

AN ABSTRACT OF THE THESIS OF

Ho-ju Fan-Chiang for the degree of Master of Science in Food Science and Technology presented on December 14, 1999

Title: Anthocyanin Pigment, Nonvolatile Acid and Sugar Composition of Blackberries.

Abstract approved Signature redacted for privacy.

Ronald E. Wrolsted

The pigments of blackberry fruit were investigated by analytical and preparative high performance liquid chromatography (HPLC) to characterize anthocyanin patterns in commercially important blackberry (*Rubus eubatus*) cultivars and selections. Five anthocyanin pigments were detected in Evergreen blackberry and they were identified as cyanidin-3-glucoside, cyanidin-3-rutinoside, pentose-cyanidin derivative and two acylated cyanidin derivatives. The monomeric anthocyanin of 52 samples ranged from 70.3 to 201 mg/100g berry (mean = 137). For commercial juice concentrates, it ranged from 12.3 to 107 mg/100g single strength juice. One of the ten commercial juice concentrates showed an atypical chromatogram.

Analyses of nonvolatile acids and sugars were conducted on 52 samples of blackberry to develop a compositional database for evaluating authenticity and quality. °Brix ranged from 6.88 to 16.83 with a mean of 10.82. Titratable acidity ranged from 0.52 to 2.24 with a mean of 1.35 g citric acid/100mL. Sucrose levels

(range, 0–12.9%; mean, 4.07%) were highly variable. The overall glucose: fructose ratio ranged from 0.81 to 1.17 with a mean of 1.01. The identities of malic, isocitric, lactoisocitric, shikimic and citric acids were confirmed. Two patterns of nonvolatile acid compositions were found in blackberries. Malic acid ranged from 5.2% to 35.3% (87.5–603 mg/100g) of total acids with a mean of 16.4% (280 mg/100g). Lactoisocitric acid ranged from 3.4–32.6% with a mean of 17.3% (mean, 293 mg/100g). Isocitric acid ranged from 79.1 to 1550 mg/100g (mean, 599 mg/100g). Citric acid ranged from 15.2 to 1892 mg/100g (mean, 572 mg/100g). Presence of fumaric acid has not previously been reported. Succinic acid was detected in some samples. Ten commercial samples were also analyzed which exhibited HPLC profiles similar to the authentic standard. It was possible to classify the commercial concentrates as to whether they were Marion, Evergreen or a mixture of the two from their acid profiles. The pigment profile may help in determination of authenticity of juice in terms of international trade. The data from pigment concentration measurement should be useful in breeding programs.

Anthocyanin Pigment, Nonvolatile Acid and Sugar Composition of Blackberries

by

Ho-ju Fan-Chiang

A THESIS
Submitted to
Oregon State University

in partial fulfillment of
the requirements for
the degree of

Master of Science

Completed December 14, 1999
Commencement June 2000

Master of Science thesis of Ho-ju Fan-Chiang presented on December 14, 1999.

Approved:

Signature redacted for privacy. *al* 1/18/00

Major Professor, representing Food Science and Technology

- Signature redacted for privacy. (R.E. Wroblestad) 1/18/00

Head of Department of Food Science and Technology

Signature redacted for privacy.

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Signature redacted for privacy. *H*

Ho-ju Fan-Chiang, Author

ACKNOWLEDGMENTS

I would like to express my gratitude to my major professor and advisor Ronald E. Wrolstad, who give me the opportunity to work on this thesis, for his support, patience and encouragement.

I am grateful for the help of Bob Durst, Lin Wen, and Brian Yorgey, both technical and personal. I want to thank Brian Ford for advice with my writing and laboratory safety during the period of my studies here. I deeply appreciate the kindness from my friendship family parents, Mr. and Mrs. Minor, who helped me along the way and let me feel Corvallis is a home away from home.

TABLE OF CONTENTS

	<u>Page</u>
Chapter 1. INTRODUCTION AND REVIEW OF LITERATURE.....	1
Introduction.....	1
Literature Review.....	3
References.....	19
Chapter 2. ANTHOCYANINS PATTERNS IN BLACKBERRIES.....	22
Abstract.....	22
Introduction.....	22
Materials and methods.....	24
Results and Discussion.....	30
Conclusion.....	45
Acknowledgement.....	45
References.....	46
Chapter 3. NONVOLATILE ACIDS AND SUGAR COMPOSITION OF BLACKBERRIES.....	48
Abstract.....	48
Introduction.....	49
Materials and methods.....	51
Results and Discussion.....	56
Conclusion.....	74

TABLE OF CONTENTS (Continued)

Acknowledgement.....	74
References.....	75
Chapter 4. CONCLUSION.....	77
BIBLIOGRAPHY.....	78

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1.1 Structure of anthocyanin pigments.....	9
1.2 Reversible transformation of anthocyanins with pH.....	16
2.1 Flowchart of blackberry anthocyanins extraction.....	27
2.2 HPLC separation of blackberry anthocyanins.....	34
2.3 Preparative HPLC chromatogram of Evergreen blackberry (<i>Rubus laciniatus</i>).....	35
2.4 HPLC separation of saponified blackberry anthocyanins.....	37
2.5 Chromatogram of international samples.....	39
2.6 Chromatogram of blackberry hybrids.....	41
2.7 Chromatogram of blackberry juice concentrate J.....	44
3.1 Sugars of blackberry.....	62
3.2 Nonvolatile acids of blackberry (pattern I).....	67
3.3 Nonvolatile acids of blackberry (pattern II).....	68

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1 Trailing, erect, and semi-erect blackberry hectarage in the Pacific Northwest at three recent ten year intervals.....	4
1.2 Soluble solid, pH, and titratable acidity of blackberries.....	7
1.3 Total anthocyanin content.....	10
1.4 The evolution of chromatography in anthocyanin analyses.....	17
2.1 Anthocyanin composition.....	32
2.2 Anthocyanin content of some common fruits.....	33
2.3 Electrospray mass spectroscopy of blackberry anthocyanins.....	36
2.4 Commercial blackberry juice concentrates.....	43
2.5 Anthocyanin composition of commecial juice concentrates.....	43
3.1 Sample table.....	58
3.2 Sugar composition.....	60
3.3 Sugar composition of commercial juice concentrates.....	64
3.4 Nonvolatile acids.....	70
3.5 Acid composition of commercial blackberry juice concentrates.....	73

ANTHOCYANIN PIGMENT, NONVOLATILE ACID AND SUGAR COMPOSITION OF BLACKBERRIES

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Anthocyanin pigments are a major focus in the search for natural food colorants. Scientists have identified anthocyanins in a wide variety of sources ranging from fruits and vegetables to flowers. Blackberries (*Rubus eubatus*) are rich in anthocyanins and have a distinctive aroma. The Pacific Northwest is a major producer of blackberries, and a significant portion of the crop is processed into juice concentrate. The product is used in the manufacture of blended juices, juice drinks, wine, and other beverages. Because of the high anthocyanin pigment content, it is also used as a colorant for brined cherries and other products. The high economic value (about \$70/gal in 1999 year) and increasing demand for blackberry juice concentrate in blended juices and drinks has made it a likely target for adulteration.

The objective of this research is to characterize the anthocyanin, sugar, and acid profiles of blackberry samples from major producing regions of the world. Fifty-five berry samples were obtained representing 18 different varieties and 21 selections from 5 different geographic locations. Ten commercial juice concentrates

were also obtained from 6 different juice processors. Blackberry fruits were characterized by one major pigment, cyanidin 3-glucoside, and, in some cases a second pigment, cyanidin 3-rutinoside in lower concentration. The minor pigments, however, have not been completely identified. Also, the total anthocyanin content of different varieties of blackberries can be helpful in terms of new variety selection and juice quality assessment. 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. Fruit juices may be adulterated by addition of foreign, cheaper juices or by addition of sugar solutions acidified with organic acids. A distinct alteration in the acid pattern of a fruit juice, accompanied by a change in acid intensities, will demonstrate the absence of the normal amount of characteristic fruit acids and the presence of foreign acids.

A more complete and extensive compositional data-base for authentic blackberry juice is needed to establish better criteria for evaluating the authenticity and quality of blackberry products in international trade. The results of these analyses were used to evaluate 10 commercial blackberry juice concentrates. The data may also be helpful in taxonomic classification, in breeding new cultivars, and in assessing processed quality.

Literature Review

VARIETY, CULTIVARS

Blackberries, in the genus *Rubus*, subgenus *Eubatus*, consist of a highly variable and complex group of plants, found throughout the world except in desert regions, but most numerous in the temperate parts of the northern hemisphere. Most species of blackberries produce biennial canes which vary from erect to procumbent in growth habit and are usually armed with sharp prickles (Moore and Skirvin, 1989). Blackberries ripen from May in the southeastern U.S. production regions to July in the Pacific Northwest. The fruiting period of individual cultivars ranges from 4 to 7 weeks at a given location.

Since present blackberry cultivars originated from the interbreeding of many genetically heterogeneous and morphologically variable species, present cultivars differ greatly in fruit and plant habit. American blackberry cultivars are often classified as erect thorny, western trailing, semierect thornless, dewberries, or raspberry-blackberry hybrids, based on gross morphology. However, there are often variations bridging two or more of these classes.

For ease of grouping, there are three types of blackberries (*Rubus spp.*) commercially grown in the United States; 1) erect, 2) semi-erect and 3) trailing (Strik, 1992). Erect and semi-erect types predominate in the Eastern and Southern regions of the United States. The Pacific Northwest (Oregon, and Washington,

USA, and British Columbia, Canada) is the principal producing area for trailing blackberries. Hectarage of erect and semi-erect type in this region is still small.

Hectarage of trailing, erect, and semi-erect blackberry cultivars in 1980, 1990, and 2000 (anticipated) is summarized in Table 1-1. Trailing blackberries account for 98% of the blackberry hectarage in the Pacific Northwest. Oregon is the leading producer with 2810 hectares in 1990, a 45% increase in hectarage since 1980.

Hectarage in Washington has increased 25% since 1980, but remains low with 50 hectares in production. Production is expected to increase in Washington in the next ten years. The trend appears to be for increased plantings of erect and semi-erect types. There is currently relatively little hectarage of erect and semi-erect types in the Pacific Northwest.

Table 1.1—Trailing, erect, and semi-erect blackberry hectarage in the Pacific Northwest, at three recent ten year intervals. (Data derived from Strik, 1992.)

		Hectares		
		1980	1990	2000 (anticipated)
Trailing:	British Columbia	0	1	?
	Washington	40	50	80
	Oregon	1950	2810	3645
	Total	1990	2861	3725
Erect:	British Columbia	0	0	?
	Washington	0	0	15
	Oregon	0	27	80
	Total	0	27	95
Semi-Erect:	British Columbia	0	2	?
	Washington	5	3	8
	Oregon	8	18	60
	Total	13	23	68
Total	2003	2911	3888	

Erect thorny cultivars (e.g., 'Cherokee,' 'Eldorado,' 'Shawnee,' 'Darrow') probably descended from hybridization of such erect native species as *R. allegheniensis*, *R. argutus*, and *R. frondosus*, with some introgression of other species (Darrow, 1967). Cultivars in this class are characterized by erect, sometimes arching, spiny canes, and prolific production of primocanes arising from adventitious buds on roots and crowns. They are large clustered and produce large, sweet fruits. Cultivars in this group are among the most winter hardy.

The western trailing blackberries (also sometimes called dewberries) of the Pacific coast of North America (e.g./ 'Logan,' 'Boysen,' 'Olallie') are largely derived from *R. ursinus*, *R. macropetalus*, and *R. loganobaccus*. Many of these cultivars also contain genes of the red raspberry, *R. idaeus*. In addition, two common cultivars of the Pacific coastal area, 'Himalaya' and 'Evergreen' were derived from *R. procerus* and *R. laciniatus*, respectively, of European origin. Cultivars in this group produce very vigorous trailing to semi-erect canes, and large, rounded to elongated, wine-colored to black fruits of distinct flavor (Moore and Skirvin, 1989).

'Marion,' 'Thornless Evergreen,' and 'Boysen' are the most important trailing blackberry cultivars in the Pacific Northwest. 'Marion' accounts for more than 50% of the hectareage in this region and continues to be widely planted. 'Thornless Evergreen' (*Rubus laciniatus*) is the second most widely planted cultivar in the Pacific Northwest. Hectareage of 'Kotata' is increasing in Oregon, as this cultivar has similar fruit quality to 'Marion' but the fruit is firmer. 'Waldo,' a genetically

thornless cultivar, is also being planted. However, although the thornless canes of this cultivar are an advantage, it is more difficult to manage because canes are less vigorous than those of 'Marion' or 'Kotata' (DeFrancesco and Martin, 1990). It is worth noting that 'Marion', 'Kotata', and 'Waldo' were originated with the U.S. Department of Agriculture and Oregon State University, Corvallis, Oregon (Brooks and Olmo, 1997).

Erect and semi-erect types are being planted because newer cultivars can be pruned mechanically, have lower training costs, produce higher average yields than trailing blackberries and produce fruit that is suitable for fresh market. In Oregon, erect blackberries, 'Cherokee' and 'Shawnee' are most widely planted with 'Cherokee' being preferred for new plantings. Growers in Oregon prefer 'Cherokee' for its superior fruit quality, yield and plant growth to 'Shawnee.' 'Hull Thornless' and 'Chester' dominate the hectarage of semi-erect blackberries in the Pacific Northwest. Both of these cultivars are the choice for new plantings in California. However in Oregon, growers seem to prefer 'Chester' as this cultivar is more productive. All of the erect and semi-erect blackberries are hand-picked in the Northwest (Strik, 1992). Oregon growers and shippers still consider the trailing blackberry to have the highest fruit quality for processing.

COMPOSITION

General Properties

Reports of titratable acidity of blackberries (Table 1.2) can go back as early as 1935 (Widdowson and McCance). Other workers reported pH and titratable acidity of blackberry in their investigation (Whiting, 1958; Jennings and Carmichael, 1978; Rommel et al., 1992). However, more complete statistical data were not available until 1985. Sapers et al. (1985) reported differences in the titratable acidity, soluble solids, total anthocyanin, and pectin content in fruit of 40 thornless blackberry cultivars and selections. The U.S. regulation for single strength blackberry juice is 10.0° Brix (Code of Federal Regulation, 1999).

Table 1.2—Soluble solid, pH, and titratable acidity of blackberries.

Author	Soluble solids	pH	Titratable acidity
Widdowson and McCance (1935)	-	-	0.97 ^a
Whiting (1958)	-	-	1.41 ^a
Jennings and Carmichael (1978) ^c	9.13-9.20	3.12-3.26 (mean= 3.18)	1.24-1.53 ^b (mean=1.36)
Sapers et al. (1985) ^d	7.7-13.9 (mean=10.8)	3.2-4.1 (mean=3.6)	0.4-1.3 ^a (mean=0.8)
Rommel et al. (1992)	12.4	3.63	1.05 ^b

^aData were calculated as citric acid, g/100g fruit.

^bData were calculated as citric acid, g/100 mL juice.

^cSample size = 3 cultivars.

^dSample size = 40 cultivars and selections.

Jennings and Carmichael (1979) have demonstrated that the color of blackberries tends to change from black to red when they are frozen—the extent of this change reflecting incomplete ripeness. They attributed these changes in color to the lowering of fruit pH caused by mixing of cellular fluids caused by cell plasmolysis.

Anthocyanins

Figure 1-1 shows a generalized structure for anthocyanin pigments. There are six common anthocyanidins (aglycons liberated from anthocyanins by acid hydrolysis). Structural variations are greatly expanded through varying patterns of glycosidic substitution on the 3 and 5 positions. Additional modifications can occur through acylation of the sugar substituents with cinnamic or organic acids (Wrolstad, 1998).

A number of workers have been identifying and characterizing blackberry anthocyanins. The distribution of individual anthocyanin pigments of *Rubus* clones was determined by TLC and densitometry (Torre and Barritt, 1977). Blackberry fruits were characterized by one major pigment, cyanidin 3-glucoside, and, in some cases a second pigment, cyanidin 3-rutinoside in lower concentration. Cyanidin-3-rutinoside was first identified and reported by Harborne and Hall (1964). Sapers et al. (1986) identified three additional anthocyanin peaks tentatively, as a xylose-cyanidin derivative and two dicarboxylic acid-acylated derivatives of cyanidin 3-glucoside, by HPLC analysis of 40 blackberry varieties.

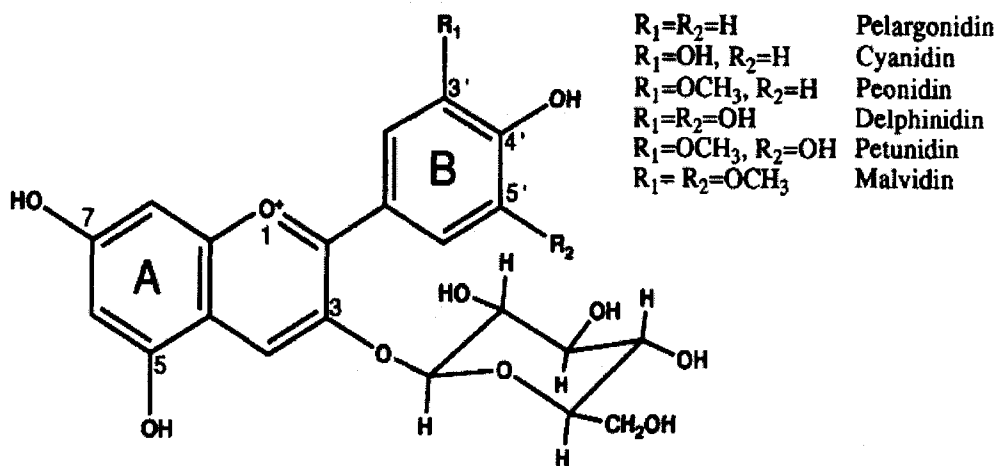


Figure 1-1 Structure of anthocyanin pigments. (derived from Wrolstad, 1998)

Rommel et al. (1992) reported seven anthocyanin pigments (cyanidin-3-glucoside, cyanidin-3-rutinoside, a xylose-cyanidin derivative, two acylated cyanidin derivatives, cyanidin and a polymeric derivative) were detected in blackberry juices and wines by HPLC. Presence of cyanidin 3-sophoroside and cyanidin 3-glycosylrutinoside were reported previously by Hong and Wrolstad (1990), and they concluded that the sample was not authentic blackberry. Torre and Barritt (1977) reported presence of pelargonidin 3-glucoside in one out of 16 species and three cultivars.

