatives and were calculated as $K = (A_a/A_{is})/(W_a/W_{is})$. XF is the dilution factor and R is the percent recovery. Recoveries were determined by putting 50 mg of each acid (dried over P_2O_5 for 36 h) through the extraction, isolation, and derivatization procedures; the R value reported is the average of two replications of duplicate trials. The factor 2 converts the 50-g sample to 100 g.

Quantities of individual sugars were calculated from an analagous formula:

$$\text{g/100 fruit} = \left(\frac{A_s}{A_{is}} \frac{W_{is}}{K} \frac{XF}{R}\right) 2$$

R and K values for the sugars had been previously determined in this laboratory (Akhavan et al., 1980).

Minimum detectable quantities of sorbitol and sucrose were determined by drying and derivatizing solutions of the following concentrations: 5.0×10^{-2} mg/mL, 5.0×10^{-3} mg/mL, and 5.0×10^{-4} mg/mL.

RESULTS AND DISCUSSION

Figure 1 is a typical chromatogram of the Me_3Si derivatives of blackberry sugars, and Table I lists the relative retention times and identities of peaks. Only fructose and glucose were found in all samples analyzed. Sucrose was not detected in any of the samples, the minimum detectable quantity with this analytical method being 0.015% on a fresh weight basis. These results are contrary to the work of Widdowson and McCance (1935) who reported 0.24% sucrose, and Lee et al. (1970) who found 0.58 and 0.60% sucrose (fresh weight) and 0.96 and 0.36% maltose (fresh weight) in two blackberry samples. No disaccharide peaks were found in the 15 samples analyzed in this study. The 96% recovery for sucrose (Table I) and the fact that glucose and fructose peaks were not evident in the sucrose standards which had been subjected to similar extraction,
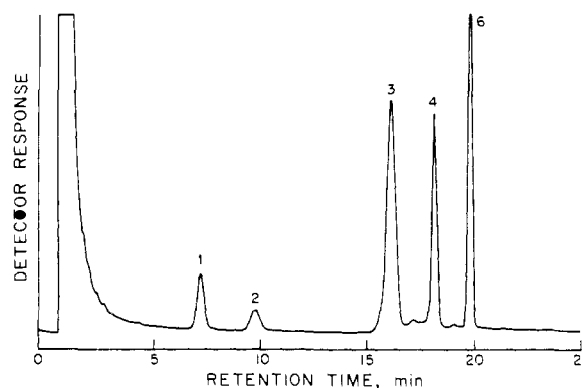
Figure 1. GC separation of Me_3Si derivatives of Evergreen blackberry sugars on SE-52 column. Peaks 1 and 2, rhamnose (internal standard); peak 3, fructose; peak 4, α-glucose; peak 6, β-glucose.

Table I. Relative Retention Times (t_R), Detector Response Response Factors (K), and Recoveries of Blackberry Sugar Sugars and Acids and Authentic Standards

compd	t_R SE-52[a]	t_R SE-30[b]	blackberry peak with coincident t_R SE-52	blackberry peak with coincident t_R SE-30	K	% recov
rhamnose[c]	0.38, 0.53		1, 2[c]			
fructose	0.88		3		0.82^d	
α-glucose	1.00		4		1.56^d	100^d
β-glucose	1.09		6		1.56^d	100^d
sorbitol	1.05				1.54^d	98
inositol	1.17					
sucrose	1.98				1.14^d	96
phosphoric		0.45		1	1.11^d	98^d
succinic		0.49		2		
malic		0.76		3	1.13^d	91^d
tartaric[c]		1.00		4^c		
lactoisocitric		1.03		5	0.50	54
shikimic		1.22				
citric		1.24		6	0.80^d	79
isocitric		1.25		6	0.80	73
quinic		1.34		7	1.53^d	98^d
α-galacturonic		1.45		9		
β-galacturonic		1.54		11		

[a] Relative to α-glucose. [b] Relative to tartaric acid. [c] Internal standard. [d] Determined by Akhavan et al. (1980).

isolation, derivatization, and separation conditions suggest that inversion of sucrose did not occur in the analytical procedure. The simple pattern for sugars suggests that the method would be appropriate for detection of adulteration with sucrose or sucrose-containing fruits. Presence of sorbitol could be indicative of adulteration with tree fruits such as apple, pear, or plum, which are of much lower economic value. Presence of maltose could suggest adulteration with corn syrup.

