Varietal Difference in Lipid Content and Fatty Acid Composition of Highbush Blueberries

Lih-Ling Wang, Andrew C. Peng* and **Andrew Proctor**
Ohio Agricultural Research and Development Center, The Ohio State University, Columbus, OH 43210

Lipids from five cultivars of highbush blueberries (*Vaccinium corymbosum* L.) were extracted and fractionated into neutral lipids (60–66%), glycolipids (20–22%) and phospholipids (14–18%). The major fatty acids in all fractions were palmitic (16:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. All lipid classes had a large concentration of C_{18} polyunsaturated acids (84–92%), indicating that blueberries are a rich source of linoleic and linolenic acids. Changes in the fatty acid composition of neutral lipids and phospholipids were not significantly different among the five cultivars, but significant differences were noted in the ratios of linoleic and linolenic acids in the glycolipids fraction.

KEY WORDS: Blueberry lipids and fatty acids.

Information on the chemical composition of blueberries is limited. Most of the available data relates to anthocyanin, sugar, acid and pectin content (1–4). With the exception of studies on the physical structure of surface wax (5,6), blueberry lipids have not been characterized.

Blueberry lipid chemistry has important implications for sensory quality, cell membrane biochemistry and post-harvest physiology. Lipids contribute to the nutritional value and characteristic aroma and flavor in fruits and berries (7). Lipids are a major component of cell membranes and as such have been related to the responses of plant tissues to environmental stress. Changes in membrane lipids have been found to respond to temperature, oxygen, chemical and radiation stresses (8–10). The determination of the component lipid classes and fatty acid composition of blueberries may be useful in understanding membrane composition, source of flavors and nutritionally important fatty acids. The objective of this research was to determine the lipid profile of blueberry cultivars, in terms of their lipid classes and fatty acid composition.

MATERIALS AND METHODS

Materials. Five commercial blueberry cultivars (*Vaccinium corymbosum* L.)—Coville, Darrow, Herbert, Jersey and Bluetta—were harvested and frozen at −10°C.

Lipid extraction. Samples were rinsed with tap water to remove detached pedicels. Lipids were extracted by an adaption of the method of Bligh and Dyer (11). Duplicate 100-gram samples were blended with 300 mL of Folch reagent (12) consisting of chloroform and methanol (2:1, v/v) for 4 min at room temperature. The homogenate was filtered through Whatman No. 1 filter paper in a Buchner funnel with suction. The insolubles were re-extracted with another 75 mL of Folch reagent and filtered. The filtrates were combined, quantitatively transferred to a separatory funnel and allowed to stand for 10 min for phase separation. The lower chloroform phase was collected, and the upper alcohol phase was re-extracted with 30 mL of chloroform. The combined chloroform extracts were washed twice with 0.9% NaCl solution. The extract was concentrated by a rotary evaporator under reduced pressure at 30°C and stored in a vacuum desiccator until a constant weight was obtained.

Separation of lipid classes—(i) Silicic acid column chromatography. Lipid mixtures were separated into three classes by a silicic acid column (13) with a loading ratio of 2:100 (wt/wt) sample: silicic acid in a 1.1 cm in diameter, 30 cm in length glass column. A 100-mg sample of crude lipid extract was dissolved in 5 mL of chloroform and applied to the column sequential eluting solvents: namely 80 mL of chloroform, 60 mL of acetone, and 60 mL of methanol were applied in that order, respectively. The flow rate was 0.5 mL per min. Solvent was removed from fractions with the aid of an evaporator at reduced pressure.

(ii) Screening of fraction purity by thin layer chromatography. The purity of each fraction was monitored by thin layer chromatography. Non-polar lipids were developed with chloroform and sprayed with phosphomolybdic acid. Polar lipids were developed by the Lepage solvent system (14). Ninhydrin reagent was used for detecting phospholipids, and 0.5% α-naphthol was used for the detection of glycolipids (15).

(iii) Fatty acid analysis by gas liquid chromatography. Methyl ester derivatives of the fatty acid components of neutral lipid, glycolipid and phospholipid fractions were prepared according to Metcalfe *et al.* (16). Each fraction was added to 5 mL of 0.5 N methanolic potassium hydroxide and heated for 4 min in a boiling water bath. Three milliliters of boron trifluoride-methanol was added to the mixture and heated in a boiling water bath for 3 min. After cooling to room temperature, the solution was transferred to a 250 mL separatory funnel with 10 mL of petroleum ether (b.p. range 68.6–69.1°C). After mixing and allowing to stand, the upper phase, containing the methyl ester derivative, was recovered. Solvent was removed by a rotary evaporator at 30°C. The methyl ester derivative was redissolved in spectro-grade hexane, and stored at 0°C.