Sapers et al. (1985) reported total anthocyanin content in fruit of 40 thornless blackberry cultivars and selections (Table 1.3). The differences of compositions

after freezing, thawing, and heating were also investigated. Red subsamples of frozen blackberries were lower than black subsamples in soluble solids and total anthocyanin contents and higher in titratable acidity. Rommel et al. (1992) reported the anthocyanin composition of blackberry juice and wine during processing and storage. The pigment content also related to preharvest condition (Naumann and Wittenburg, 1980). Total anthocyanin content is of limited value in detecting adulteration, however, it is very useful for quality assessment (Wrolstad et al., 1995). Total anthocyanin content may be a useful index of color quality to the beverage processing industry. It would be also helpful in selection of new cultivars.

Table 1.3—Total anthocyanin content.

	Method ^a	Total anthocyanin (mg/100g fruit)
Torre and Barritt (1977)	TLC	82.5-326 (mean = 180.6)
Sapers et al. (1985)	pH differential	33.1-82.8 (mean = 56.3)
Rommel et al. (1992)	pH differential	26.2

^a Pigment content may vary due to different extraction method.

Sugar

Widdowson and McCance (1935) reported glucose and fructose to be the principle 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.

Glucose and fructose were the only sugars detected, reported by Wrolstad et al (1980), by gas chromatographic analysis of trimethylsilyl (Me₃Si) derivatives. Twenty-nine samples representing 8 varieties, one wild variety, and 3 hybrids were investigated. The overall glucose/fructose ratio was 0.710, with a standard deviation of 0.170. This value may be a useful index in adulteration investigations. There was less variation in the glucose/fructose ration for the blackberry samples than for the glucose and fructose content. There were 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 were not noticeably different from the whole fruit samples in this investigation.

Workers in Europe and the United States (Fitelson, 1970; Benk and Bergmann, 1978; Kline et al., 1970) have reported that blackberries contain only glucose and fructose and do not contain sorbitol or sucrose.

Acids

Bigelow and Dunbar (1917), in their study of the acid content of fruits, stated that "some samples of blackberries appeared to contain only citric acid, some contained malic acid in trace amounts with no citric, while others gave no reactions for either malic or citric." Nelson (1925) discovered that isocitric was the dominant

and characteristic acid in blackberries. Isocitric acid constituted about five-sixths of the total acids of the fruit. The presence of lactoisocitric acid in blackberries was first suggested by Nelson on the basis of titration results. The organic acids of a number of berry fruits have been investigated qualitatively and quantitatively using the methods of paper chromatography, silica gel partition chromatography and ion exchange by Whiting (1958), who 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. A distinct alteration in the acid pattern of a fruit juice, accompanied by a change in acid intensities, will demonstrate the absence of the normal amount of characteristic fruit acids and the presence of foreign acids. For example, addition of grape juice to dark colored juices can readily be detected by the presence of tartaric acid. He reported R_f values for common fruit acids by a proposed paper chromatographic method. The blackberry juice showed the same R_f value for isocitric acid obtained for citric acid and also showed an unidentified acid spot with a high R_f value.

The detection of phosphoric, malic, lactoisocitric, citric-isocitric, and quinic was reported by Wrolstad et al (1980) by gas chromatographic analysis of trimethylsilyl (Me_3Si) derivatives. The results showed considerable variation among the 15 samples with lactoisocitric being absent in six of the samples. With this analytical method, a mixture of citric and isocitric acid could not be resolved because of

relatively close retention time. Presence of shikimic acid was not confirmed in this investigation.

Adulteration

The sugar composition of different fruits has been helpful in determining the authenticity of fruit juices and concentrates (Wrolstad & Shallenberger, 1981). That publication gives the glucose, fructose, sucrose, and sorbitol contents of apple, pear, grape, cherry, blackberry, raspberry, strawberry, plum, and peach fruit as compiled from the literature.

The simple pattern for sugars suggests that the method would be appropriate for detection of adulteration with sucrose or sucrose-containing fruits (Wrolstad et al., 1980). 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. Wrolstad et al (1982) reported that some commercial blackberry juice concentrates and wines were adulterated based on detection of their sorbitol content. Sorbitol is not fermented by yeast and can be detected in wines made from blackberry juice concentrates that contain sorbitol.

Sorbitol is present in cheaper concentrates such as apple, pear, and plum (Wrolstad and Shallenberger, 1981), which are readily available, and its presence could be an indication of adulteration with those fruits.

Fruit juices are characterized by simple major organic acid patterns, so that addition of a foreign acid or cheaper fruit juices can frequently be detected by significant changes in the major organic acid pattern. Fitelson (1969) reported seven of the eight collaborators reported correctly that the unknown blackberry juice was adulterated, although the acid pattern of this juice is more complicated and the addition of citric acid is more difficult to detect. Adulteration of blackberry juice concentrates and wines with juice of sorbitol-containing fruits was detected by determining carbohydrates by high performance liquid chromatography, gas-liquid chromatography, and thin layer chromatography (Wrolstad et al., 1982). Sorbitol is not fermented by yeast and can be detected in wines made from blackberry juice concentrates that contain sorbitol. High levels of sorbitol and quinic and malic acids suggested that an imported blackberry concentrate may have been adulterated with plum. Degradation of anthocyanin pigments during processing and storage limits the utility of pigment analyses in detecting adulteration.

García-Viguera et al.(1997) reported the determination of adulteration in blackberry jam when mixed with strawberry, cherry and raspberry jams by anthocyanin pigment profile. In general, a single qualitative anthocyanin pattern of a fruit is found regardless of the variations in total amount of anthocyanins. The predominant anthocyanin in blackberry was cyanidin 3-glucoside. It would be easy to detect presence of other fruits anthocyanins such as pelargonidin 3-glucoside

(strawberry), or peonidin 3-glucoside (cherry), or cyanidin 3-sophoroside and cyanidin 3-glucosylrutinoside (red raspberry), etc.

MEASUREMENT OF ANTHOCYANINS

Quantitative Spectral Methods (pH differential method)

The quantity of total monomeric anthocyanin pigment in a fruit juice is accurately and easily measured by the pH differential method (Francis, 1982; Wrolstad, 1976). Total anthocyanin content is of limited value in detecting adulteration, however, it is very useful for quality assessment (Wrolstad et al., 1995)

The structural transformations which pelarginidin-3-glucoside undergoes with change in pH are illustrated in Figure 1-2. Anthocyanin pigments can be described as being indicators, that is, their hue and intensity change with pH. At pH 1.0, anthocyanins exist in the highly colored oxonium or flavylium form and at pH 4.5 they are predominately in the colorless carbinol form.

The quantitative procedure for determining anthocyanin content which will be described is based on those facts. One aliquot of an aqueous anthocyanin solution is adjusted to pH 1.0 and another to pH 4.5. The difference in absorbance at the wavelength of maximum absorption will be proportional to anthocyanin content (Wrolstad, 1976).

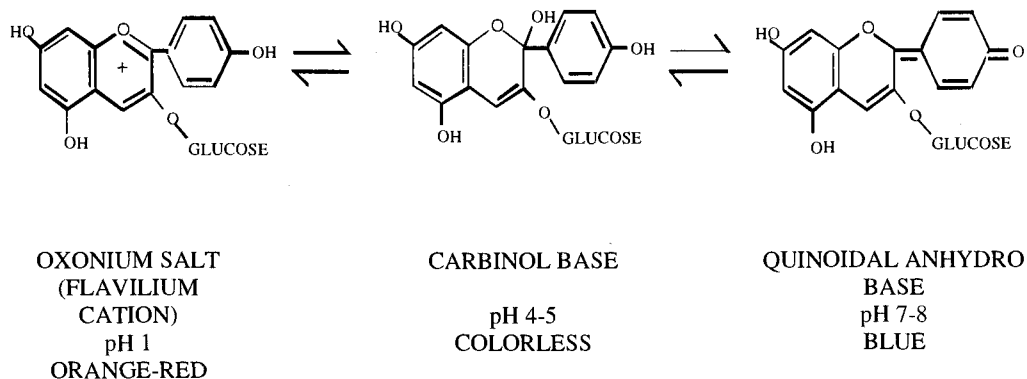


Figure 1.2—Reversible transformation of anthocyanins with pH. (derived from Wrolstad, 1998)

Determination of anthocyanin content is based on Lambert-Beer's Law: $A = \epsilon CL$. A stands for absorbance which is measured with a spectrophotometer. ϵ stands for molar absorbance, a physical constant for a molecular species in a given solvent system at a given wavelength. Molar absorbance values for purified pigments taken from the literature can be used, making it unnecessary to determine them. Since the molar absorbance for many of the anthocyanins are unknown, the value for a known pigment may arbitrarily be used for calculation. For example, an accurate molar absorbance for cyanidin-3-sophoroside, the major pigment of red raspberries, has not been determined. Workers calculated the pigment content as cyanidin-3-glucoside in the investigations on red raspberry juice (Spanos and Wrolstad, 1987; Rommel et al., 1990; Boyles and Wrolstad, 1993). To measure absorbance, the juice, concentrate, or wine needs to be diluted with buffer. The

order of dilution must be such that the sample at pH 1.0 will have an absorbance less than 1.0 and preferably in the range of 0.4-0.6. The dilution strength should be the same for both pH 1 and pH 4.5 samples. The diluted samples should be clear and not contain haze or sediment. Any sediment should be removed by centrifuging or filtering the sample. If the sample is free of haze, the absorbance at 700 nm should be 0. Turbidity (haze) can be corrected for by measuring the absorbance at 700 nm and subtracting this from the absorbance at the wavelength of maximum adsorption (510-540) (Wrolstad, 1976).

Separation of anthocyanin Pigments (chromatography)

The development of chromatography in separating anthocyanin pigments is shown in Table 1-4.

Table 1.4—The evolution of chromatography in anthocyanin analyses. (derived from Wrolstad, 1998)

Method	Advantage	Disadvantage	Year
Paper Chromatography	1. Simple 2. Economic	1. Time-consuming 2. Low sensitivity	1950's & 60's
Column Chromatography	1. Good for preparative work		1950's & 60's
Thin-layer Chromatography	1. Faster than paper 2. More useful information	1. Rf values not very reproducible	1950's & 60's
High Performance Liquid Chromatography (HPLC)	1. Good resolution 2. Rapid (20 min to 1 hr) 3. Small sample size 4. Minimal sample preparation 5. Retention times predictable	1. High operating costs	current
Capillary Electrophoresis (CE)	1. Small sample size 2. Low operating costs	1. High capital investment	future

Developments in paper and thin-layer chromatographic techniques during 1950's and 1960's permitted the separation of anthocyanin pigments and stimulated numerous investigations. Over 300 different anthocyanin pigments have been found in nature (Harborne and Turner, 1984).

Greater resolution, shorter analysis time, and easy measurement of relative amounts through integration of total peak area are advantages which have made High Performance Liquid Chromatography (HPLC) the method of choice for anthocyanin analyses. HPLC analysis of anthocyanin pigments is very useful for screening anthocyanin-containing fruit juices for authenticity and quality (Wrolstad et al., 1995).

The development of reversed-phase chromatography and gradient elution paved the way for the introduction of modern column chromatography (Robards et al., 1994). These procedures represent variants where the mobile phase is more polar than the stationary phase, or the polarity of the mobile phase is continuously varied throughout the analysis (gradient elution). An attractive feature of reversed-phase chromatography in anthocyanin analyses is the predictability of elution order based on polarity. Many investigations of anthocyanins in fruits and vegetables have done by HPLC.

References

- Benk, V.E., and Bergmann, R. 1978. Contributions to the knowledge of blackberry mother juice. *Flussige Obst* 45: 14-17.
- Boyles, M.J., and Wrolstad, R.E. 1993. Anthocyanin composition of red raspberry juice: influences of cultivar, processing and environmental factors. *J. Food Sci.* 58, 1135-1141.
- Brooks, R.M., and Olmo, H.O. 1997. The Brooks and Olmo register of fruit & nut varieties. 3rd ed. ASHS Press, Alexandria, Virginia.
- Code of Federal Regulation. 1999. 21 CFR parts 101.30. The office of the federal register national archives and records administration, Washington, DC.
- Darrow, G.M. 1967. The cultivated raspberry and blackberry in North America: Breeding and improvement. *Am. Hortic. Mag.* 46, 203-218.
- DeFrancesco, J.T., and Martin, L.W. 1990. Characteristics and management of Kotata and Waldo blackberries. *Ore. Hort. Soc. Proc.* 81, 183-186.
- Fitelson, J. 1970. Detection of adulteration in fruit juices by qualitative determination of carbohydrates by gas-liquid chromatography. *J. Assoc. Off. Anal. Chem.* 53: 1193-1197.
- Francis, F.J. 1982. Analysis of anthocyanins. *In Anthocyanins as food colors*, P. Markakis (Editor). Academic Press, New York.
- García-Viguera, C., Zafrilla, P., and Tomás-Barberán. 1997. Determination of authenticity of fruit jams by HPLC analysis of anthocyanins. *J. Sci. Food Agric.* 73: 207-213.
- Harborne, J.B and Turner, B.L. 1984. *Plant chemosystematics*. Academic Press, New York.
- Jennings, D. L., and Carmichael, E. 1979. Colour changes in frozen blackberry. *HortScience* 9:246.
- Kline, D.A., Fernanadez-Flores, E., and Johnson, A.R. 1970. Quantitative determination of sugars in fruits by GLC separation of TMS derivatives. *J. Assoc. Off. Anal. Chem.* 53: 1198-1202.

- Moore, J.N., and Skirvin, R.M. 1989. Blackberry management *In* Small fruit crop management. Galletta, G.J and Himelrick, D.G. (Editor). Prentice-Hall, Inc. Englewood Cliffs, New Jersey.
- Naumann, W.D. and Wittenburg, U. 1980. Anthocyanins, soluble solids, and titratable acidity in blackberries as influenced by preharvest temperatures. *Acta Hort.* 112: 183-190.
- Nelson, E.K. 1925. The non-volatile acids of the blackberry. *J. Am. Chem. Soc.* 47, 568-572.
- Robards, K., Haddad, P.R., and Jackson, P.E. 1994. Principles and practice of modern chromatographic methods. Academic Press Inc. San Diego, California.
- Rommel, A., Heatherbell, D.A., and Wrolstad, R.E. 1990. Red raspberry juice and wine: effect of processing and storage on anthocyanin pigment composition, color and appearance. *J. Food Sci.* 55, 1011-1017.
- ✓ Sapers, G. M., Burgher, A.M., Phillips, J.G., and Galletta, G.J. 1985. Composition and color of fruits and juice of thornless blackberry cultivars. *J. Amer. Soc. Hort. Sci.* 110:243-248.
- Sapers, G.M., Hicks, K.B., Burgher, A.M., Hargrave, D.L., Sondey, S.M., Bilyk, A. 1986. Anthocyanin patterns in ripening thornless blackberries. *J. Am. Soc. Hortic. Sci.* 11, 945-950.
- Spanos, G.A., and Wrolstad, R.E. 1987. Anthocyanin pigment, nonvolatile acid, and sugar composition of red raspberry juice. *J. Assoc. Off. Anal. Chem.* 70: 1036-1046.
- Strik, B.C. 1992. Blackberry cultivars and production trends in the Pacific Northwest. *Fruit Varieties Journal.* 46(4): 202-206.
- Torre, L.C. and Barritt, B.H. 1977. Quantitative evaluation of *Rubus* fruit anthocyanin pigments. *J. Food Sci.* 42, 488-490.
- Whiting, G.C. 1958. The non-volatile organic acids of some berry fruits. *J. Sci. Food Agric.* 9, 244-248.
- Widdowson, E.M., McCance, R.A. 1935. Determination of glucose, fructose, sucrose and starch. *Biochem. J.* 29, 151-156.

- Wrolstad, R.E. 1976. Color and pigment analyses in fruit products. Oregon St. Univ. Agric. Exp. Stn. Bull. No. 624.
- Wrolstad, R.E., Culbertson, J.D., Cornwell, C.J., and Mattick, L.R. 1982. Detection of adulteration in blackberry juice concentrates and wines. J. Assoc. Off. Anal. Chem. Vol. 65, No. 6.
- Wrolstad, R.E., Hong, V., Boyles, M.J., and Durst, R.W. 1995. Use of anthocyanin pigment analyses for detecting adulteration in fruit juices. *In* Methods to detect adulteration of fruit juice beverages. Nagy, S., and Wade, R. L. (Editor). Agscience, Inc. Florida.
- Wrolstad, R. E. 1998. 'Pigments and color evaluation' FST 561 class handout. Food Sceince and Technology Department, Oregon State University. Corvallis, Oregon.

Chapter 2

ANTHOCYANIN PIGMENT COMPOSITION OF BLACKBERRIES

Abstract

The pigments of blackberry fruit were investigated by analytical and preparative high performance liquid chromatography (HPLC) to characterize anthocyanin patterns in commercially important cultivars and selections. Five anthocyanin pigments were detected in Evergreen blackberry and they were identified as cyanidin-3-glucoside, cyanidin-3-rutinoside, xylose-cyanidin derivative and two acylated cyanidin derivatives. The monomeric anthocyanin content of 52 samples ranged from 70.3 to 201 mg/100g berry (mean = 137). For commercial juice concentrates, it ranged from 12.3 to 107 mg/100g single strength juice. One of the ten commercial juice concentrates showed an atypical chromatogram. The pigment profile may help in determination of authenticity of juice in terms of international trade. The data from pigment concentration measurement would be useful in breeding programs.