Table II lists the quantitative results for the blackberry sugars. The range given for an individual sample (such as Bedford Giant) indicates the variation between GC determinations of a single sample preparation and is quite low. Several samples were subjected to duplicate, triplicate, or quadruplicate analyses, the range for their mean value indicating the variation of the analytical method. Again, reproducibility is quite acceptable. The overall mean, range, and standard deviation for the 15 samples are reported. Fructose is present in largest amounts for all samples except the unripe (2.05% total sugars) Evergreen sample. The overall glucose/fructose ratio is 0.710,

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Table II. Free Sugars in Blackberries[a]

berry sample	fructose, g/100 g[b]	% fructose	glucose, g/100 g[b]	% glucose	total sugars ratio glu/fru	total sugars g/100 g[b]
Cherokee						
1	6.11 ± 0.07	56.3	4.74 ± 0.06	43.6	0.774	10.85
2	5.76 ± 0.94	64.3	3.20 ± 0.10	35.7	0.555	8.96
3	4.90 ± 0.57	59.2	3.38 ± 0.12	40.8	0.689	8.28
4	4.71 ± 0.0	58.1	3.40 ± 0.01	41.9	0.721	8.11
$\overline{X}$	5.37 ± 0.66	59.5	3.68 ± 1.06	40.5	0.681	9.18
Dirksen thornless						
1	4.04 ± 0.07	57.8	2.95 ± 0.03	42.2	0.730	6.99
2	3.76 ± 0.02	58.6	2.65 ± 0.13	41.3	0.705	6.41
3	3.98 ± 0.53	59.8	2.67 ± 0.51	40.1	0.671	6.65
$\overline{X}$	3.93 ± 0.17	58.8	2.76 ± 0.19	41.4	0.704	6.68
Western Mountain Trailing						
1	6.00 ± 0.10	61.5	3.75 ± 0.01	38.4	0.624	9.75
2	6.38 ± 0.01	61.0	4.07 ± 0.01	38.9	0.638	10.45
$\overline{X}$	6.19 ± 0.19	61.2	3.91 ± 0.16	38.6	0.631	10.10
Olallie						
1	4.89 ± 0.05	61.2	3.10 ± 0.03	38.8	0.634	7.99
2	4.00 ± 0.21	60.9	2.56 ± 0.15	39.0	0.640	6.56
$\overline{X}$	4.45 ± 0.45	61.0	2.83 ± 0.27	38.9	0.638	7.27
Bedford Giant	3.57 ± 0.17	61.6	2.22 ± 0.18	38.3	0.622	5.79
Santiam	3.70 ± 0.70	53.3	3.24 ± 0	46.7	0.876	6.94
Himalaya	6.53 ± 0.11	61.7	4.06 ± 0.09	38.3	0.621	10.59
Evergreen (ripe)	6.11 ± 0.01	60.6	3.98 ± 0.07	39.4	0.650	10.09
Evergreen (unripe)	0.90	43.9	1.15	56.1	1.28	2.05
Evergreen pulp						
1	4.93 ± 0.16	61.0	3.15 ± 0.09	39.0	0.639	8.08
2	4.85 ± 0.05	61.1	3.09 ± 0.02	38.9	0.637	7.94
3	5.28 ± 0.02	61.0	3.38 ± 0.11	39.0	0.639	8.66
$\overline{X}$	5.03 ± 0.25	61.0	3.21 ± 0.17	39.0	0.639	8.23
Marion pulp						
1	4.81 ± 0.15	61.0	3.07 ± 0.07	39.0	0.639	7.88
2	4.90 ± 0.12	61.5	3.07 ± 0.09	38.5	0.626	7.97
3	4.98 ± 0.01	61.5	3.12 ± 0.01	38.5	0.626	8.10
$\overline{X}$	4.90 ± 0.11	61.4	3.09 ± 0.03	38.7	0.630	7.98
domestic blackberry concentrate						
1	5.19 ± 0.16	59.4	3.54 ± 0.87	40.5	0.682	8.73
2	5.28 ± 0.05	63.2	3.08 ± 0.02	36.8	0.582	8.36
3	5.40 ± 0.02	62.7	3.21 ± 0.04	37.3	0.595	8.61
$\overline{X}$	5.29 ± 0.11	61.7	3.29 ± 0.33	38.2	0.619	8.57
Boysenberry	3.70 ± 0.15	59.9	2.48 ± 0.11	40.1	0.669	6.18
Loganberry	3.91 ± 0.07	58.5	2.77 ± 0.08	41.5	0.709	6.68
Young						
1	4.67 ± 0.30	59.7	3.15 ± 0.18	40.3	0.675	7.82
2	4.65 ± 0.16	59.7	3.14 ± 0.14	40.3	0.675	7.79
$\overline{X}$	4.66 ± 0.01	59.7	3.14 ± 0.01	40.3	0.675	7.80
$S_{\overline{X}}$	1.39	4.66	0.75	4.68	0.170	2.13
$\overline{X}$	4.54	58.9	3.05	41.1	0.710	7.61
R	0.90-6.53	43.9-61.7	1.15-4.06	38.2-56.1	0.619-1.28	2.05-10.59