Fatty acid composition was determined using a gas liquid chromatograph (Hewlett Packard model 5890A) with a flame ionization detector (FID) and a Hewlett Packard model 3390A integrator. The stainless-steel column of 305 × 0.32 cm was prepared with 15% (w/w) diethyleneglycol succinate and 1% (w/w) phosphoric acid in a solid support phase of acid-washed Chromosorb W, 80–100 mesh. The column temperature was programmed at the rate of 10°C per

*To whom correspondence should be addressed at the Department of Horticulture, The Ohio State University, 2001 Fyffe Court, Columbus, Ohio 43210-1096.

min from 80°C to 190°C. Detector temperature was 230°C and the injection port temperature was 220°C. The carrier gas was nitrogen with a flow rate of 20 mL per min.

Fatty acid composition of each lipid fraction was identified by comparing the retention time with that of reference compounds. Retention time on the chromatogram was plotted vs carbon number on semilog paper for supplementing unknown methyl esters on the same column under the same conditions.

Statistical analysis. Data were analyzed by one way analysis of variance (17) to detect any significant varietal difference in the lipid fractions and fatty acid compositions among five cultivars ($\alpha = 0.05$). When significant differences were found, Tukey pairwise comparison (18) was used to detect the significance between any two cultivars ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Determination of lipid content. The data in Table 1 show that the crude lipid content of five blueberry cultivars ranged from 0.43 to 0.55 g for 100 g blueberries (2.77-3.74% on a dry weight basis). These results are comparable with those of Watt and Merrill (2) who reported highbush blueberries contained 0.5% lipids in fresh blueberries.

Lipid classes of blueberries. Table 2 compares the total lipids, neutral lipids, glycolipids and phospholipids of five blueberry cultivars. Overall, lipids isolated from blueberry fruit were predominantly neutral lipids (60.6-66.0%), with considerably less glycolipids (20.2-21.6%) and phospholipids (13.7-17.8%). Blueberries, according to Bell's studies (19), have an average of 64 seeds per berry, which may explain the predominance of neutral lipids. Another source of neutral lipids in blueberries is probably waxes. Phospholipids are typical membrane lipids. There was no significant varietal differences in total lipids or lipid classes (Table 2), which may be explained by the similar genetic background of the five cultivars.

Fatty acid composition of blueberries—fatty acid composition of blueberry total lipids. The data in Table 3 show that myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) were the principal fatty acids. In particular, the polyunsaturated acids—linoleic (40.1-45.1%), oleic (17.6-22.5%) and linolenic (16.6-23.6%)—accounted for 82-89% of all fatty acids. The blueberry appears to be a rich source of linoleic and linolenic acids, and may serve as a good source of essential fatty acids. Some minor fatty acids—capric (10:0), lauric (12:0), palmitoleic (16:1), heneicosanoic (21:0), behenic (22:0) and lignoceric (24:0)—were present in a combined concentration of less than 1% of total lipids. The patterns of major fatty acid composition of total crude lipids among the five blueberry cultivars were fairly similar. The only varietal difference observed was that palmit-

TABLE 1

Crude Lipid Content of Five Blueberry Cultivars[a]

Cultivar	Percentage (%) of crude lipid[b] on fresh weight basis	Percentage (%) of crude lipid[b] on dry weight basis
Coville	0.502 ± 0.032[c]	3.41 ± 0.22[c]
Darrow	0.512 ± 0.025[c]	3.44 ± 0.17[c]
Herbert	0.545 ± 0.029[c]	3.74 ± 0.20[c]
Jersey	0.428 ± 0.036[d]	2.77 ± 0.23[d]
Bluetta	0.455 ± 0.047[c,d]	2.97 ± 0.31[c,d]

[a]Data are reported as means ± SD.
[b]Means with the same superscript in the same column are not significantly different at = 0.05 level by Tukey's pairwise comparison (18).
[c]See footnote b.
[d]See footnote b.