Introduction

A number of workers have been identifying and characterizing blackberry anthocyanins. Bate-Smith (1948) investigated anthocyanin pigments in blackberries by paper chromatography, reported a monoglycoside of cyanidin and an unidentified pigment. Fouassin (1956) and Blundstone and Crean (1966) also reported cyanidin 3-glucoside and other unidentified pigments. Cyanidin-3-

rutinoside was first identified and reported by Harborne and Hall (1964). The distribution of individual anthocyanin pigments of *Rubus* clones was determined by TLC and densitometry (Torre and Barritt, 1977). Blackberry fruits were characterized by one major pigment, cyanidin 3-glucoside, and, in some cases a second pigment, cyanidin 3-rutinoside in lower concentration. Sapers et al. (1986) tentatively identified three additional anthocyanin peaks as a xylose-cyanidin derivative and two dicarboxylic acid-acylated derivatives of cyanidin 3-glucoside, by HPLC analysis of many blackberry varieties. Rommel et al. (1992) reported seven anthocyanin pigments (cyanidin-3-glucoside, cyanidin-3-rutinoside, a xylose-cyanidin derivative, two acylated cyanidin derivatives, cyanidin and a polymeric derivative) in blackberry juices and wines by HPLC. Pelargonidin 3-glucoside has been reported in one out of 16 species and three cultivars. (Torre and Barritt, 1977). Hong and Wrolstad (1990) reported presence of cyanidin 3-sophoroside and cyanidin 3-glycosylrutinoside in a commercial blackberry juice sample by HPLC based on retention time, and concluded the sample was not authentic.

Sapers et al. (1986) reported that during ripening, cyanidin 3-glucoside increased to a greater extent than the other pigments, and that the accumulation of individual anthocyanins was linearly related to the total anthocyanin content and to the soluble solid:acidity ratio.

The growing hectareage of blackberries in the Pacific Northwest has been increasing dramatically for the past 20 years (Strik, 1992). The high economic value (about \$70/gal in 1999 year) and increasing demand for blackberry juice

concentrate in blended juices and drinks has made it a likely target for adulteration. García-Viguera et al.(1997) reported adulteration of blackberry jam when mixed with strawberry, cherry and raspberry jams by pigment profile. In general, a single qualitative anthocyanin pattern of a fruit is found regardless of the variations in total amount of anthocyanins.

The objective of this research is to characterize the anthocyanins profile of blackberry samples from major producing regions of the world. A more complete and extensive compositional data-base for authentic blackberry juice is needed to establish better criteria for evaluating the authenticity and quality of blackberry products in international trade. The data may also be helpful in taxonomic classification, in breeding new cultivars, and in assessing processed quality.

Materials and Methods

SAMPLES

For the '96, '97 and '98 seasons, 55 samples were obtained representing 18 different varieties and 21 selections from 5 different geographic locations.

Blackberry fruit samples were obtained from North Willamette Research and Extension Center (NWREC), Aurora, OR; Hurst Berry Farm, Sheridan, OR; ARS SCARL Research Center (USDA), Lane, OK. Three European samples were supplied by EUROFINS Laboratories (Nantes, France), originating from France and Macedonia; two samples from Central America (Mexico, unknown variety) and South America (Chilean, unknown variety) were supplied by Hortifrut. In

addition to 52 authentic berry samples, wild cultivars and hybrids such as Boysenberry and Loganberry, which have blackberry parentage, were also included in this investigation. Fruit samples were individually quick-frozen (IQF), and stored frozen at $-12\text{ }^{\circ}\text{C}$ until needed.

Nine commercial juice concentrates and one puree sample were supplied by six juice processing companies, two of the samples originated from Chile and Yugoslavia. In soliciting the samples, the companies were assured that individual sample identities would be kept confidential. These samples were coded A through J. After arrival, samples were stored frozen at $-12\text{ }^{\circ}\text{C}$. Before analysis, they were thawed and adjusted to single strength juice.

PIGMENT EXTRACTION

Extraction of anthocyanins from blackberries followed procedures outlined from Giusti (1996b). Fifty grams of frozen blackberries were liquid nitrogen powdered using a stainless steel Waring Blender (Figure 2.1). The powdered samples were then blended for 3 minutes with 100 mL of acetone and filtered using Whatman No. 4 filter paper on a Büchner funnel. The filter cake remnant was reextracted with an aqueous solution of acetone: water, 70/30 v/v, until a clear solution appeared. The filtrates were combined and shaken in a separatory funnel with a solution of acetone: chloroform, 1:2 v/v, and stored overnight at $1\text{ }^{\circ}\text{C}$. The aqueous portion was separated and put onto a Büchi rotary evaporator at $42\text{ }^{\circ}\text{C}$ for 10 minutes to rid the sample of residual acetone. The extract was then brought to 200

mL in a volumetric flask with deionized water. The procedure of extraction was replicated for each sample.

TOTAL MONOMERIC ANTHOCYANIN CONTENT

Monomeric anthocyanin content of the extract was determined using a pH differential method (Wrolstad, 1982). A Shimadzu 200 UV spectrophotometer and 1 cm pathlength disposable cells were used for spectral measurements. The absorbences were read at 420, 510 and 700 nm. Pigment content was calculated as Cyanidin-3-glucoside using an extinction coefficient (ϵ) of 29,600 L/cm-mg and molecular weight of 445.2g/L (Wrolstad, 1976). All analyses were duplicated with means being reported.

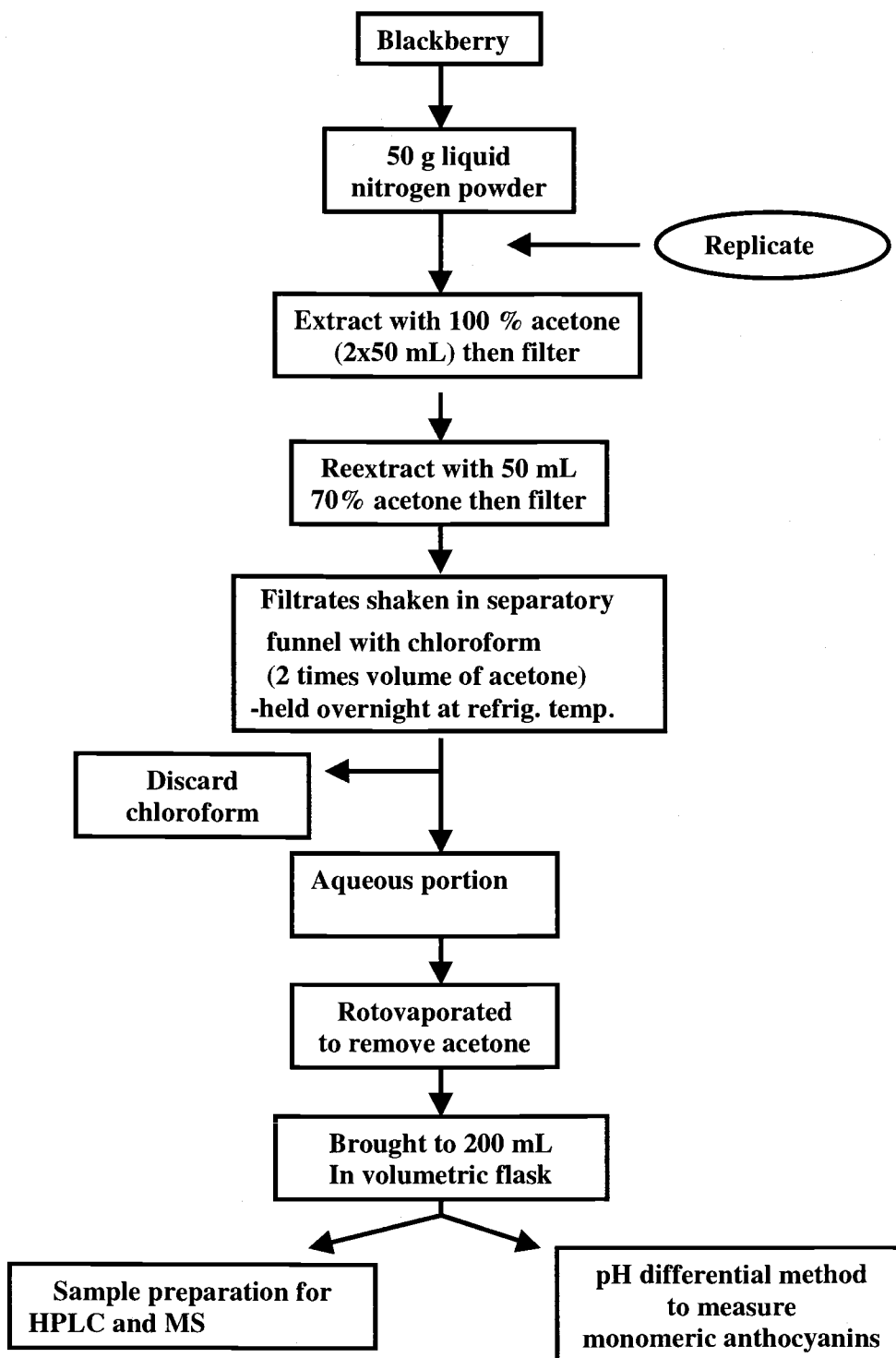


Figure 2.1 —Flowchart of blackberry anthocyanins extraction.

HPLC SEPARATION OF ANTHOCYANINS

An analytical HPLC Perkin-Elmer Series 400 and a Semi-preparative Dynamax Rainin Model SD-300 Liquid Chromatograph, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with Hewlett-Packard HPLC^{2D} ChemStation software was used.

The analytical HPLC system used a 250 x 4.6 mm id Prodigy 5 μ ODS-3 column (Phenomenex). Two mobile phases used were solvent A (100% acetonitrile) and solvent B (5% acetonitrile, 10% acetic acid and 1% Phosphoric acid). The following solvent gradient program was used: 100% B through 5 min, then 20%/80% A/B through 20 min, then 40%/60% A/B through 25 min, then 5 min back to 100% B. Flow rate 1 mL/min. Injection volume: 50 μ L. Samples obtained from liquid nitrogen extraction were filtered through a 0.45 μ L Millipore filter type HA (Millipore Corp., Bedford, MA). Commercial juice concentrate was diluted to single strength juice prior to analysis.

The preparative HPLC system used a Semi-prep microsorb C-18 reversed phase column (5 micron) 250 x 21.4 mm i.d. (Rainin Instrument Co., Inc., Emeryville, CA), fitted with a 50 x 21.4 mm i.d. guard module (Rainin Instrument Co., Inc., Emeryville, CA). Two solvents used were solvent A (100% acetonitrile) and solvent B (5% acetonitrile, 10% acetic acid and 1% phosphoric acid). Isocratic 7% A and 93% B through 20 min. Flow rate: 20 mL/min. Injection volume: 1 mL. Solvents and samples were filtered through a 0.45 μ L Millipore filter type HA (Millipore Corp., Bedford, MA).

Preparative chromatography of Evergreen blackberry (*Rubus laciniatus*) yielded fractions of peaks 1, 2, 3, 4, and 5. Each fraction was again concentrated by rotary evaporation then passed through a C-18 mini-column to remove phosphate buffer; anthocyanin was subsequently recovered with methanol containing 0.01% HCl (v/v). Part of eluate was treated then subjected to analytical HPLC to determine purity of the individual peaks, another part of eluate was subjected to mass spectroscopy to determine individual pigment's molecular weight

ALKALINE HYDROLYSIS OF ANTHOCYANINS

Purified pigment was hydrolyzed (saponified) in a screw-cap test tube with 10 mL of 10% aqueous KOH for 8 min at room temperature (c.a. 23°C) in the dark, as described by Hong and Wrolstad (1990). The solution was neutralized using 2N HCl, and the hydrolysate was purified using a C₁₈ minicolumn, filtered through 0.45µm Millipore filter (type HA), and injected onto LC system.

ELECTRO-SPRAY MASS SPECTROSCOPY (MS) OF BLACKBERRY ANTHOCYANINS

Low-resolution MS was done using electrospray MS. The instrument was a Perkin-Elmer SCIEX API III + Mass Spectrometer, equipped with an Ion Spray source (ISV = 47000, orifice voltage of 80) and loop injection. Partially purified blackberry anthocyanin extract was injected directly into the system (Giusti and Wrolstad, 1996a).

Results and Discussion

MONOMERIC ANTHOCYANIN CONTENT

The monomeric anthocyanin of the samples ranged from 70.3 to 200.9 mg/100g berry (mean = 136.9) (table 2.1). Torre and Barritt (1977) reported 82.5 to 326 mg/100g berry (mean = 180.6) from 19 blackberry species and cultivars by TLC. However, our data showed higher pigment content than reports from Sapers et al. (1985) and Rommel et al. (1992) most likely because of a different extraction method. The advantage of liquid nitrogen powdering extraction is increased extraction efficiency from the surface area. Acetone is very effective in extracting anthocyanins from fruit tissues. The nitrogen atmosphere and low temperature also prevented pigment degradation.

Table 2.2 lists the anthocyanin content of a number of anthocyanin-rich fruits taken from the literature. Blackberries are generally intermediate in anthocyanin content between red and black raspberries. Bilberry, black chokeberry, elderberry and black raspberry had the higher anthocyanin content, over 200 mg/100g of fruit, and red raspberry had the lowest, less than 60 mg/100g.

Total anthocyanin content is of limited value in detecting adulteration, however, it is very useful for quality assessment (Wrolstad et al., 1995). The information obtained from monomeric anthocyanin content of experimental selections may also be helpful in breeding program.

CHARACTERIZATION OF BLACKBERRY ANTHOCYANINS

HPLC of typical blackberry extracts revealed 1 major (peak 1), and 4 minor (peaks 2-5) detected at 520nm (Figure 2.2). Preparative chromatography of Evergreen blackberry (*Rubus laciniatus*) yielded fractions of peaks 1, 2, 3, 4, and 5 (Figure 2.3). The results of mass spectroscopy of individual pigment's molecular weight are shown in Table 2.3.

The identification of anthocyanin pigments in this investigation was both confirmed by retention time from HPLC chromatogram and molecular weight from Electro-Spray Mass Spectroscopy (ESMS). An attractive feature of reversed-phase chromatography is the the predictability of elution order based on polarity. The anthocyanin profile of blackberries is characterized by cyanidin 3-glucoside (peak 1) and lesser quantities of cyanidin 3-rutinoside (Peak 2). Cyanidin-3-glucoside and cyanidin-3-rutinoside were reported as the two major anthocyanins in blackberries by several workers (Torre and Barritt, 1977; Jennings and Carmichael, 1980; Sapers et al., 1986; Hong and Wrolstad, 1990a). Cyanidin 3-glucoside is the major pigment in all blackberry samples analyzed with a range between 43.6% to 95.2% of total pigment (mean = 82.9%). Cyanidin 3-rutinoside range from trace to 53.4% of total pigment (mean = 10.2%). Cyanidin 3-glucoside and cyanidin 3-rutinoside accounted for 89.5% and 18.4% (mean) respectively in an investigation of 19 blackberry species (Torre and Barritt, 1977). Sapers et al. (1986) investigated pigment changes in cultivars with ripening. The percentage of cyanidin 3-glucoside

Table 2.1 — Anthocyanin composition.

#	Year Sample	peak1 (%)	Peak2 (%)	Peak3 (%)	Peak4 (%)	Peak5 (%)	Monomeric Anthocyanin mg/100g berry ^a
Oregon samples (n = 40)							
1	96 Marion	69.7	26.5	trace	1.1	2.7	144.1
2	96 Kotata	91.6	8.4	trace	trace	trace	141.4
3	96 Waldo	88.0	8.6	0.4	0.6	2.5	132.5
4	96 Sikiyou	79.0	19.7	ND	1.3	trace	131.9
5	96 1112-2	70.4	27.5	0.5	1.5	ND	110.4
6	96 N-71	89.2	8.0	ND	2.9	ND	117.2
7	97 Marion	76.8	19.7	trace	1.2	2.3	167.1
8	97 Kotata	91.9	8.1	trace	trace	trace	146.8
9	97 Waldo	89.5	7.5	trace	0.8	2.2	153.2
10	97 Evergreen	79.8	4.1	7.0	5.1	4.0	116.6
11	97 Sikiyou	68.8	30.1	ND ^c	1.1	trace	107.6
12	97 1111-1	75.1	23.3	trace	1.6	trace	108.3
13	97 1112-2	67.8	30.1	0.2	0.6	1.4	161.1
14	97 N-71	89.2	7.0	trace	3.8	trace	120.5
15	98 Marion	77.8	19.4	trace	0.9	1.9	197
16	98 Kotata	95.2	4.8	trace	trace	trace	200.9
17	98 Waldo	91.3	6.1	trace	0.8	1.8	151.6
18	98 Lochness	92.2	4.1	3.1	0.6	trace	160.9
19	98 Black Douglas	90.1	3.5	0.5	1.9	4.0	149.5
20	98 1299-1	87.3	10.9	1.3	0.6	trace	127.6
21	98 1316-1	76.6	23.4	ND	trace	trace	171.2
22	98 1324-1 ^b						126.7
23	98 1325-1	43.6	53.4	0.3	1.5	1.1	137.7
24	98 1332-10	84.0	14.9	trace	1.1	ND	118.6
25	98 1361-1	92.9	6.8	ND	0.3	ND	123.2
26	98 1363-1	92.1	7.4	ND	0.5	ND	197.1
27	98 1369-3	68.7	30.4	ND	1.0	trace	191.5
28	98 1370-2	67.3	32.7	trace	ND	ND	135.7
29	97 Hull	75.9	1.0	11.3	3.8	7.9	74.5
30	97 Chester	83.4	1.1	4.1	3.6	7.8	107.7
31	97 Lochness	90.4	5.2	3.6	0.7	trace	117.7
32	97 Arapaho	87.0	4.8	4.8	1.6	1.9	102.2
33	97 Evergreen	83.2	3.1	7.2	3.7	2.7	99.7
34	97 Kotata	89.7	10.3	trace	trace	trace	119.6
35	98 Hull	77.8	1.0	11.2	3.2	6.8	102.2
36	98 Chester	86.9	trace	3.7	3.2	6.2	131.9
37	97 Wild Himalaya	83.2	1.1	8.9	2.7	4.1	174.0
38	97 Wild Evergreen	83.4	2.5	6.7	4.1	3.3	142.1
39	98 Wild Himalaya	82.9	1.0	9.1	2.9	4.1	183.4
40	98 Wild Evergreen	81.1	3.7	7.4	4.6	3.2	117.2

Table 2-1 —Continued

Oklahoma samples (n = 6)								
41	96	Navaho	87.5	0.8	7.3	1.9	2.5	130.4
42	96	Shawnee	92.2	4.3	0.3	1.8	1.5	113.5
43	96	Arapoho	87.5	4.7	3.6	2.2	2.0	90.9
44	96	Choctan	90.7	4.6	trace	4.7	trace	84.0
45	96	Chester	88.6	trace	3.0	2.6	5.8	140.7
46	97	Kiowa	91.6	4.7	trace	2.2	1.4	153.7
International samples (n = 6)								
47	96	Lochness (France)	92.8	3.8	3.4	trace	trace	191.5
48	96	Smoothstem (France)	84.1	0.6	6.3	1.5	7.4	93.9
49	98	(Macedonia sample)	77.0	0.7	5.4	2.0	14.9	111.3
50	98	(Chilean sample)	83.7	trace	3.7	3.7	8.9	139.1
51	98	(Chilean sample)	83.0	trace	3.5	4.1	9.3	144.1
52	98	(Mexico sample)	78.7	17.4	trace	3.9	trace	70.3
		Minimum	43.6	0.6	0.2	0.3	1.1	70.3
		Maximum	95.2	53.4	11.3	5.1	14.9	200.9
		Mean	82.9	10.2	2.5	1.9	2.5	136.9
		STD.	9.5	11.5	3.3	1.4	3.2	31.7

^a Berry samples are expressed on a fresh weight basis.