[a] Values reported are the mean and range for two-four GLC determinations. [b] Berry samples are expressed on a fresh weight basis; pulp and concentrate are expressed on a fresh juice (10° brix) basis.

with a standard deviation of 0.170. This value may be a useful index in adulteration investigations. Whiting (1970) stated in his review that glucose exceeds the fructose concentration in most fruits; in apples and pears fructose exceeds glucose by an order of three times. There is less variation in the glucose/fructose ratio for the blackberry samples than for the glucose and fructose content. There are no differences evident in the sugar composition of the wild varieties, the European sample, or the hybrids (Boysenberry, Loganberry, and Young). The sugar composition of the concentrate and pulp samples are not noticeably different from the whole fruit samples.

A typical chromatogram of blackberry Me_3Si acid derivatives is shown in Figure 2, and Table I lists relative retention times and peak identities. These results confirm earlier reports of the presence of lactoisocitric, malic, and quinic acids in blackberries (Nelson, 1925; Whiting, 1958; Fitelson, 1969). Although citric acid had a slightly lower retention than isocitric acid, mixtures of the two compounds could not be resolved; hence the quantitative data

for peak 6 is presented in Table III as citric acid plus isocitric acid. Whiting (1958) and Nelson's (1925) earlier identification work would suggest that the peak would be principally, if not entirely, isocitric acid. Acids present in relatively small amounts that have not been previously reported in blackberry include phosphoric, succinic, and α- and β-galacturonic acids. Peak 8 is a phthalic ester artifact, possibly arising from GC column degradation. Whiting's (1958) report of presence of trace amounts of shikimic acid was not confirmed.

Table III gives the quantitative results for the five major acids in the blackberry samples. Results are given in both milliequivalents and mg/100 g, and the weight percentage of the individual acids is given as well. The range for the milliequivalent value of a single data point represents the variation between GC determinations of a single sample preparation and is generally quite low. The range for the samples which were analyzed in duplicate or triplicate indicated that the reproducibility of the analytical procedure is quite good: one exception is that 2.4% lacto-

Table III. Nonvolatile Acids in Blackberries[a]