TABLE 2

Lipid Classes of Five Blueberry Cultivars Eluted by Column Chromatography (g/100 g)*

	Total lipids	Neutral lipids		Glycolipids		Phospholipids	
Cultivar	(wt)	(wt)	(%)	(wt)	(%)	(wt)	(%)
Coville	0.499[a]	0.325[a]	65.1[a]	0.101[a]	20.2[a]	0.073[a]	14.6[a]
Darrow	0.496[a]	0.326[a]	65.7[a]	0.100[a]	20.2[a]	0.070[a]	14.1[a]
Herbert	0.523[a]	0.345[a]	66.0[a]	0.106[a]	20.3[a]	0.072[a]	13.7[a]
Jersey	0.416[a,b]	0.252[a]	60.0[a]	0.090[a]	21.6[a]	0.074[a]	17.8[a]
Bluetta	0.431[a]	0.267[a]	61.9[a]	0.089[a]	20.6[a]	0.075[a]	17.4[a]

*Means with the same superscript in the same column are not significantly different at $\alpha = 0.05$ level by Tukey pairwise comparison (18). Percentages represent the fraction of a given lipid class with respect to total lipid content within a cultivar.

TABLE 3

Major Fatty Acids of Total Lipids Among Five Blueberry Cultivars (%)*

Fatty acid	Blueberry cultivar				
	Coville	Darrow	Herbert	Jersey	Bluetta
14:0	1.22^a	2.27^a	2.31^a	1.09^a	1.22^a
16:0	12.95^a	12.61^a	12.13^a	9.44^b	9.65^b
18:0	2.35^a	2.31^a	3.38^a	2.05^a	1.73^a
18:1	17.60^a	18.87^a	22.51^a	20.44^a	21.86^a
18:2	44.44^a	40.12^a	40.39^a	45.05^a	41.57^a
18:3	19.62^a	23.48^a	16.56^a	20.15^a	23.55^a

*Means with the same superscript in the same row are not significantly different at $\alpha = 0.05$ level by Tukey pairwise comparison (18).

TABLE 4

Major Fatty Acids of Neutral Lipids Among Five Blueberry Cultivars (%)*

Fatty acid	Blueberry cultivar				
	Coville	Darrow	Herbert	Jersey	Bluetta
14:0	2.04^a	1.22^a	1.75^a	0.57^a	0.54^a
16:0	7.02^a	7.33^a	9.24^a	7.35^a	6.67^a
18:0	2.67^a	2.09^a	2.37^a	1.93^a	1.58^a
18:1	22.96^a	24.33^a	23.17^a	23.36^a	25.42^a
18:2	36.87^a	37.73^a	37.43^a	43.77^b	43.04^b
18:3	23.85^a	24.84^a	24.14^a	20.67^a	23.51^a

*Means with the same superscript in the same row are not significantly different at $\alpha = 0.05$ level by Tukey pairwise comparison (18).

TABLE 5

Major Fatty Acids of Glycolipids Among Five Blueberry Cultivars (%)*

Fatty acid	Blueberry cultivar				
	Coville	Darrow	Herbert	Jersey	Bluetta
14:0	0.98^a	1.94^a	1.64^a	2.31^a	2.24^a
16:0	18.86^a	21.82^a	18.31^a	22.18^a	18.05^a
18:0	3.39^a	4.42^a	4.56^a	5.36^a	3.29^a
18:1	7.44^a	9.50^a	8.59^a	7.18^a	8.10^a
18:2	47.66^b	28.74^a	43.66^b	27.94^a	32.64^a
18:3	20.06^a	31.62^b	21.43^a	31.82^b	31.64^b

*Means with the same superscript in the same row are not significantly different at $\alpha = 0.05$ level by Tukey pairwise comparison (18).

TABLE 6

Major Fatty Acids of Phospholipids Among Five Blueberry Cultivars (%)*

Fatty acid	Blueberry cultivar				
	Coville	Darrow	Herbert	Jersey	Bluetta
14:0	2.01^a	0.47^a	3.75^a	1.86^a	0.51^a
16:0	21.24^a	21.23^a	21.76^a	16.89^b	16.99^b
18:0	2.77^a	3.01^a	3.68^a	2.82^a	2.18^a
18:1	8.01^a	11.41^a	10.22^a	7.35^a	8.95^a
18:2	49.60^a	48.88^a	43.70^a	51.86^a	52.84^a
18:3	14.04^a	14.19^a	14.64^a	16.87^a	16.18^a

*Means with the same superscript in the same row are not significantly different at $\alpha = 0.05$ level by Tukey pairwise comparison (18).

ic acid in Jersey and Bluetta was significantly lower than that in Coville, Darrow and Herbert.

Fatty acid composition of blueberry neutral lipids. There were little varietal differences in the fatty acid composition of neutral lipids of blueberries (Table 4), being similar to that of total lipids. The significant difference in the content of individual fatty acids was observed in linoleic acids, with Jersey and Bluetta having higher linoleic acid content than the other three cultivars.