^b Selection with unidentified pigments.

^c Nondetectable

Table 2-2 —Anthocyanin content of some common fruits. (partially derived from Giusti and Wrolstad, 1999)

Source	Pigment content (mg/100g fresh weight)
Apple (Scugog)	10
Bilberries	300-320
Blackberries	70-201
Black currants	130-400
Blueberries	25-495
Black Chokeberries	560
Cherries	4-450
Cranberries	60-200
Elderberry	450
Kiwi	100
Black Raspberries	300-400
Red Raspberries	20-60
Strawberries	15-35

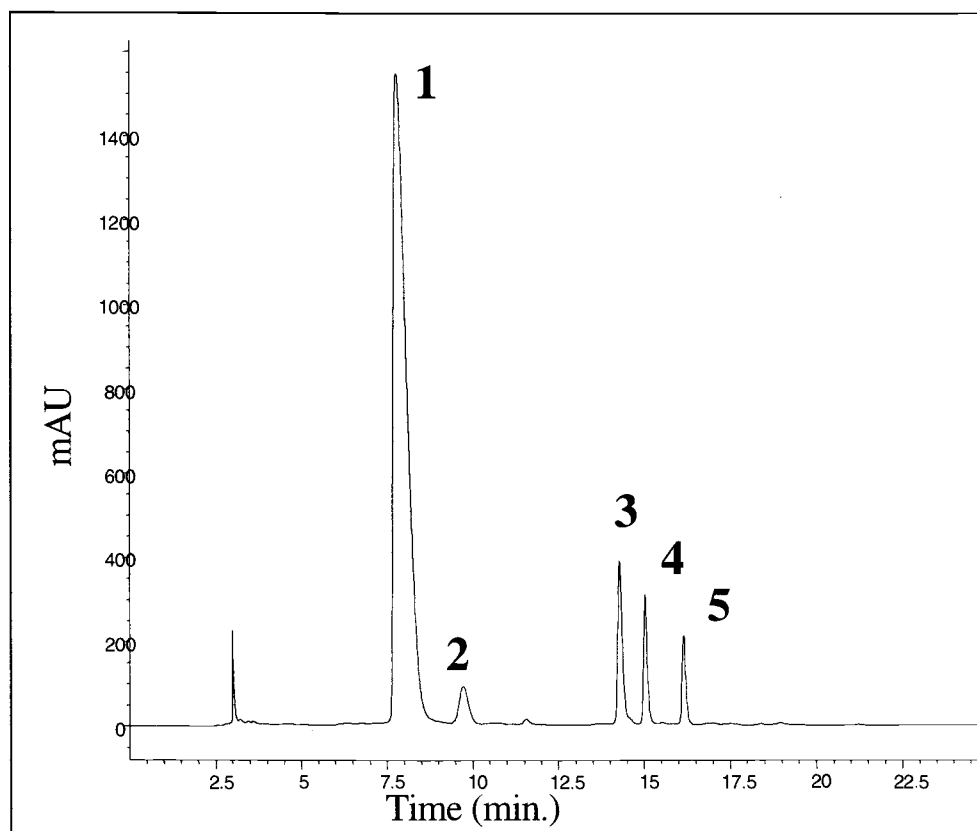


Figure 2.2 — HPLC separation of blackberry anthocyanins. 250 x 4.6 mm id Prodigy 5 μ ODS 3 column, two solvents used were solvent A (100% acetonitrile) and solvent B (5% acetonitrile, 10% acetic acid and 1% Phosphoric acid). The following solvent gradient program was used: 100% B through 5 min, then 20%/80% A/B through 20 min, then 40%/60% A/B through 25 min.

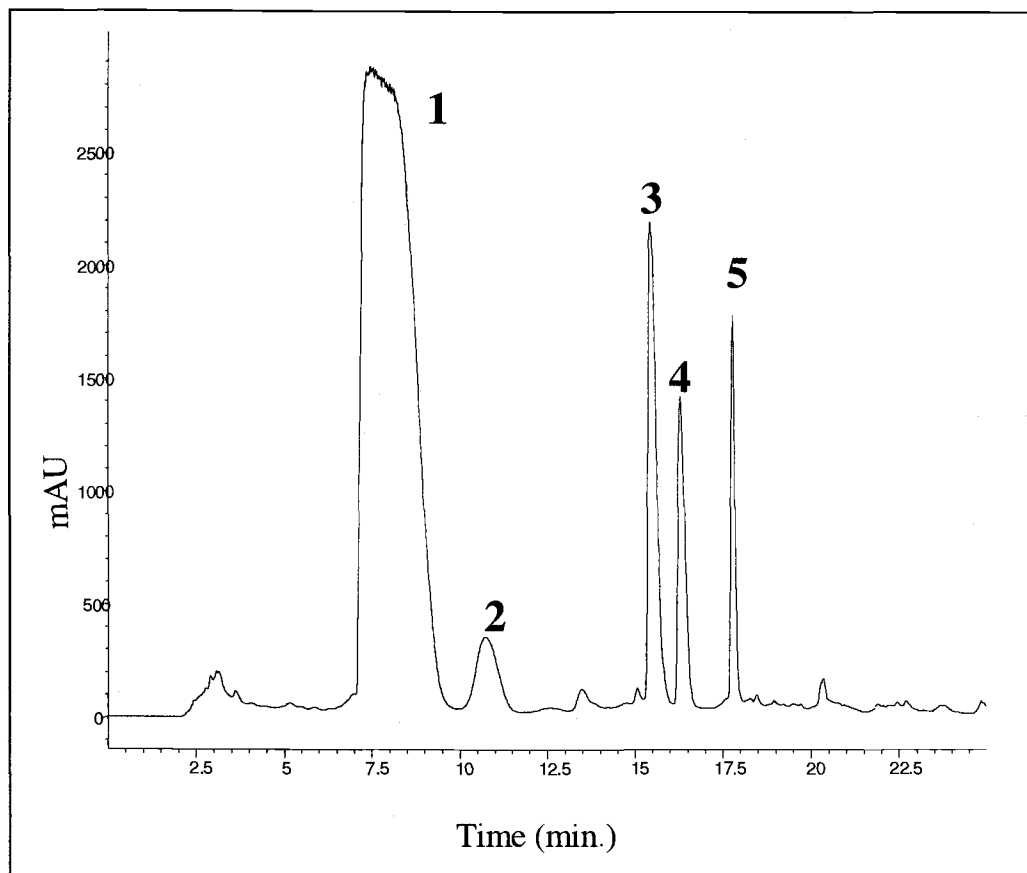


Figure 2.3 —Preparative HPLC chromatogram of Evergreen blackberry (*Rubus laciniatus*). Pigments were isolated with a Semi-prep microsorb C-18 column (Rainin, Inc., MA), two solvents used were solvent A (100% acetonitrile) and solvent B (5% acetonitrile, 10% acetic acid and 1% Phosphoric acid). Isocratic 7% A and 93% B through 20 min.

Table 2.3 — Electrospray mass spectroscopy of blackberry anthocyanins.

Peak	Characteristic	Molecular weight	Peak identification
1	non-acylated Acn.	449.0	cyanidin-3-glucoside
2	non-acylated Acn.	595.1	cyanidin-3-rutinoside
3	non-acylated Acn.	419.0	xylose-cyanidin derivative
4	acylated Acn.	535.1	malonic acid acylated cyanidin-3-glucoside
5	acylated Acn.	593.0	

increased with maturity for two cultivars; with one of the minor pigments decreasing substantially with ripening which correlated to the increase of cyanidin 3-glucoside.

A typical anthocyanin chromatogram of the 54 samples in this study showed that no peak eluted earlier than cyanidin 3-glucoside except for blackberry hybrids Loganberry and Boysenberry. Presence of cyanidin 3-sophoroside and cyanidin 3-glycosylrutinoside in blackberry juice concentrate reported previously by Hong and Wrolstad (1990) was not observed in this study. Since these two compounds had not been reported before in blackberry, the authors explained that the sample might have contamination from other species of *Rubus* (red raspberry or its clones). Our data supports their speculation of the sample being mixed with non-blackberry fruit or its origin being of a blackberry hybrid.

Peaks 3, 4, and 5 represented minor concentration as total pigment was concerned. In some cases, one or two or all of these three peaks presented trace

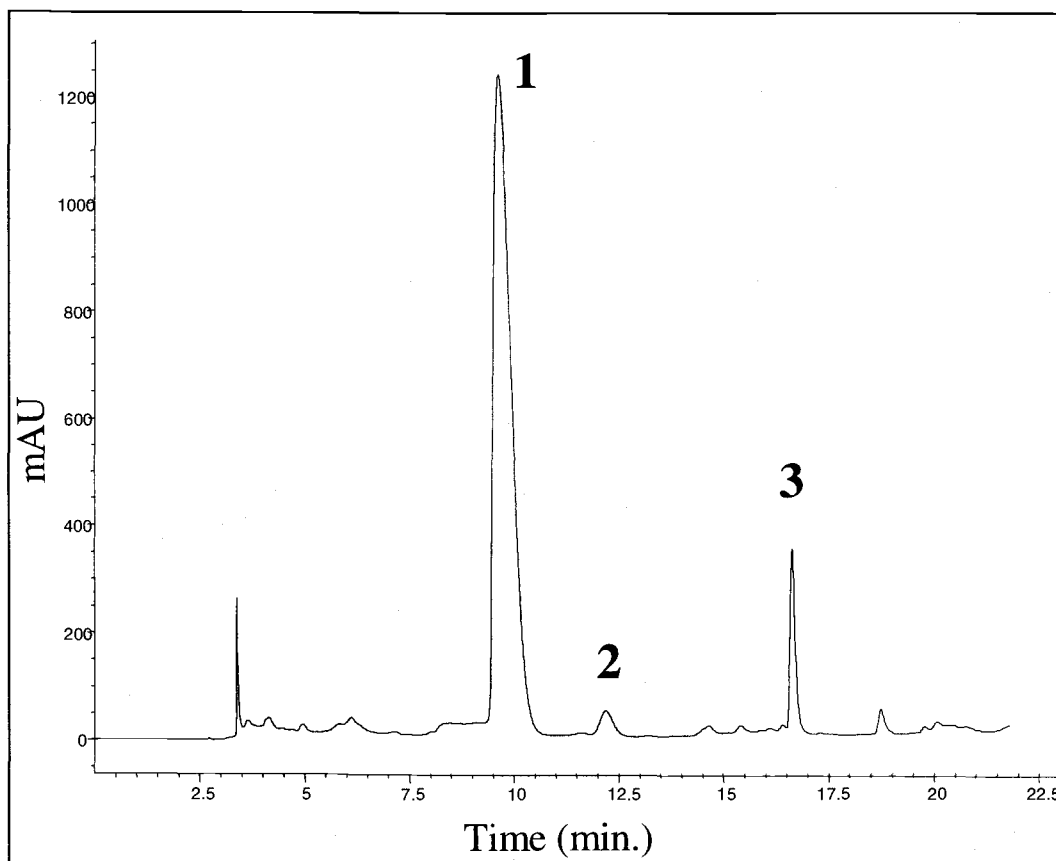


Figure 2.4 — HPLC separation of saponified blackberry anthocyanins. 250 x 4.6 mm id Prodigy 5 μ ODS 3 column, two solvents used were solvent A (100% acetonitrile) and solvent B (5% acetonitrile, 10% acetic acid and 1% Phosphoric acid). The following solvent gradient program was used: 100% B through 5 min, then 20%/80% A/B through 20 min, then 40%/60% A/B through 25 min.

ANTHOCYANIN PATTERNS IN BLACKBERRY

Sapers et al. (1986) proposed 5 patterns of the proportions of individual anthocyanins in different cultivars and selections from thornless blackberries. They are: (A) a large peak 1, a trace peak 2, and small peaks 3, 4, and 5; (B) a pattern similar to pattern A but with trace amounts of peak 3; (C) a smaller proportion of peak 1 and a larger proportion of peak 3 than is obtained with the other patterns; (D) resembles pattern A but with larger proportion of peak 5; (E) a pattern characterized by the elevation of peak 2 and disappearance of peak 3 and 5.

Four patterns were observed from this investigation mostly based on the proportion of cyanidin 3-rutinoside and minor pigments. 'Chester' and 'Smoothstem' resembled pattern A. 'Hull', 'Wild Himalaya' and 'Wild evergreen' resembled pattern C. Two Chilean samples and Yugoslavia sample were similar to pattern D. Most of the experimental selections fits to pattern E. In some cases, the percentage of peak 2 can count up to 53.4% of total pigment ('Sikiyou', 1112-2, 1369-3, 1370-2). Pattern B was not observed in this investigation. A large portion of the samples investigated in this study were trailing blackberries, they were characterized by smaller amounts of peaks 3, 4, and 5 than those from erect or semi-erect varieties.

In general, blackberries were characterized with five anthocyanin pigments. As international trade may concern, Figure 2.5 shows the chromatograms of blackberry from France, Yugoslavia, Chile and Mexico, which shared the same

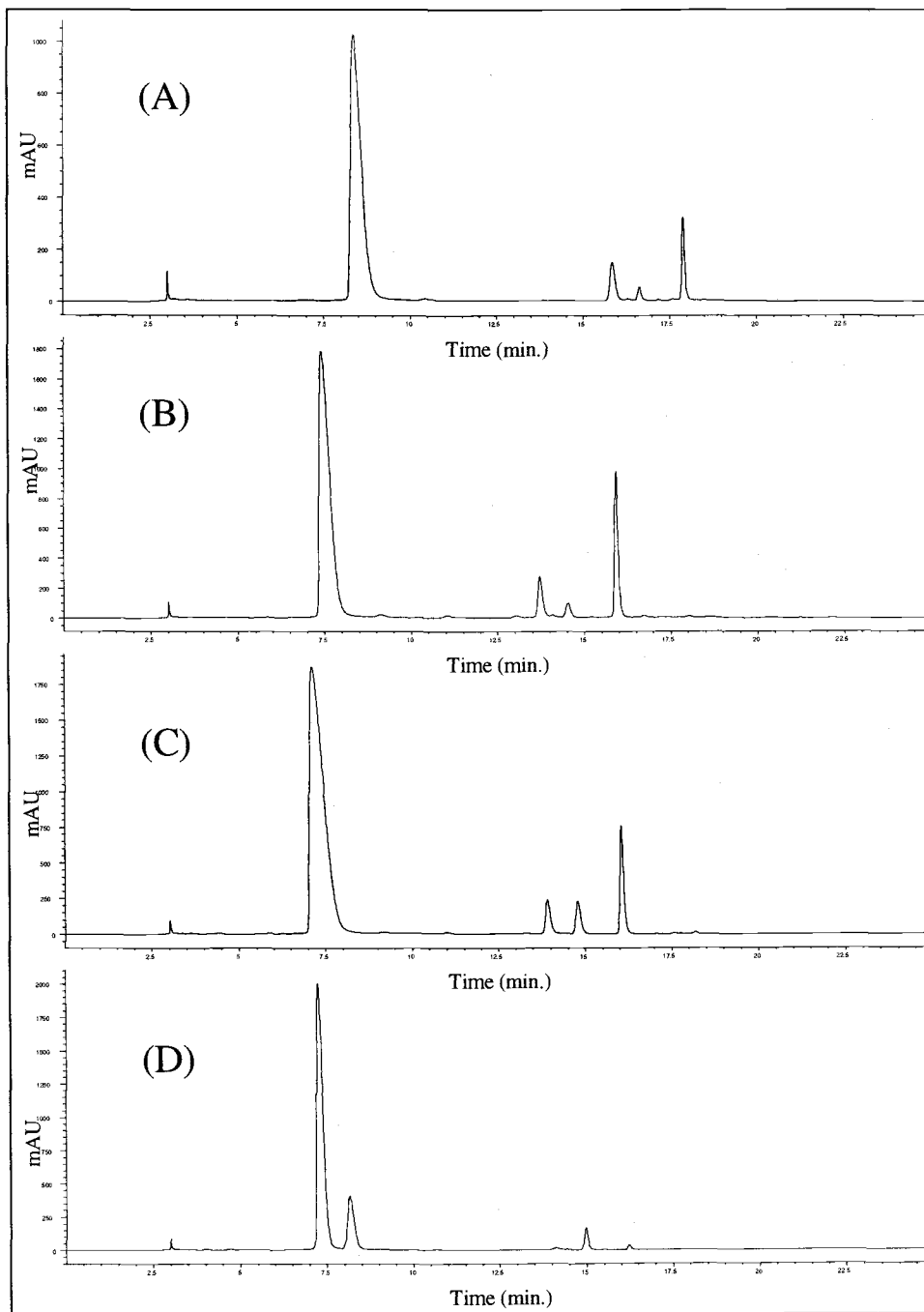


Figure 2.5 — Chromatogram of international samples: (a) France, (b) Yugoslavia, (c) Chile, and (d) Mexico.

anthocyanin profile as domestic samples. Blackberry hybrids show distinctive different anthocyanin profiles (Figure 2.6). Loganberry (*Rubus loganbaccus*) is a most important hybrid between blackberry and raspberry, but it is often considered a red variety of blackberries. Boysenberry is very likely a cross between the loganberry (*Rubus loganbaccus*) and a trailing blackberry (*Rubus baileyanus* Britt.) (Mazza and Miniati, 1993). Boysenberry and Loganberry fruit are characterized by the presence of cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside, and cyanidin 3-rutinoside. The pigment profile is an effective method for identifying these blackberry hybrids and detecting adulteration with other anthocyanin fruits, juices or colorants.