berry sample		phosphoric acid			malic acid			lactoisocitric acid	
		mequiv/100 g[b]	mg/100 g[b]	%	mequiv/100 g[b]	mg/100 g[b]	%	mequiv/100 g[b]	mg/100 g[b]
Cherokee		1.45 ± 0.15	47.4	13.7	1.64 ± 0.25	110	31.8		
Dirksen Thornless		1.02 ± 0.10	33.4	8.6	1.26 ± 0.02	84.4	21.7		
Western Mountain Trailing		3.00 ± 0.50	98.1	8.5	0.826 ± 0.029	55.3	4.8		
		2.94 ± 0.50	96.1	9.6	0.729 ± 0.029	48.8	4.8		
	$\overline{X}$	2.97 ± 0.03	97.1	9.0	0.778 ± 0.049	52.0	4.8		
Olallie		1.10 ± 0.11	36.0	3.2	3.61 ± 0.21	242	21.7		
		1.04 ± 0.02	34.0	3.0	5.06 ± 0.13	339	29.6		
	$\overline{X}$	1.07 ± 0.03	35.0	3.1	4.34 ± 0.72	290	25.7		
Bedford Giant		2.03 ± 0.60	66.4	3.3	8.84 ± 0.60	592	29.1	0.227 ± 0.069	19.9
Himalaya		0.818 ± 0.04	26.8	5.6	1.10 ± 0.07	73.7	15.2	2.62 ± 0.70	229
Evergreen (ripe)		1.06 ± 0.15	34.6	4.9	1.68 ± 0.03	112	16.0	2.19 ± 1.62	191
		1.09 ± 0.05	35.6	5.6	1.65 ± 0.18	110	17.4	1.56 ± 0.74	135
	$\overline{X}$	1.08 ± 0.01	35.1	5.3	1.66 ± 0.01	111	16.7	1.88 ± 0.32	163
Evergreen (unripe)		0.074 ± 0.04	2.4	0.14	0.050 ± 0.05	3.35	0.19	14.8 ± 0.28	1284
Evergreen Pulp		1.38 ± 0.01	45.1	3.8	2.91 ± 0.01	195	16.6	5.07 ± 0.16	439
		1.32 ± 0.04	43.2	3.7	2.96 ± 0.03	198	17.0	5.00 ± 0.06	435
		1.29 ± 0.03	42.2	3.6	2.79 ± 0.03	187	15.8	5.51 ± 0.29	480
	$\overline{X}$	1.33 ± 0.05	43.5	3.8	2.89 ± 0.10	193	16.4	5.19 ± 0.22	451
Marion Pulp		0.785 ± 0.61	25.7	2.1	0.785 ± 0.04	52.6	4.2	3.09 ± 0.21	270
		0.801 ± 0.04	26.2	2.2	0.784 ± 0.13	52.6	4.5	2.62 ± 0.15	229
		0.784 ± 0.06	25.6	2.2	0.740 ± 0.05	49.6	4.4	1.93 ± 0.26	168
	$\overline{X}$	0.790 ± 0.01	25.8	2.2	0.770 ± 0.03	51.6	4.4	2.55 ± 0.44	222
domestic blackberry concentrate		1.89 ± 0.03	61.9	6.2	2.75 ± 0.03	184	18.5	3.21 ± 0.25	280
		1.85 ± 0.03	60.5	6.2	2.74 ± 0.01	184	18.7	3.02 ± 0.07	263
		1.89 ± 0.03	61.8	6.8	2.66 ± 0.05	178	19.5	2.75 ± 0.04	240
	$\overline{X}$	1.88 ± 0.03	61.4	6.4	2.72 ± 0.06	182	18.9	2.99 ± 0.23	260
Boysenberry		0.535 ± 0.05	17.5	1.5	2.98 ± 0.04	200	16.9		
		0.840	27.5	1.6	2.62	176	10.5		
	$\overline{X}$	0.688 ± 0.15	22.5	1.6	2.80 ± 0.18	188	13.2		
Loganberry		0.781 ± 0.08	25.5	2.6	0.534 ± 0.06	35.8	3.6		
Young		1.52 ± 0.12	49.7	4.6	1.25 ± 0.06	85.1	7.9	0.292 ± 0.368	25.4
		1.91 ± 0.09	62.5	6.2	1.44 ± 0.15	96.5	9.5		
	$\overline{X}$	1.72 ± 0.19	56.1	5.4	1.36 ± 0.08	90.8	8.7	0.146 ± 0.15	12.7
R		0.074-2.97	2.4-97.1	0.14-13.7	0.050-8.84	3.35-592	0.19-31.8	0.0-14.8 14.8	0.0-1284
$S_{\overline{x}}$		0.715	23.4	3.55	2.23	149	9.93	3.98	346
$\overline{X}$		1.26	41.3	5.05	2.20	146	15.0	2.17	189