Fatty acid composition of blueberry glycolipids. In glycolipids (Table 5), the predominant fatty acids were linoleic (27.9–47.7%), linolenic (20.1–31.8%) and palmitic (18.1–22.2%) acids. The glycolipids were more saturated than the neutral lipids due to the abundance of palmitic acid. The total content of linoleic and linolenic acids was similar in all five cultivars, but the ratios of linolenic acid (18:3) to linoleic acid (18:2) were slightly higher in Darrow and Jersey. The pattern of fatty acid composition had two distinguished types. Darrow, Jersey and Bluetta had an approximately equal amount of linoleic and linolenic acids; whereas Coville and Herbert showed approximately a 2:1 ratio of linoleic and linolenic acids.

Fatty acid composition of blueberry phospholipids. Although phospholipids were characterized by high linoleic acid content (43.7–52.8%), the overall pattern of fatty acid composition of phospholipids among the five cultivars was similar (Table 6). The only significant difference was that Coville, Darrow and Herbert contained a higher content of palmitic acid than Jersey and Bluetta. The predominant fatty acids were linoleic (43.7–52.8%), palmitic (16.9–21.8%), and linolenic (14.0–16.9%) acids.

Varietal differences in short-chain and medium-chain fatty acids. The major fatty acids of blueberry lipids were palmitic, oleic, linoleic and linolenic acids. The short-chain fatty acids were in relatively small quantity among the five cultivars. However, there was a fair amount of myristic acid (14:0). Hirvi and Kanen (20) have reported that myristicine was an aroma component of a highbush blueberry. Myristic acid may be a precursor of myristicine. The presence of these short-chain and medium-chain fatty acids likely contributed to the characteristic flavor and aroma of blueberries.

ACKNOWLEDGMENT

Research support provided by The Ohio Agricultural Research and Development Center, The Ohio State University. Journal Article No. 400-89.

REFERENCES

1. Woodruff, R.E., D.H. Dewey and H.M. Shell, *Proc. Am. Soc. Hort. Sci. 75*:387 (1960).
2. Watt, B.K., and A.L. Merrill, *U.S. Dept. Agric. Handbook 8*, 1964, U.S. Government, Washington D.C.
3. Eck, P., *Blueberry Science*, Rutgers University Press, New Brunswick, NJ 1988.
4. Proctor, A., and L.C. Peng, *J. Food Sci. 54*:385 (1989).
5. Schonherr, J., *Planta (Berl.) 131*:159 (1976).
6. Albrigo, L.G., P.M. Lyrene and B. Freeman, *J. Am. Soc. Hort. Sci. 105*:230 (1980).
7. Supran, M.K., *Lipids as a Source of Flavor*, ACS Symposium Series 75, American Chemical Society, Washington, D.C., 1978, pp. 11–12.

8. Hitchcock, C., and B.W. Nichols, in *Plant Lipid Biochemistry,* Academic Press, New York, NY, 1971, pp. 86–94.
9. Geduspan, H.S., and A.C. Peng, *J. Sci. Food Agric. 40*:333 (1987).
10. Ramarathnam, N., T. Osawa, M. Namiki and S. Kawaskishi, *J. Am. Oil Chem. Soc. 66*:105 (1989).
11. Bligh, E.G., and W.J. Dyer, *Can. J. Biochem. Physiol. 37*:911 (1959).
12. Folch. J., M. Lees and G.H. Sloane-Stanley, *J. Biol. Chem. 226*:497 (1957).
13. Rouser, G., G. Kritchevsky, G. Simon and J. Nelson, *Lipids 2*:37 (1967).
14. Lepage, M., *Ibid. 2*:244 (1967).
15. Mangold, H.K., *J. Am. Oil Chem. Soc. 38*:708 (1961).
16. Metcalfe, L.D., A.A. Schmitz and J.R. Pelka, *Anal. Chem. 38*:514 (1966).
17. Devore, J., and R. Peck, *Statistics: The Exploration and Analysis of Data,* West Publishing Co., St. Paul, MN, 1986, pp. 558–605.
18. Keppel, G., in *Design and Analysis: A Researcher's Book,* Prentice-Hall Inc., Englewood Cliffs, NJ, 1982, p. 148.
19. Bell, H.P., *Can. J. Bot. 35*:139–153 (1957).
20. Hirvi, T., and E.H. Kanen, in *Instrumental Analysis of Food,* edited by G. Charalambous, and G. Inlett, Academic Press, New York, NY, 1983, pp. 132–138.

[Received January 3, 1990; accepted March 20, 1990]