ANALYSIS OF COMMERCIAL BLACKBERRY JUICE CONCENTRATES

The monomeric anthocyanin content of commercial juice concentrates ranged from 12.3 to 106.6 mg/100g juice (Table 2.4). The pigment contents were low because of inefficient juice extraction and possible degradation of pigments during processing. The general lower monomeric anthocyanin content of commercial juice concentrates reveals that there is room for modification and improvement for pigment extraction.

Table 2.5 shows the anthocyanin composition of 10 commercial juice concentrates. The two most common cultivars in Oregon for processing are 'Evergreen' and 'Marion'. Samples A, B and E were labeled as Evergreen blackberry and their anthocyanin pattern is consistent with pattern C as

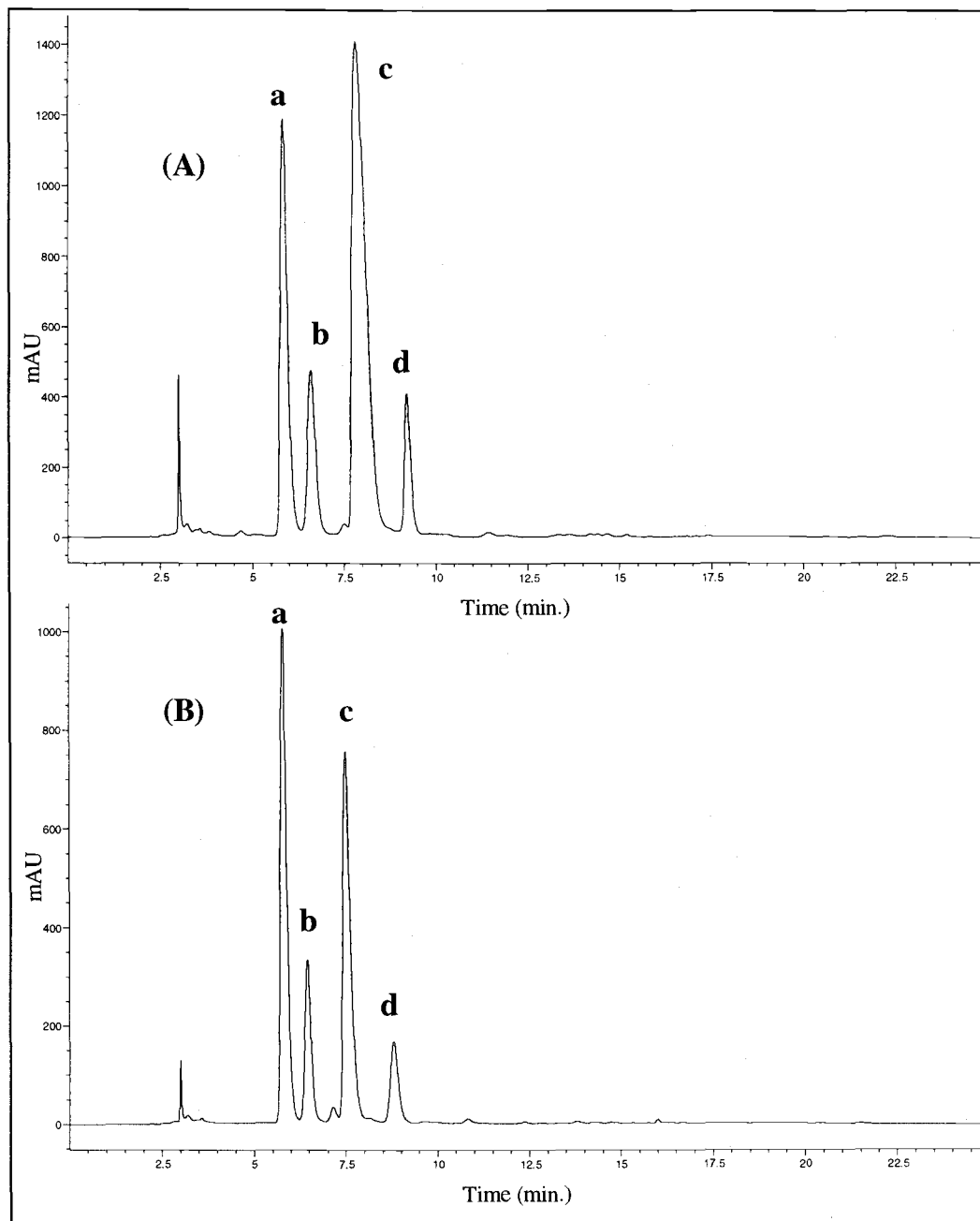


Figure 2.6 — Chromatogram of blackberry hybrids: (A) Boysenberry and (B) Loganberry. Peak characterization: (a) cyanidin 3-sophoroside, (b) cyanidin 3-glucosylrutinoside, (c) cyanidin 3-glucoside, and (d) cyanidin 3-rutinoside

mentioned above. The pattern was characterized by smaller peak 1 and larger portion of peak 3. Sample C is labeled as Marion blackberry and matched anthocyanin profile for its large portion of peak 2. Three unknown cultivar samples from Oregon (F, G and I) were most likely made from 'Marion'.

Sample J had an atypical pigment profile from patterns observed (Figure 2.7). Four minor pigments were eluted earlier than cyanidin 3-glucoside and more than three additional minor pigments were detected after cyanidin 3-glucoside. Alien peaks suggest this juice concentrate may contain other fruit juice. The early peaks may be contaminated with another species of *Rubus* (red raspberry, boysenberry, and loganberry etc.)

Table 2.4 — Commercial blackberry juice concentrates.

Year	Sample ^a	Cultivar	Brix	Diluted Brix	pH	ACN (mg/100g)	Normalized ACN (mg/100g)
94	A	Evergreen	68	10.06	3.85	13.00	12.29
98	B	Evergreen	68	10.07	3.37	45.28	44.96
98	C	Marion	65	10.06	3.23	65.61	65.22
98	D	Unknown (Chilean)	65	10.07	3.31	30.80	30.58
-	E	Evergreen	65	10.15	3.89	28.22	27.81
95	F	Unknown	60	10.02	3.44	69.69	69.55
97	G	Unknown(puree)	20	9.78	3.08	104.28	106.62
98	H	Unknown (Chilean)	67	10.46	3.35	37.56	35.91
98	I	Unknown (Oregon)	65	10.26	3.29	40.27	39.25
98	J	Unknown(Yugoslavia)	65	10.57	3.19	24.39	23.07

^a samples were from 6 juice processors.

Table 2.5 — Anthocyanin composition of commercial juice concentrates.

Sample	Peak1 (%)	Peak2 (%)	Peak3 (%)	Peak4 (%)	Peak5 (%)
A	71.7	4.3	11.7	7.2	5.1
B	80.1	2.5	5.5	4.5	7.4
C	71.2	25.0	Trace	1.1	2.7
D	81.6	1.5	7.8	2.5	6.6
E	76.1	3.7	8.1	6.7	5.4
F	76.1	15.9	2.6	2.5	2.9
G	68.1	27.4	0.7	1.3	2.5
H	86.5	1.4	3.8	1.8	6.5
I	75.9	18.3	1.9	1.3	2.6
J ^a	51.2	0.9	2.9	1.8	7.0

^a sample with unidentified

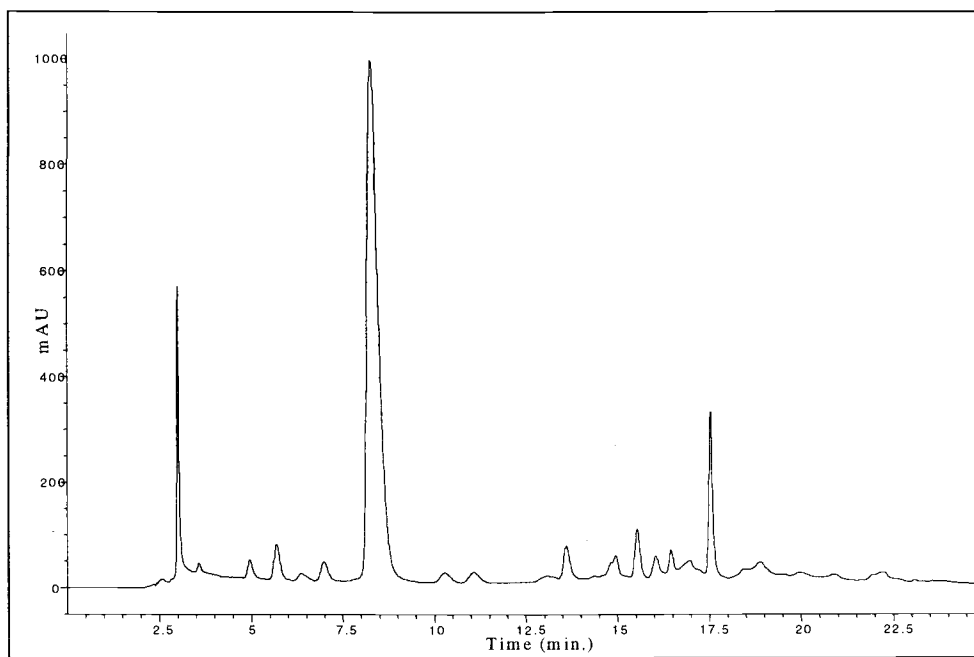


Figure 2.7 — Chromatogram of blackberry juice concentrate J.

Conclusion

The Anthocyanin pigment composition of 18 different varieties and 21 selections blackberries showed four distinctive patterns. There were no significant qualitative pigment differences between domestic and international samples collected. Five anthocyanin pigments were detected in blackberry and they were identified as cyanidin 3-glucoside, cyanidin 3-rutinoside, pentose-cyanidin derivative and two acylated cyanidin derivatives. The patterns are based on cyanidin 3-rutinoside and other minor pigments. The distinctive anthocyanin profile may be a helpful index in determination of authenticity of blackberry juice. The information obtained from monomeric anthocyanin content may be helpful in breeding program. The general lower monomeric anthocyanin content of commercial juice concentrates show that there is opportunity for modification and improvement of pigment extraction.

Acknowledgement

This project is being supported by the Northwest Center for Small Fruits Research. We thank Brian Arbogast of the Dept. of Agricultural Chemistry, Oregon State Univ., for the mass spectroscopy analyses, and Bob Durst and Brian Yorgey for laboratory and technical assistance and the following individuals for help in sample procurement: Chad Finn, NCSFR, Corvallis; Penny Perkins-Veazie, USDA,

Lane, Oklahoma; Mark Hurst, Hursts Berry Farm, Sheridan, OR; Silvia Cuadrado, EUROFINS Laboratories, Nantes, France; Victor Moller, Hortifrut S.A. Chili.

References

- Bate-Smith, E.C. 1948. Paper chromatography of anthocyanins and related substances in petal extracts. *Nature (London)*, 161, 835.
- Blundstone, H.A.W., and Crean, D.E.C. 1966. The pigments of red fruits, fruit vegetable. Preservation Research Association. Chipping Campden, England. 37.
- Fouassin, A. 1956. Identification par chromatographie des pigments anthocyanins des fruits et des legumes. *Rev. Ferment. Ind. Aliment.* 11, 173.
- García-Viguera, C., Zafrilla, P., and Tomás-Barberán. 1997. Determination of authenticity of fruit jams by HPLC analysis of anthocyanins. *J. Sci. Food Agric.* 73: 207-213.
- Giusti, M.M. and Wrolstad, R.E. 1996(a). Characterization of red radish anthocyanin. *J. Food Sci.* 61(2): 322-326.
- Giusti, M.M. and Wrolstad, R.E. 1996(b). Red radish anthocyanins as natural red colorant for maraschino cherries. *J. Food Sci.* 61(4): 688-694.
- Giusti, M.M. and Wrolstad, R.E. 2000. Unit F2.2 Characterization and measurement with UV-visible spectroscopy. Chap. F2: Anthocyanin. *In* Current protocols in food analytical chemistry. John Wiley and Sons. Inc. New York.
- Haborne, J.B. and Hall, E. 1964. Plant Polyphenols. XIII. The systematic distribution and origin of anthocyanins containing branched trisaccharides, *phytochemistry*, 3, 453.
- Hong, V. and Wrolstad, R.E. 1990a. Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection. *J. Agric. Food Chem.* 38(3): 698.
- Jennings, D. L. and Carmichael, E. 1980. Anthocyanin variation in the genus *Rubus*. *New. Phytol.* 84: 505.

- Mazza, G. and Miniati, E. (Ed.). 1993. Anthocyanins in fruits, vegetables and grains. CRC Press, Inc., Boca Raton, Florida.
- Rommel, A., Wrolstad, R.E. and Heatherbell, D.A. 1992. Blackberry juice and wine: processing and storage effects on anthocyanin composition, color and appearance. *J. Food Sci.* 57(2): 385-391.
- Strik, B.C. 1992. Blackberry cultivars and production trends in the Pacific Northwest. *Fruit Varieties Journal.* 46(4): 202-206.
- Sapers, G.M., Hicks, K.B., Burgher, A.M., Hargrave, D.L., Sondey, S.M. and Bilyk, A. 1986. Anthocyanin patterns in ripening thornless blackberries. *J. Am. Soc. Hortic. Sci.* 11: 945-950.
- Torre, L.C. and Barritt, B.H. 1977. Quantitative evaluation of *Rubus* fruit anthocyanin pigments. *J. Food Sci.* 42: 488-490.
- Wrolstad, R.E. 1976. Color and pigment analyses in fruit products. Oregon St. Univ. Agric. Exp. Stn. Bull. No. 624.
- Wrolstad, R.E., Culbertson, J.D., Cornwell, C.J., and Mattick, L.R. 1982. Detection of adulteration in blackberry juice concentrates and wines. *J. Assoc. Off. Anal. Chem.* Vol. 65, No. 6.
- Wrolstad, R.E., Hong, V., Boyles, M.J., and Durst, R.W. 1995. Use of anthocyanin pigment analyses for detecting adulteration in fruit juices *In* Methods to detect adulteration of fruit juice beverages. Volume I. Nagy, S. and Wade, R.L.(Editor). AGSCIENCE, INC., Auburndale, Florida.

Chapter 3

NONVOLATILE ACID AND SUGAR COMPOSITION OF BLACKBERRIES

Abstract

Analyses of nonvolatile acids and sugars were conducted on 52 samples of blackberry (*Rubus eubatus*) to develop a compositional database for evaluating authenticity and quality. °Brix ranged from 6.88 to 16.83 with a mean of 10.82. Titratable acidity ranged from 0.52 to 2.24 with a mean of 1.35 g citric acid/100mL. Sucrose levels (range, 0–12.9%; mean, 4.07%) were highly variable. The overall glucose: fructose ratio ranged from 0.81 to 1.17 with a mean of 1.01. Two patterns of nonvolatile acid compositions were found in blackberries. The identities of malic, isocitric, lactoisocitric, shikimic and citric acids were confirmed. Malic acid ranged from 5.2% to 35.3% (87.5–603 mg/100g) of total acids with a mean of 16.4% (280 mg/100g). Lactoisocitric acid ranged from 3.4–32.6% with a mean of 17.3% (mean, 293 mg/100g). Isocitric acid ranged from 79.1 to 1554 mg/100g (mean, 599 mg/100g). Citric acid ranged from 15.2 to 1892 mg/100g (mean, 572 mg/100g). Presence of fumaric acid has not previously been reported. Succinic acid was detected in some samples. Ten commercial samples were also analyzed which exhibited HPLC profiles similar to the authentic standard. It was possible to classify the commercial concentrates as to whether they were Marion, Evergreen or a mixture of the two from their acid profiles.

Introduction

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 (Wrolstad et al., 1980). Fruit juices may be adulterated by addition of foreign, cheaper juices or by addition of sugar solutions acidified with organic acids (Fitelson, 1969).

Widdowson and McCance (1935) reported glucose and fructose to be the principle sugars of blackberry. The percentage of glucose, fructose and sucrose were 3.24, 2.88 and 0.24, respectively. Workers in Europe and the United States (Fitelson, 1970; Benk and Bergmann, 1978; Kline et al., 1970) have reported that blackberries contain only glucose and fructose and do not contain sorbitol or sucrose. Fitelson (1970) reported qualitative analysis of the sugars by gas-liquid chromatography (GLC) to detect adulteration of blackberry and other fruit juice concentrates. Wrolstad et al (1980) reported that the sugar composition of blackberries was quite uniform, with only glucose and fructose detected. Sorbitol is not fermented by yeast and can be detected in wines made from blackberry juice concentrates that contain sorbitol (Wrolstad et al., 1982). In that investigation, high levels of sorbitol and quinic and malic acids suggest that an imported blackberry concentrate may have been adulterated with plum.