[a] Values reported are the mean and range for two-four GLC determinations. [b] Berry samples are expressed on a fresh

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%	citric and isocitric acid			quinic acid			total	
	mequiv/100 g^b	mg/100 g^b	%	mequiv/100 g^b	mg/100 g^b	%	mequiv/100 g^b	mg/100 g^b
	2.94 ± 0.44	188	54.3	0.0041 ± 0.0006	0.78	0.2	6.03	346
	4.21 ± 0.66	269	69.1	0.001	1.9	0.5	6.50	389
	15.38 ± 0.16	984	85.3	0.082 ± 0.01	15.7	1.4	19.29	1153
	13.15 ± 3.01	842	83.8	0.099 ± 0.02	19.0	1.7	16.92	1005
	14.26 ± 1.11	913	84.6	0.090 ± 0.09	17.3	1.6	18.10	1079
	13.03 ± 0.21	834	74.9	0.009 ± 0.001	1.7	0.1	17.7	1114
	12.09 ± 0.47	774	67.5				18.2	1147
	12.56 ± 0.47	804	71.2	0.004	0.8	0.1	18.0	1130
1.0	20.9 ± 0.5	1338	65.8	0.092 ±	17.7	0.9	32.9	2034
47.2	2.42 ± 0.33	155	32.0	0.005 ± 0.001	0.9	0.2	6.96	485
27.3	5.57 ± 0.26	356	50.9	0.034 ± 0.01	6.5	0.9	10.5	700
	5.44 ± 0.49	348	54.9	0.027 ± 0.002	5.2	0.8	9.8	634
24.4	5.50 ± 0.07	352	52.8	0.031 ± 0.004	5.8	0.9	10.2	667
72.4	7.58 ± 0.48	485	27.3				23.2	1775
37.3	7.53 ± 0.57	482	41.0	0.078 ± 0.002	15.0	1.2	17.7	1176
37.4	7.40 ± 0.21	474	40.7	0.070 ± 0.003	13.4	1.2	17.4	1164
40.5	7.21 ± 0.78	461	38.9	0.073 ± 0.013	14.0	1.2	17.5	1184
38.4	7.38 ± 0.17	472	40.2	0.074 ± 0.004	14.1	1.2	17.5	1174
21.8	13.83 ± 0.82	885	71.4	0.035 ± 0.022	6.7	0.5	18.5	1240
19.4	13.49 ± 0.18	885	73.3	0 033 ± 0.077	6.3	0.5	17.7	1178
14.7	13.89 ± 0.98	889	78.0	0.042 ± 0.01	8.1	0.7	17.4	1140
18.7	13.74 ± 0.25	879	74.2	0.037 ± 0.01	7.0	0.6	17.9	1185
28.2	6.98 ± 0.12	447	45.0	0.111 ± 0.002	21.3	2.1	14.9	994
26.8	7.08 ± 0.09	453	46.1	0.116 ± 0.001	22.3	2.3	14.8	983
26.3	6.50 ± 0.10	416	45.5	0.092 ± 0.04	17.7	1.9	13.9	914
27.0	6.85 ± 0.35	439	45.6	0.106 ± 0.14	20.4	2.1	14.5	963
	15.01 ± 0.60	961	81.0	0.014 ± 0.001	7.87	0.7	18.5	1186
	22.86	1463	87.5	0.029	5.57	0.3	26.3	1672
	18.94 ± 3.9	1212	84.8	0.021 ± 0.008	6.72	0.5	22.4	1429
	14.27 ± 1.04	913	92.2	0.017 ± 0.007	3.26	0.3	15.6	990
2.4	14.26 ± 0.45	913	84.8	0.022 ± 0.008	4.2	0.4	17.4	1077
	13.26 ± 0.56	849	83.7	0.031 ± 0.021	6.0	0.6	16.6	1014
1.2	13.76 ± 0.50	881	84.3	0.026 ± 0.005	5.1	0.5	17.0	1045
0.0–72.4	2.42–20.9	155–1338	27.3–92.2	~0–0.106	0–20.4	0–2.1	6.03–32.9	346–2034
22.92	5.89	375	20.98	0.106	7.11	0.604	7.34	487
13.7	10.38	664	59.9	0.065	7.26	0.69	16.2	1049