Fruits can be grouped according to their glucose:fructose ratio. Pear and apple contain much more fructose than glucose while raspberry, blackberry, grape, strawberry, and cherry have essentially invert sugar patterns. On the other hand,

peach and plum contain more glucose than fructose (Wrolstad and Shallenberger, 1981).

Whiting (1958) reported malic to be the predominant acid in blackberries with large amounts of isocitric and lactoisocitric; trace amounts of quinic and shikimic acids were also shown to be present.

A distinct alteration in the acid pattern of a fruit juice, accompanied by a change in acid intensities, will demonstrate the absence of the normal amount of characteristic fruit acids and the presence of foreign acids. For example, addition of grape juice to dark colored juices can readily be detected by the presence of tartaric acid (Fitelson, 1969)

The detection of phosphoric, malic, lactoisocitric, citric-isocitric, and quinic was reported by Wrolstad et al (1980) by gas chromatographic analysis of trimethylsilyl (Me₃Si) derivatives. The results showed considerable variation among the 15 blackberry varieties with lactoisocitric being absent in six of the samples. From this method, the major difficulty is that the mixture of citric and isocitric acids could not be resolved because of relatively close retention time.

The literature provides limited compositional data for authentic blackberry juice. Compositional data-base as RSK values list "guide values" for raspberry, black currant, sour cherry, etc., but there are no reported standard RSK values for blackberries which would be helpful in determination of the authenticity of blackberry juice.

The purpose of this investigation is to develop a statistical data-base which encompasses samples from the principal blackberry producing regions of the world. A more complete and extensive compositional data-base for authentic blackberry juice is needed to establish better criteria for evaluating the authenticity and quality of blackberry products in international trade.

Materials and Methods

SAMPLES

For the '96, '97 and '98 seasons, 55 samples were obtained representing 18 different varieties and 21 selections from 5 different geographic locations. Blackberry fruit samples were obtained from North Willamette Research and Extension Center (NWREC), Aurora, OR; Hurst Berry Farm, Sheridan, OR; ARS SCARL Research Center (USDA), Lane, OK. Three European samples were supplied by EUROFINS Laboratories Nantes, France, originating from France and Macedonia; two samples from Central America (Mexico, unknown variety) and South America (Chilean, unknown variety) were supplied by Hortifrut. In addition to the 52 authentic berry samples, wild cultivars and hybrids such as Boysenberry and Loganberry, which have blackberry parentage, were also included in this investigation. Fruit samples were individually quick-frozen (IQF), and stored frozen at -12°C until needed.

Nine commercial juice concentrates and one puree sample were supplied by six juice processing companies, two of the samples originated from Chile and

Yugoslavia. In soliciting the samples, the companies were assured that individual sample identities would be kept confidential. These samples were coded A through J. After arrival, samples were stored frozen at $-12\text{ }^{\circ}\text{C}$. Before analysis, they were thawed and adjusted to single strength juice.

REAGENTS

- (a) HPLC mobile phase for organic acids.-Phosphate buffer pH 2.4. Dissolve 27.2 g KH_2PO_4 in 1000 mL deionized water and adjusted pH to 2.4 with concentrated phosphoric acid. Filter through 0.45 μm Millipore filter, type HA (Millipore Corp., Bedford, MA), and degas.
- (b) HPLC mobile phase for sugars.-200 mg $\text{Ca}(\text{NO}_3)_2/1000\text{mL}$ water.-Dissolve 200 mg $\text{Ca}(\text{NO}_3)_2$ in 1000mL deionized water. Filter through 0.45 μm Millipore filter, type HA (Millipore Corp., Bedford, MA), and degas.
- (c) Organic acid standards.- 1% solutions of reagent grade malic, isocitric, lactoisocitric, shikimic, citric, succinic and fumaric acid were made individually and accurately weighed. Standards were then diluted and combined to the appropriate concentrations that cover the range of the juice samples according to preliminary runs.
- (d) Sugar standards.- 1% of reagent grade sucrose, fructose, glucose and sorbitol were made individually and accurately weighed. Mannitol was added as internal standard. Standards were then diluted and added together to the

appropriate concentration which cover the range of the juice samples according to preliminary runs.

EXTRACTION PROCEDURE

Extraction of acids and sugars from blackberries followed procedures outlined from Giusti (1996b). Fifty grams of frozen blackberries were liquid nitrogen powdered using a stainless steel Waring Blender. The powdered samples were then blended for 3 minutes with 100 mL of acetone and filtered using Whatman No. 4 filter paper on a Büchner funnel. The filter cake remnant was reextracted with an aqueous solution of acetone: water, 70/30 v/v, until a clear solution appeared. The filtrates were combined and shaken in a separatory funnel with a solution of acetone: chloroform, 1:2 v/v, and stored overnight at 1 °C. The aqueous portion was separated and put onto a Büchi rotovapor at 42 °C for 10 minutes to rid the sample of residual acetone. The extract was then brought to 200 mL in a volumetric flask with deionized water. The procedure of extraction was replicated for each sample. Samples were stored frozen at -12°C. before analyses.

ORGANIC ACIDS —HPLC DETECTION

Individual nonvolatile acids were determined by using the AOAC Official Method **986.13**.

Sample preparation

5 mL of sample was passed through a previously activated C₁₈ minicolumn. The first 2 mL was discarded and the remaining eluate collected, filtered through 0.45µm Millipore filter (type HA), and injected onto the LC system.

Juice sample preparation

1.5 mL Bio-Rex 5 resin bed was prepared in Poly-Prep column and the bed was rinsed with 5 mL deionized water. 3 mL of sample was carefully applied to the resin bed and the bed was washed with 3 mL water. A test tube was placed under column and acids were eluted with 3.5 mL 10% sulfuric acid followed by 3.5 mL deionized water. The eluate was mixed and passed through activated C₁₈ minicolumn. The first 3 mL was discard, remaining eluate was collected, filtered through 0.45µm Millipore filter (type HA), and injected onto LC system.

HPLC determination

High performance liquid chromatography was operated under the following conditions: columns, Spherisorb ODS-2, 5 µm, 250 × 4.6 mm id, and Spherisorb ODS-1, 5 µm, 250 × 4.6 mm id in series (Alltech Associates, Deerfield, IL) fitted with ODS-10 Micro-Guard column (Bio-Rad); mobile phase, 0.2 M KH₂PO₄ pH 2.4; flow rate, 0.7 mL/min; injection volume, 50 µL. The program is 100% solvent for 27 minute. Each sample was dual injected for replication. Different

concentration standards were dual injected between every two samples to monitor the performance of instrument, peak shift and standard curve calculation.

SUGARS—HPLC DETECTION

The detection followed the procedure described by Spanos and Wrolstad (1987).

Sample preparation

5 mL liquid nitrogen extract or diluted single strength juice concentrate was mixed with 3 mL sugar standard solution and mixture was passed through activated Sep-Pak C18 cartridge. The first 3 mL was discarded and remaining elute was collected. 4 mL eluant was applied to 1.2 mL Bio-Rex 5 resin bed. The first 2 mL was discarded, the remaining eluant was collected, mixed, filtered through 0.45 μm Millipore filter (type HA), and injected onto HPLC.

HPLC determination

Liquid chromatograph (Varian 5000) was operated under following condition: column, Bio-Rad Aminex HPX-87; mobile phase, 200 mg $\text{Ca}(\text{NO}_3)_2/1000$ mL water; flow rate, 0.7 mL/min; elution temperature, 85°C; detection, refractive index, 4X attenuation; detection temperature, 25°C; integrator, Perkin-Elmer LCI 100 laboratory computing integrator; injection volume, 50 μL . Each sample was dual injected for replication.

°Brix

Soluble solids were determined with AOAC Official Method **932.14C**, using refractometer without correction for acidity. Soluble solids were measured by using an Auto Abbe refractometer Model 10500 (Leica Inc., Buffalo, NY). All readings were corrected to standard temperature at 20°C.

Titrateable Acidity

Titrateable acidity was determined by using AOAC Official Method **942.15B** glass electrode method. Results are expressed as anhydrous citric acid per 100 mL juice.

Results and Discussion**GENERAL PROPERTIES**

Table 3.1 summarizes the characteristics of 52 blackberry samples grown in 1996, 1997, and 1998. °Brix of the blackberries ranged from 6.88 – 16.83 with a mean of 10.82 which is very close to the USA single strength standard 10.0°. Most of the experimental selections showed lower soluble solids than the mean value. The results of soluble solid were similar to Sapers et al. (1985) investigation of 40 varieties and selections of thornless blackberry (range, 7.7-13.9; mean = 10.8).

The pH ranged from 2.65 – 3.61 with a mean of 3.19. Titrateable acidity ranged from 0.52 – 2.24 (mg citric/100g) with a mean of 1.35. Blackberry hybrids

(Boysenberry and Loganberry) were not included in the statistical summary for any of the compositional tables.

SUGAR ANALYSES

The results of the sugar analyses are shown in Table 3.2. The individual sugar was identified by comparison of retention time with standards. Nonvolatile acids were removed by anion exchange minicolumn to prevent coelution with sugars. Sucrose, glucose and fructose were found in blackberry. Sucrose ranged from 0 to 12.9% of total sugars, with a mean of 4.07%. Widdowson and McCance (1935) reported sucrose representing 3.7% of total sugars and Lee et al. (1970) reported 10%.

Glucose and fructose were dominant sugars present in nearly equal quantities. The overall Glucose:fructose ratio ranged of 0.81 to 1.17 with a mean of 1.01. No sorbitol was detected in authentic samples. There were less variation in sugar composition than acid composition in blackberries. There is considerable variation in the individual and total sugar contents, but the glucose-to-fructose ratio and the individual sugar content (with the exception of sucrose) expressed as percent of total show much less variation. The blackberry sugar profile, as compiled from the literature by Wrolstad and Shallenberger (1981), showed a narrower range but higher total sugar content. The sucrose content and the glucose-to-fructose ratio (mean = 1.0) are in agreement with our data.

Table 3.1—Sample table

#	Sample	Year	Growing location	Brix	pH	TA (Citric acid, g/100mL)
Oregon samples (n = 40)						
1	MARION	96	NWREC. ^a	13.80	2.93	2.02
2	KOTATA	96	NWREC.	10.80	3.28	2.00
3	WALDO	96	NWREC.	16.50	3.46	1.89
4	Sikiyou	96	NWREC.	10.80	3.21	1.36
5	1112-2	96	NWREC.	12.10	3.19	1.77
6	N-71	96	NWREC.	10.80	3.01	2.09
7	MARION	97	NWREC.	13.50	3.09	1.68
8	KOTATA	97	NWREC.	10.50	3.04	2.12
9	WALDO	97	NWREC.	11.85	3.30	1.88
10	EVERGREEN	97	NWREC.	15.62	3.57	0.83
11	Sikiyou	97	NWREC.	11.55	3.11	1.72
12	1111-1	97	NWREC.	9.05	3.04	1.28
13	1112-2	97	NWREC.	10.43	3.45	1.15
14	N-71	97	NWREC.	9.60	3.01	2.04
15	MARION	98	NWREC.	11.80	3.17	1.62
16	KOTATA	98	NWREC.	9.03	3.29	1.58
17	WALDO	98	NWREC.	12.43	3.27	1.59
18	LOCH NESS	98	NWREC.	9.95	2.99	1.40
19	BLACK DOUGLAS	98	NWREC.	9.92	3.12	2.24
20	1299-1	98	NWREC.	6.88	3.41	0.93
21	1316-1	98	NWREC.	7.24	3.24	1.45
22	1324-1	98	NWREC.	8.49	3.22	0.97
23	1325-1	98	NWREC.	10.22	3.16	1.25
24	1332-10	98	NWREC.	6.97	3.02	1.57
25	1361-1	98	NWREC.	7.82	3.28	1.50
26	1363-1	98	NWREC.	7.69	3.16	1.95
27	1369-3	98	NWREC.	9.57	3.09	1.66
28	1370-2	98	NWREC.	7.69	3.04	1.54
29	HULL	97	Hurst Berry Farm, Sheridan.	10.82	3.27	0.80
30	CHESTER	97	Hurst Berry Farm, Sheridan.	9.98	2.71	1.37
31	LOCH NESS	97	Hurst Berry Farm, Sheridan.	11.03	2.65	1.36
32	ARAPAHO	97	Hurst Berry Farm, Sheridan.	16.83	3.55	0.52
33	EVERGREEN	97	Hurst Berry Farm, Sheridan.	11.52	3.36	0.95
34	KOTATA	97	Hurst Berry Farm, Sheridan.	14.29	3.29	1.04
35	HULL	98	Hurst Berry Farm, Sheridan.	11.15	3.52	0.66
36	CHESTER	98	Hurst Berry Farm, Sheridan.	11.57	3.15	1.57
37	Wild HIMALAYA	97	Sheridan.	13.38	3.21	0.97
38	Wild EVERGREEN	97	Wrolstad Farm, Molalla.	12.92	3.61	0.71
39	Wild HIMALAYA	98	Corvallis.	12.38	3.21	1.26
40	Wild EVERGREEN	98	Wrolstad Farm, Molalla.	13.48	3.60	0.84
Oklahoma samples (n = 6)						
41	NAVAHO	96	ARS SCARL Research Center, Lane.	13.63	3.45	0.74
42	SHAWNEE	96	ARS SCARL Research Center, Lane.	10.52	3.35	0.81
43	ARAPAHO	96	ARS SCARL Research Center, Lane.	10.52	3.30	0.81
44	CHOCTAN	96	ARS SCARL Research Center, Lane.	8.96	3.15	0.70
45	CHESTER	96	ARS SCARL Research Center, Lane.	9.20	3.19	0.80
46	KIOWA	97	ARS SCARL Research Center, Lane.	9.32	2.86	0.71

Table 3.1—continued

International samples (n = 6)					
47 LOCH NESS	96	France.	9.31	2.94	0.96
48 SMOOTHSTEN	96	France.	11.33	3.14	0.91
49 Unknown	98	Macedonia.	9.96	3.03	1.83
50 Unknown	98	Chile	9.20	3.09	1.79
51 Unknown	98	Chile	9.00	3.09	1.55
52 Unknown	98	Mexico	9.80	3.11	1.31
Min.			6.88	2.65	0.52
Max.			16.83	3.61	2.24
Mean			10.82	3.19	1.35
Std.			2.28	0.21	0.47
Others (Oregon samples) (n = 3) ^b					
53 BOYSEN	98	Hurst Berry Farm, Sheridan.	13.74	3.16	1.21
54 BOYSEN	98	Corvallis.	12.64	3.41	1.07
55 LOGAN	98	-	11.12	3.13	1.91

^a North Willamette Research & Extension Center (NWREC)

^b Non-blackberry samples were not included in statistic analysis.

Figure 3.1 shows the chromatograms of blackberry sugars from pressed juice sample and sample extracted by liquid nitrogen. Liquid nitrogen extraction procedure avoided sucrose hydrolysis by native invertase. Low temperature and nitrogen atmosphere should inhibit enzyme activity. Sucrose was not detectable in commercial juice samples, which was presumed to be degraded by invertase during processing. Glucose to fructose ratio shift may indicate the degradation. Some of the international and domestic berry samples had low or no sucrose content. Storage conditions may have permitted sucrose hydrolysis from native invertase.

Table 3.2 —Sugar composition

#	Year	Sample	Concentration, g/100g berry ^a				Percent of total sugar			
			Sucrose	Glucose	Fructose	Total	Sucrose	Glucose	Fructose	Glucose/Fructose
Oregon samples (n = 40)										
1	96	Marion	0.17	2.68	2.63	5.48	3.0	48.9	48.1	1.02
2	96	Kotata	0.20	2.48	2.45	5.12	3.8	48.4	47.8	1.01
3	96	Waldo	0.36	2.87	2.90	6.13	5.9	46.8	47.3	0.99
4	96	Sikiyou	0.26	2.90	2.98	6.14	4.2	47.2	48.6	0.97
5	96	1112-2	0.19	2.43	2.35	4.97	3.9	48.9	47.3	1.03
6	96	N-71	0.32	2.45	2.65	5.42	5.9	45.2	48.9	0.92
7	97	Marion	0.35	2.85	2.81	6.02	5.9	47.4	46.8	1.01
8	97	Kotata	0.39	2.09	2.14	4.61	8.4	45.2	46.4	0.98
9	97	Waldo	0.06	2.97	2.89	5.92	0.9	50.3	48.8	1.03
10	97	Evergreen	0.02	5.01	4.87	9.90	0.2	50.6	49.2	1.03
11	97	Sikiyou	0.21	2.56	2.70	5.47	3.8	46.8	49.4	0.95
12	97	1111-1	0.15	2.15	2.25	4.55	3.3	47.3	49.5	0.96
13	97	1112-2	0.36	3.01	2.97	6.34	5.7	47.4	46.9	1.01
14	97	N-71	0.20	1.17	1.25	2.62	7.7	44.7	47.6	0.94
15	98	Marion	0.06	3.21	3.22	6.50	1.0	49.4	49.6	1.00
16	98	Kotata	0.06	2.46	2.50	5.02	1.1	49.0	49.8	0.98
17	98	Waldo	0.20	3.95	3.78	7.93	2.6	49.8	47.7	1.04
18	98	Lochness	0.30	2.50	2.50	5.30	5.6	47.2	47.3	1.00
19	98	Black Douglas	0.36	2.34	2.22	4.91	7.3	47.6	45.1	1.05
20	98	1299-1	0.18	1.78	1.68	3.64	5.0	48.9	46.1	1.06
21	98	1316-1	0.14	1.67	1.71	3.52	3.9	47.4	48.7	0.97
22	98	1324-1	0.31	2.30	2.29	4.91	6.4	46.9	46.7	1.00
23	98	1325-1	0.22	3.02	3.07	6.30	3.4	47.9	48.7	0.98
24	98	1332-10	0.08	1.52	1.47	3.07	2.5	49.7	47.8	1.04
25	98	1361-1	0.16	1.52	1.49	3.17	5.0	48.0	47.0	1.02
26	98	1363-1	0.16	1.48	1.31	2.95	5.5	50.3	44.3	1.14
27	98	1369-3	0.18	2.42	2.39	4.99	3.6	48.5	47.9	1.01
28	98	1370-2	0.17	1.82	1.72	3.71	4.5	49.1	46.4	1.06
29	97	Hull	0.48	2.95	3.13	6.57	7.3	45.0	47.7	0.94
30	97	Chester	0.37	2.19	2.35	4.91	7.5	44.6	47.9	0.93
31	97	Lochness	0.72	2.17	2.69	5.58	12.9	38.9	48.2	0.81
32	97	Arapaho	1.07	6.34	6.45	13.86	7.7	45.7	46.5	0.98
33	97	Evergreen	0.68	2.73	2.42	5.83	11.7	46.8	41.5	1.13
34	97	Kotata	0.56	4.45	4.53	9.54	5.9	46.6	47.5	0.98
35	98	Hull	0.08	3.46	3.41	6.95	1.2	49.8	49.0	1.02
36	98	Chester	0.16	2.32	2.36	4.85	3.3	47.9	48.8	0.98
37	97	Wild Himalaya	0.51	4.30	3.66	8.47	6.0	50.8	43.2	1.17
38	97	Wild Evergreen	0.79	3.76	3.62	8.17	9.7	46.0	44.3	1.04
39	98	Wild Himalaya	0.31	4.04	3.99	8.35	3.7	48.4	47.8	1.01
40	98	Wild Evergreen	0.40	4.39	4.21	8.99	4.4	48.8	46.8	1.04
Oklahoma samples (n = 6)										
41	96	Navaho	0.04	4.38	4.06	8.48	0.5	51.7	47.9	1.08
42	96	Shawnee	0.03	3.46	3.09	6.58	0.5	52.6	47.0	1.12
43	96	Arapaho	0.21	3.15	2.74	6.10	3.4	51.6	44.9	1.15
44	96	Choctan	ND	3.26	3.29	6.55	ND	49.8	50.2	0.99
45	96	Chester	ND	2.47	2.38	4.85	ND	50.9	49.1	1.04
46	97	Kiowa	0.16	2.94	3.13	6.23	2.6	47.2	50.2	0.94

Table 3.2 —continued

International samples (n = 6)										
47	96	Lochness	ND	2.86	2.84	5.7	ND	50.2	49.8	1.01
48	96	Smoothstem	ND	3.29	3.56	6.85	ND	48.0	52.0	0.92
49	98	(Macedonia sample)	0.01	2.68	2.75	5.43	0.1	49.3	50.6	0.98
50	98	(Chilean sample)	0.19	2.30	2.39	4.88	3.9	47.1	49.0	0.96
51	98	(Chilean sample)	0.42	2.19	2.35	4.95	8.5	44.1	47.4	0.93
52	98	(Mexico sample)	0.06	2.97	3.09	6.12	1.0	48.6	50.5	0.96
		Minimum	—	1.17	1.25	2.62	—	38.9	41.5	0.81
		Maximum	1.07	6.34	6.45	13.86	12.9	52.6	52.0	1.17
		Mean	0.24	2.91	2.89	6.04	4.1	48.1	47.8	1.01
		STD.	0.22	0.95	0.91	1.94	2.9	2.3	1.9	0.06
Others (Oregon samples) (n = 3)										
53	98	Boysenberry	0.42	3.80	3.68	7.90	5.3	48.0	46.6	1.03
54	98	Boysenberry	0.37	3.96	3.92	8.25	4.5	48.0	47.5	1.01
55	98	Logan	0.02	3.50	3.28	6.80	0.27	51.49	48.24	1.07

*Berry samples are expressed on a fresh weight basis.

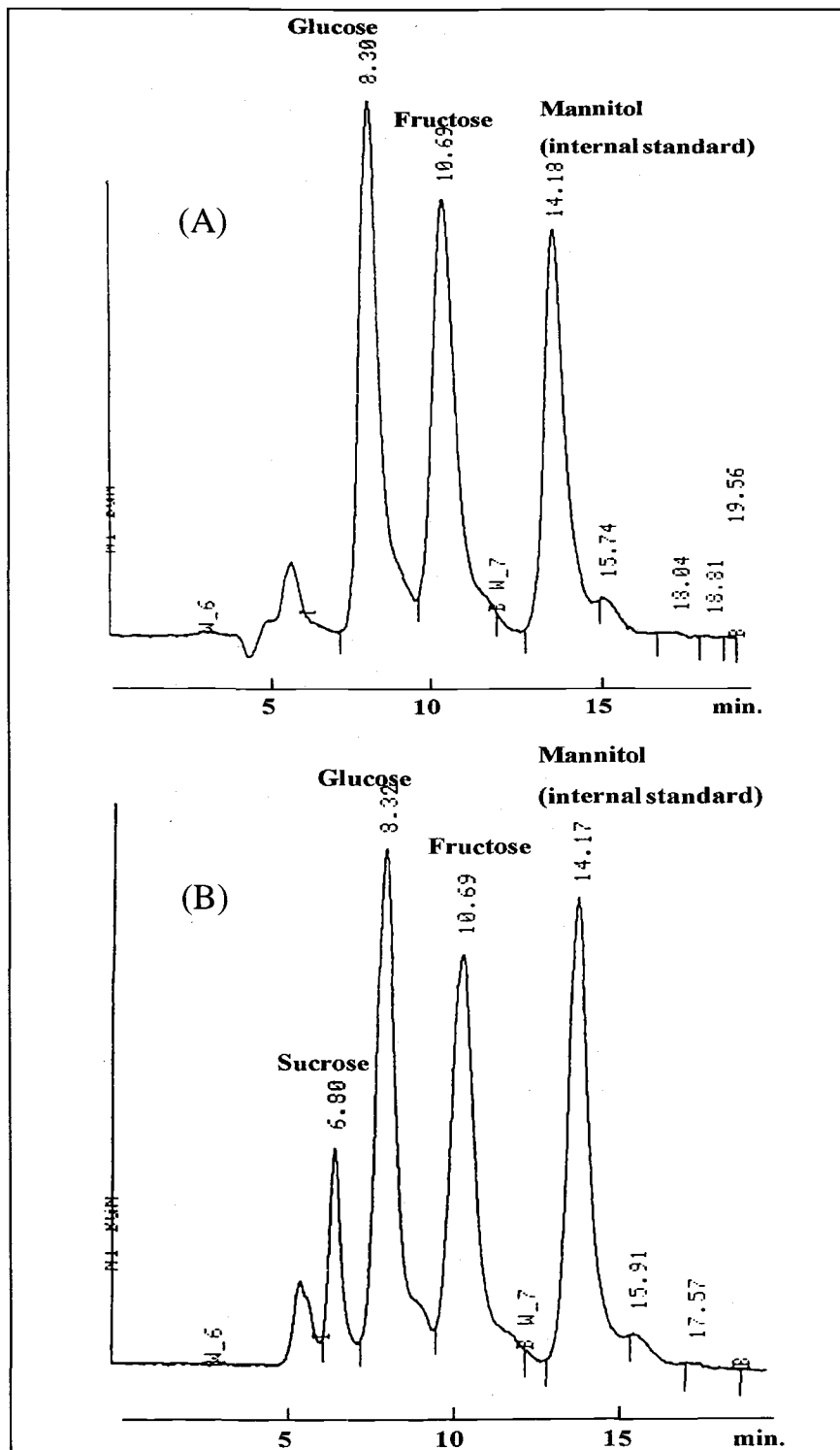


Figure 3.1—Sugars of blackberry. (A) juice sample. (B) liquid nitrogen extract sample.

Results for the commercial juice concentrates are shown in Table 3.3. Sucrose was detected in samples D, H, I, and J. The glucose:fructose ratio of all commercial samples are within the range of data obtained from authentic samples, no extreme value was observed. However, sorbitol was detected in some of the samples, which was not detected in any of the authentic samples. Sorbitol is sometimes used as an osmotic agent in commercial pectolytic enzyme preparations and could be a source of sorbitol in fruit juice (Durst et al., 1995). In that study, nine of 33 commercial pectinase contained sorbitol. The sorbitol contribution would be 0.005-0.015 g/100mL, if the juice was processed at highest recommended dosage. Thus, commercial enzyme preparations are a potential source of trace amounts of sorbitol. It is worth noting that sample I had unusually high content (0.214 g/100 mL, 3.2 % of total sugar) which is most likely indicative of contamination with sorbitol containing fruit. The simple pattern of the sugar profile of blackberry is not unique or distinctive. However, presence of trace amounts of alien sugars may be an indication of adulteration. Presence of sorbitol is a very useful indicator

Table 3.3— Sugar compositions of commercial juice concentrates.

Year	Sampl	Cultivar	Concentration, g/100g ^a				Total	Percent of total sugar				Glucose/Fructose
			Sucrose	Glucose	Fructose	Sorbitol		Sucrose	Glucose	Fructose	Sorbitol	
94	A	Evergre	0.00	3.8	4.0	ND	7.8	0.0	48.	51.	ND	0.9
98	B	Evergre	0.00	3.1	3.2	Trace	6.4	0.0	49.	50.	Trace	0.9
98	C	Mario	0.00	3.3	3.2	Trace	6.6	0.0	50.	49.	Trace	1.0
98	D	Unknown	0.02	3.3	3.5	ND	6.8	0.3	48.	51.	ND	0.9
?	E	Evergre	0.00	3.3	3.3	Trace	6.6	0.0	49.	50.	Trace	0.9
95	F	Unkno	0.00	3.1	3.1	ND	6.3	0.0	50.	49.	ND	1.0
97	G	Unknown(puree)	0.00	3.3	3.1	ND	6.5	0.0	51.	48.	ND	1.0
98	H	Unknown	0.02	3.3	3.5	ND	6.8	0.4	48.	51.	ND	0.9
98	I	Unknown	0.03	3.1	3.3	0.21	6.6	0.4	46.	49.	3.2	0.9
98	J	Unknown(Yugoslavia)	0.03	3.2	3.3	Trace	6.6	0.4	48.	50.	Trace	0.9

^aJuice concentrates are expressed on a fresh juice

of dilution with a sorbitol-containing juice as cherry, plum, apple, or pear (Spanos and Wrolstad, 1987).

NONVOLATILE ACID ANALYSES

Identification of individual peaks was done by matching retention time with standards. Presence of malic, isocitric, lactoisocitric, shikimic, citric, and fumaric acids were identified in this investigation. Succinic acid was detected in some of the samples. Presence of fumaric acid has not previously been reported. C-18 minicolumn was applied to remove anthocyanins and phenolic compounds during sample preparation, which dramatically decreased interference. The difficulty of not been able to separate citric and isocitric by gas chromatographic analysis of trimethylsilyl (Me₃Si) derivatives was resolved by applying HPLC separation. The acid resolution is highly related to column performance and mobile phase composition.

Two distinct patterns of nonvolatile acids in blackberry were found in this investigation. Chromatograms of trailing, semi-erect and erect blackberries are shown in Figures 3.2. and 3.3. Cultivars with high proportions of citric acid have smaller proportions of isocitric and lactoisocitric, and vice-versa. Pattern I is characterized by citric acid as the dominant acid in this pattern. Trailing blackberries, 'Marion', 'Kotata', 'Waldo', 'Sikiyou' and all the experimental selections, are pattern I except those with European origin ('Evergreen', 'wild Evergreen', and 'wild Himalaya'). Pattern II is characterized by isocitric acid as the

dominant acid. Semi-erect blackberries 'Hull', 'Chester', 'Lochness', and 'Smoothstem' and erect blackberries 'Arapaho', 'Navaho', 'Shawnee', 'Choctaw', and 'Kiowa' belong to pattern II. The results from this large data- base clarified the long-term debate about whether citric or isocitric acid is the dominant acid in blackberries.

The results of the nonvolatile organic acid analyses are shown in Table 3.4, .87.5-603 mg/100g (mean, 280 mg/100g) malic acid, 55.6-633 mg/100g (mean, 293 mg/100g) lactoisocitric acid, 79.1-1554 mg/100g (mean, 599 mg/100g) isocitric acid, 15.2-1892 mg/100g (mean, 572 mg/100g) citric acid.

Lactoisocitric is a marker compound in blackberry. Lactoisocitric is present in trace amounts in some fruits and vegetables, but it rarely accumulates to significant amounts. All blackberry samples contain lactoisocitric in substantial quantities. It ranged from 3.4 to 32.6 % of total acids with a mean of 17.3%. Blackberry hybrids, Boysenberry and Loganberry, were low in Lactoisocitric.

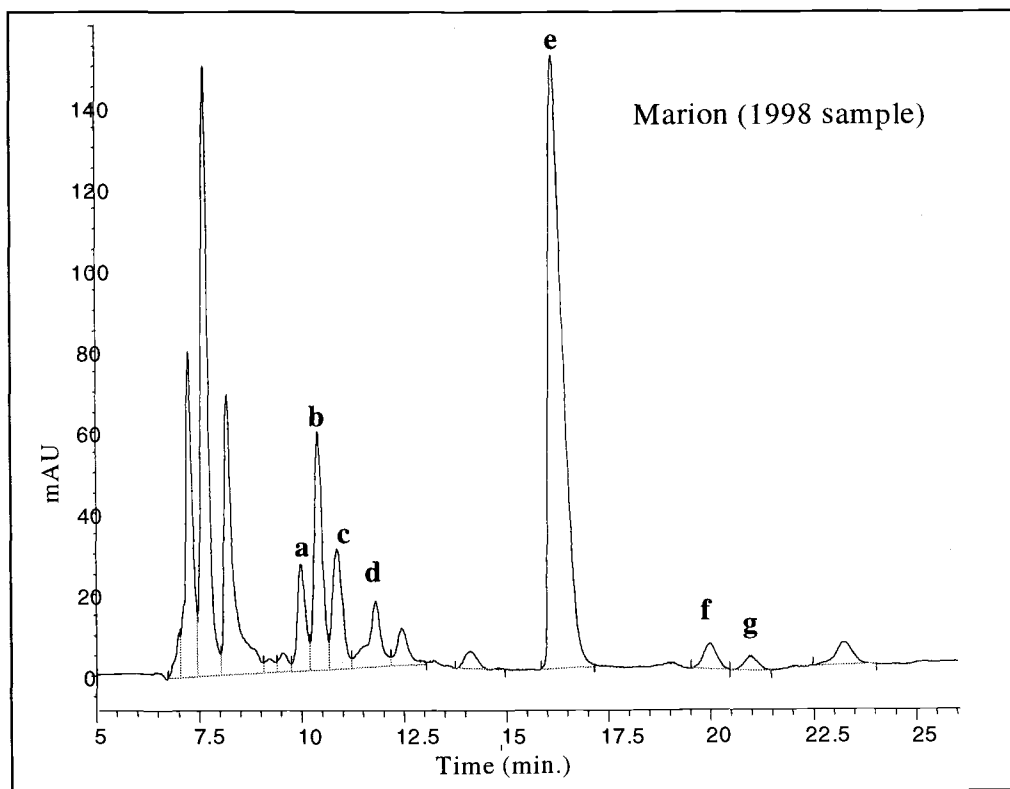


Figure 3.2—Nonvolatile acids of blackberry (pattern I). Peak identification: a—malic, b—isocitric, c—lactoisocitric, d—shikimic, e—citric, f—succinic, and g—fumaric.

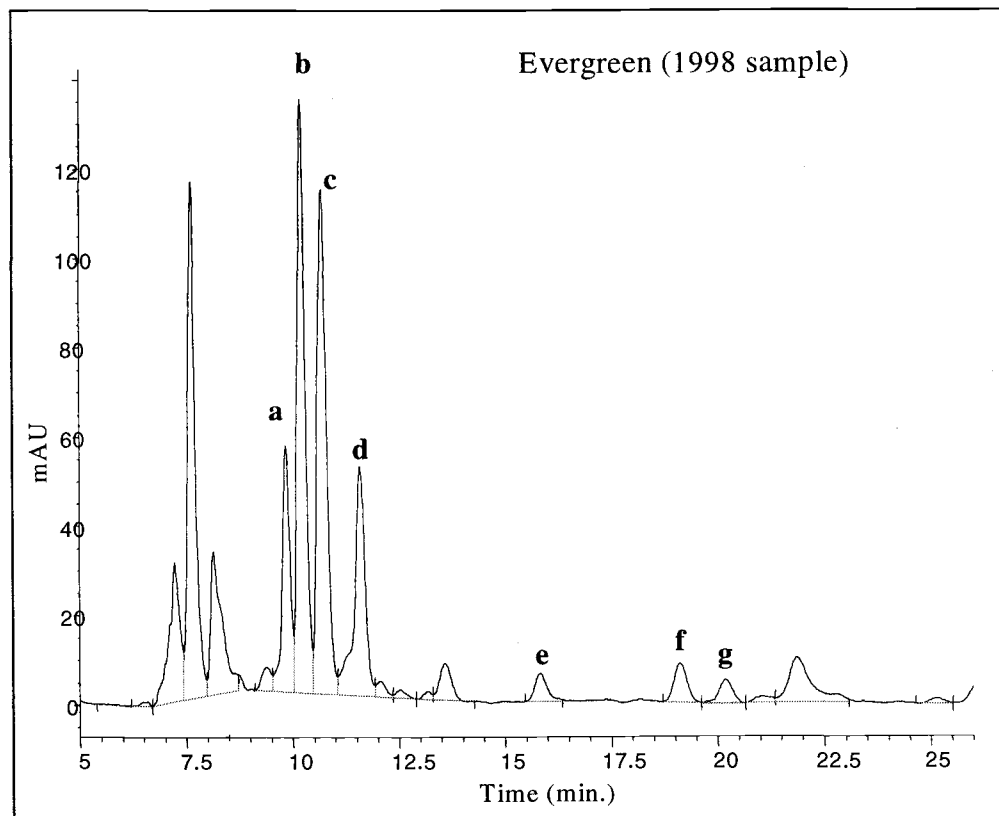


Figure 3.3—Nonvolatile acids of blackberry (pattern II). Peak identification: a—malic, b—isocitric, c—lactoisocitric, d—shikimic, e—citric, f—succinic, and g—fumaric.