weight basis; pulp and concentrate are expressed on a fresh juice (10° brix) basis.
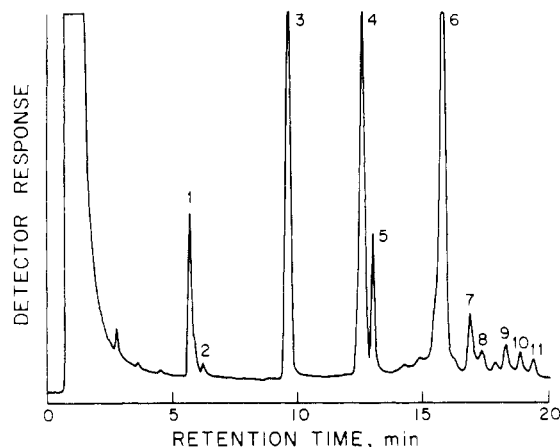
Figure 2. GC separation on Me_3Si derivatives of Evergreen blackberry acids on SE-30 column. Peak 1, phosphoric; peak 2, succinic; peak 3, malic; peak 4, tartaric (internal standard); peak 5, lactoisocitric; peak 6, citric + isocitric; peak 7, quinic; peak 9, α-galacturonic; peak 11, β-galacturonic.

isocitric acid was detected in one Young preparation and none in another.

In comparing the acid composition of the different samples, the most striking result is the absence of lacto-isocitric acid in several of the samples. Analyses for those samples were repeated omitting the tartrate internal standard as it was considered that small amounts of iso-citric might not be resolved from the tartrate peak. There was no evidence of lactoisocitric acid in those determinations. Another consideration was that there may be hydrolysis of lactoisocitric acid to isocitric acid. No isocitric acid was detected, however, in GC analyses of lactoisocitric acid standard preparations. Similarly, no lactoisocitric acid peaks were evident in GC analyses of isocitric acid samples. The detector response (K) values and the percent recovery for lactoisocitric acid (Table I) are lowest of all acids analyzed. The possibility of hydrolysis of lactoisocitric acid or its Me_3Si derivative should not be ignored. The reproducibility for lactoisocitric acid content of replicate samples is reasonably good with the exception of the Young berry sample. Two replicates of duplicate trials were run in determination of the percent recovery of lactoisocitric acid; the range for these determinations were 52.9–56.4%, again indicating the reproducibility of the method. Maturity appears to affect the lactoisocitric acid content as unripe Evergreen fruit contained 72.4% lactoisocitric acid (1284 mg/100 g), while ripe Evergreen samples contained 24.4% (163 mg/100 g). Processing does not appear to affect lactoisocitric acid content to a large degree

as Evergree and Marion Pulp, and the domestic concentrate (which most likely would have been manufactured from Evergree or Marion fruit) contains quantities comparable to the fruit. Lactoisocitric acid is absent or present in low quantities in the blackberry hybrids, Boysenberry, Loganberry, and Young; one wild variety (Himalaya) contained lactoisocitric acid, while the other (Western Mountain Trailing) did not.

Peak 6, which would presumably be mostly isocitric acid based on other worker's reports, is the major acid for all samples except the unripe Evergreen samples. The large range for peak 6 would make it difficult to detect adulteration with added citric and/or isocitric acids. The within sample variation for malic and phosphoric acids is low; however, the range for malic and phosphoric acids content for the different samples is quite large. Our results are contradictory to Whiting (1958) who reported malic acid to be the dominant acid in blackberry. Quinic acid was detected in low amounts in all but the unripe Evergreen sample.

The large qualitative and quantitative variation in the acid composition of these blackberry samples would suggest that using their concentrations in determining authenticity of blackberry products has severe limitations.

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