Table 3.5 shows the nonvolatile acids composition of commercial juice concentrates procured. Sample A, B, and E were labeled as 'Evergreen' blackberry juice concentrates. Their acids composition matches pattern II, which is characterized by isocitric acid as the dominant acid and relatively low in citric acid. Sample C was labeled as 'Marion' blackberry juice concentrates. Its acid composition matches pattern I, which was characterized by citric acid as the dominant acid and relatively low in isocitric acid. Unlabelled samples F, G, and I were previously proposed to be sourced from cultivar 'Marion' from their anthocyanin pattern. The nonvolatile acid pattern does not confirm that they are exclusively 'Marion'. The quantities of citric and isocitric were equally prevalent in sample F and I, which suggests these two juice concentrates were made from both cultivars. This may also explain why these two samples have lower concentration of peak 2 and more significant peak 3 of anthocyanin profile than typical Marion blackberry juice should be (chapter 2). For Evergreen blackberry, pigment peak 2 presented lower concentration and pigment peak 3 higher than the one in Marion blackberry. Mixing of two or more cultivars in juice concentrate production could easily take place. The two Chilean samples had different acid patterns which suggested that they were made from different varieties. Because of the large variation in citric and isocitric content, adulteration with citric acid would be difficult to detect.

Table 3.4 —Nonvolatile acids

#	Year	Sample	Type	Malic		Isocitric		Isocitric Lacton		Citric		Shikimic mg/100g ^a	Fumaric mg/100g ^a	Citric/ isocitric
				mg/100g ^a	%	mg/100g ^a	%	mg/100g ^a	%	mg/100g ^a	%			
Oregon samples (n = 40)														
1	96	Marion	Trailing	91.7	8.2	296.9	26.6	166.1	14.9	560.9	50.3	0.68	0.54	1.89
2	96	Kotata	Trailing	266.6	12.3	164.0	7.6	108.5	5.0	1626.3	75.1	0.32	0.22	9.92
3	96	Waldo	Trailing	204.7	7.0	529.2	18.2	284.0	9.8	1891.8	65.0	0.40	0.22	3.57
4	96	Sikiyou	Trailing	424.4	22.8	278.0	15.0	233.3	12.5	923.4	49.7	0.52	0.36	3.32
5	96	1112-2	Trailing	240.3	18.9	292.8	23.0	273.2	21.5	464.8	36.6	0.32	0.38	1.59
6	96	N-71	Trailing	311.0	12.8	422.4	17.3	194.4	8.0	1507.6	61.9	0.30	0.14	3.57
7	97	Marion	Trailing	87.5	5.2	438.8	25.9	200.6	11.9	965.0	57.0	0.26	0.34	2.20
8	97	Kotata	Trailing	311.3	16.0	91.3	4.7	97.0	5.0	1448.0	74.3	0.45	0.28	15.86
9	97	Waldo	Trailing	120.4	6.3	274.3	14.3	181.8	9.5	1339.7	69.9	0.27	0.36	4.88
10	97	Evergreen	Trailing	231.7	15.6	833.3	56.2	381.1	25.7	36.2	2.4	0.43	0.31	0.04
11	97	Sikiyou	Trailing	552.6	35.2	153.9	9.8	161.6	10.3	703.1	44.7	0.68	0.15	4.57
12	97	1111-1	Trailing	276.7	22.2	103.7	8.3	128.1	10.3	737.3	59.2	0.37	0.16	7.11
13	97	1112-2	Trailing	464.4	16.6	301.7	10.8	235.9	8.4	1803.7	64.3	0.81	0.49	5.98
14	97	N-71	Trailing	363.8	18.9	216.1	11.2	110.5	5.7	1236.6	64.2	0.23	0.28	5.72
15	98	Marion	Trailing	100.4	6.0	372.5	22.2	137.8	8.2	1070.6	63.7	0.19	0.28	2.87
16	98	Kotata	Trailing	154.8	9.3	117.0	7.1	55.6	3.4	1329.5	80.2	0.30	0.29	11.36
17	98	Waldo	Trailing	136.1	7.5	299.2	16.6	151.2	8.4	1217.0	67.5	0.22	0.27	4.07
18	98	Lochness	Semi-Erect	184.8	9.5	1207.9	62.4	510.8	26.4	33.3	1.7	0.12	0.24	0.03
19	98	Black Douglas	Trailing	216.5	10.6	256.1	12.5	240.8	11.7	1338.7	65.2	0.36	0.34	5.23
20	98	1299-1	Trailing	124.5	11.2	98.2	8.9	88.1	8.0	796.3	71.9	0.12	0.36	8.11
21	98	1316-1	Trailing	421.8	25.9	79.1	4.9	75.7	4.6	1053.3	64.6	0.07	0.44	13.32
22	98	1324-1	Trailing	218.1	18.6	270.8	23.1	272.3	23.3	408.8	34.9	0.27	0.21	1.51
23	98	1325-1	Trailing	461.8	27.6	267.5	16.0	185.3	11.1	757.9	45.3	0.44	trace	2.83
24	98	1332-10	Trailing	423.3	26.9	217.7	13.9	216.1	13.8	713.8	45.4	0.31	0.37	3.28
25	98	1361-1	Trailing	406.1	18.3	313.9	14.2	279.4	12.6	1214.2	54.9	0.54	trace	3.87
26	98	1363-1	Trailing	323.1	12.9	524.2	21.0	464.1	18.6	1186.8	47.5	0.29	trace	2.26
27	98	1369-3	Trailing	602.7	31.5	227.1	11.9	241.3	12.6	844.2	44.1	0.48	trace	3.72

Table 3.4 —continued

28	98	1370-2	Trailing	354.8	23.7	150.3	10.0	99.6	6.6	895.2	59.7	0.28	0.26	5.96
29	97	Hull	Semi-	152.4	15.2	600.6	59.8	213.6	21.3	38.2	3.8	0.18	0.32	0.06
30	97	Chester	Semi-	470.7	17.2	1554.1	56.7	632.5	23.1	84.6	3.1	0.21	0.32	0.05
31	97	Lochnes	Semi-	182.7	9.4	1283.4	66.2	442.9	22.8	29.5	1.5	0.08	0.25	0.02
32	97	Arapah	Erect	291.5	17.8	753.9	46.0	530.0	32.3	64.5	3.9	0.35	0.34	0.09
33	97	Evergree	Trailing	295.3	23.2	616.9	48.5	337.0	26.5	21.7	1.7	0.31	0.41	0.04
34	97	Kotata	Trailing	102.5	10.2	89.4	8.9	92.1	9.1	725.4	71.9	0.25	0.26	8.11
35	98	Hull	Semi-	183.2	14.3	766.9	59.9	286.3	22.4	44.3	3.5	0.32	0.36	0.06
36	98	Chester	Semi-	253.8	10.7	1528.1	64.3	510.1	21.5	86.0	3.6	0.25	0.37	0.06
37	97	Wild	Trailing	228.8	13.9	985.9	59.9	363.0	22.1	67.7	4.1	0.43	0.46	0.07
38	97	Wild	Trailing	268.0	19.3	694.3	50.0	405.3	29.2	20.1	1.4	0.32	0.27	0.03
39	98	Wild	Trailing	269.7	14.8	1091.2	60.0	427.9	23.5	31.3	1.7	0.32	0.21	0.03
40	98	Wild	Trailing	210.3	14.7	828.0	57.8	358.1	25.0	37.0	2.6	0.50	0.47	0.04
Oklahoma samples (n = 6)														
41	96	Navaho	Erect	129.5	8.9	908.2	62.7	390.6	26.9	21.3	1.5	0.12	0.38	0.02
42	96	Shawne	Erect	311.8	23.4	676.1	50.8	343.8	25.8	Trace		0.08	0.32	
43	96	Arapoh	Erect	223.4	15.7	723.3	50.9	453.2	31.9	19.8	1.4	0.20	0.19	0.03
44	96	Chocta	Erect	354.7	35.3	394.0	39.3	229.4	22.9	25.5	2.5	0.09	0.56	0.06
45	96	Chester	Semi-	147.8	9.2	1022.8	63.6	416.5	25.9	21.4	1.3	0.09	0.42	0.02
46	97	Kiowa	Erect	111.1	11.3	617.3	63.1	235.4	24.0	15.2	1.5	0.16	0.24	0.02
International samples (n = 6)														
47	96	Lochnes	Semi-	162.0	9.1	1270.3	71.6	311.8	17.6	28.9	1.6	0.08	0.38	0.02
48	96	Smoothste	Semi-	412.0	24.3	900.0	53.1	330.6	19.5	53.2	3.1	0.29	0.29	0.06
49	98	(Macedonia	unknow	526.5	21.6	1380.6	56.5	469.8	19.2	64.7	2.6	0.21	0.35	0.05
50	98	(Chilean	unknow	405.3	15.8	1512.5	58.9	607.5	23.6	44.4	1.7	0.17	0.32	0.03
51	98	(Chilean	unknow	288.7	12.7	1445.9	63.6	499.8	22.0	38.4	1.7	0.14	0.25	0.03
52	98	(Mexico	unknow	481.7	27.2	680.2	38.4	578.4	32.6	32.7	1.8	0.20	0.16	0.05

Table 3.4—continued

			Minimum	87.5	5.2	79.1	4.7	55.6	3.4	15.2	1.3	0.07	0.14	0.02
			Maximum	602.7	35.3	1554.1	71.6	632.5	32.6	1891.8	80.2	0.81	0.56	15.86
			Mean	279.6	16.4	598.5	34.7	293.1	17.3	571.5	31.6	0.30	0.32	3.08
			STD.	132.5	7.5	439.9	22.6	152.5	8.3	589.2	29.8	0.16	0.10	3.78
Others (Oregon samples) (n = 3)														
53	98	Boysenberry	Trailing	208.7	13.5	116.4	7.5	55.2	3.6	1170.9	75.5	0.41	0.11	10.06
54	98	Boysenberry	Trailing	159.2	14.0	105.3	9.3	48.2	4.2	823.6	72.5	0.33	0.28	7.82
55	98	Logan	Trailing	183.93	13.7	110.85	8.2	51.72	3.8	997.22	74.2	0.40	0.42	9.00

^aBerry samples are expressed on a fresh weight

^bNon-blackberry samples were not included in statistic

Table 3.5 —Acid composition of commercial blackberry juice concentrates.

Year	Sample	Cultivar	Malic		Isocitric		Ioscitric Lactone		Citric		Shikimic mg/100 ^a g	Fumaric mg/100 ^a g
			mg/100 ^a g	%	mg/100 ^a g	%	mg/100 ^a g	%	mg/100 ^a g	%		
94	A	Evergreen	109.6	16.7	283.3	43.2	214.6	32.7	46.6	7.1	0.28	1.99
98	B	Evergreen	165.2	16.6	541.6	54.5	261.4	26.3	25.1	2.5	0.20	0.91
98	C	Marion	124.3	8.6	374.5	25.8	176.2	12.1	776.2	53.5	0.28	0.42
98	D	Unknown	60.0	6.3	274.2	28.9	150.4	15.8	463.6	48.8	0.55	0.56
?	E	Evergreen	89.2	7.6	621.8	53.3	335.3	28.7	118.8	10.2	0.33	1.33
95	F	Unknown	89.2	7.0	514.2	40.6	257.7	20.4	403.8	31.9	0.43	0.03
97	G	Unknown (puree)	81.1	6.2	361.6	27.6	156.4	11.9	709.6	54.2	0.28	0.31
98	H	Unknown	124.7	10.3	703.8	58.3	270.0	22.4	108.1	9.0	0.51	0.57
98	I	Unknown	102.2	8.9	457.1	39.6	144.8	12.5	449.4	38.9	0.32	0.40
98	J	Unknown (Yugoslavia)	297.8	17.7	1028.3	61.1	241.8	14.4	115.2	6.8	0.51	0.64

^a Berry samples are expressed on a fresh weight

Conclusion

Two patterns of acid profiles of blackberry were identified in this investigation. The method we used has the optimum separation of acids in berry fruit juices. It was possible to classify the commercial concentrate as to whether they were Marion, Evergreen or a mixture of the two from their acid profiles. The simple pattern of sugar profile limits its utility in detecting adulteration. Not a single index, anthocyanin profiles or acid or sugar composition can alone reveal the quality and authenticity of commercial juice concentrate.

Acknowledgement

This project is being supported by the Northwest Center for Small Fruits Research. We thank Bob Durst and Brian Yorgey for laboratory and technical assistance and the following individuals for help in sample procurement: Chad Finn, NCSFR, Corvallis, OR; Penny Perkins-Veazie, USDA, Lane, OK; Mark Hurst, Hursts Berry Farm, Sheridan, OR; Silvia Cuadrado, EUROFINS Laboratories, Nantes, France; Victor Moller, Hortifrut S.A. Chili.

References

- Benk, V.E., and Bergmann, R. 1978. Contributions to the knowledge of blackberry mother juice. *Flussige Obst* 45: 14-17.
- Bielig, H.J., Faethe, w., Fuchs, G., Koch, J., Wallrauch, S., and Wucherpfenning, K. 1987. RSK values-the complete manual, verband der deutschen Fruchtsaftindustrie E.V., Bonn, Germany
- Durst, R.W., Wrolstad, R.E., and Krueger, D.A. 1995. Sugar, nonvolatile acid, $^{13}\text{C}/^{12}\text{C}$ ratio, and mineral analysis for determination of the authenticity and quality of red raspberry juice composition. *Journal of AOAC International*. 78: 1195-1204.
- Fitelson, J. 1969. Paper Chromatographic Detection of major organic acid in fruit juices. *J. Assoc. Off. Anal. Chem.* 52: 646.
- Fitelson, J. 1970. Detection of adulteration in fruit juices by qualitative Determination of carbohydrates by gas-liquid chromatography. *J. Assoc. Off. Anal. Chem.* 53: 1193-1197.
- Kline, D.A., Fernanadez-Flores, E., and Johnson, A.R. 1970. Quantitative determintation of sugars in fruits by GLC separation of TMS derivatives. *J. Assoc. Off. Anal. Chem.* 53: 1198-1202.
- Lee, C.Y., Shallenberger, R.S., and Vittum, M.T. 1970. Free sugar in fruits and vegetables. *New York Food and Life Science Bulletin* No. 1. N.Y. State Agriculture Experiment Station, Geneva, NY.
- Official Methods of Analysis (1990) 15th Ed., AOAC, Arlington, VA, Vol. II
- Spanos, G.A., and Wrolstad, R. E. 1987. Anthocyanin pigment, nonvolatile acid, and sugar composition of red raspberry juice. *J. Assoc. Off. Anal. Chem.* 70: 1036-1046.
- Whiting, G.C. 1958. The non-volatile organic acids of some berry fruits. *J. Sci. Food Agric.* 9, 244.
- Wrolstad, R.E., Culbertson, J.D., Nagaki, D.A., and Madero, C.F. 1980. Sugars and nonvolatile acids of blackberries. *J. Agric. Food Chem.* 28: 553-558.

Wrolstad, R.E., Culbertson, J.D., Cornwell, C.J., and Mattick, L.R. 1982. Detection of adulteration in blackberry juice concentrates and wines. *J. Assoc. Off. Anal. Chem.* V. 65(6): 1417-1423.

Wrolstad, R.E., and Shallenberger, R.S. 1981. Free sugar and sorbitol in fruits- A compilation from the literature. *J. Assoc. Off. Anal. Chem.* 64(1): 91-103.

Chapter 4

CONCLUSION

The Anthocyanin pigment composition of 18 different varieties and 21 selections blackberries showed four distinctive patterns. There were no significant qualitative pigment differences between domestic and international samples collected. Five anthocyanin pigments were detected in blackberry and they were identified as cyanidin 3-glucoside, cyanidin 3-rutinoside, pentose-cyanidin derivative and two acylated cyanidin derivatives. The patterns are based on cyanidin 3-rutinoside and other minor pigments. The distinctive anthocyanin profile may be a helpful index in determination of authenticity of blackberry juice. The information obtained from monomeric anthocyanin content may be helpful in breeding program. The general lower monomeric anthocyanin content of commercial juice concentrates show that there is opportunity for modification and improvement of pigment extraction.

Two patterns of acid profiles of blackberry were identified in this investigation. The method we used has the optimum separation of acids in berry fruit juices. It was possible to classify the commercial concentrate as to whether they were Marion, Evergreen or a mixture of the two from their acid profiles. The simple pattern of sugar profile limits its utility in detecting adulteration. Not a single index, anthocyanin profiles or acid or sugar composition can alone reveal the quality and authenticity of commercial juice concentrate.

BIBLIOGRAPHY

- Bate-Smith, E.C. 1948. Paper chromatography of anthocyanins and related substances in petal extracts. *Nature (London)*, 161, 835.
- Benk, V.E., and Bergmann, R. 1978. Contributions to the knowledge of blackberry mother juice. *Flussige Obst* 45: 14-17.
- Bielig, H.J., Faethe, w., Fuchs, G., Koch, J., Wallrauch, S., and Wucherpfenning, K 1987. RSK values-the complete manual, verband der deutschen Fruchtsaftindustrie E.V., Bonn, Germany
- Blundstone, H.A.W., and Crean, D.E.C. 1966. The pigments of red fruits, fruit vegetable. Preservation Research Association. Chipping Campden, England. 37.
- Boyles, M.J., and Wrolstad, R.E. 1993. Anthocyanin composition of red raspberry juice: influences of cultivar, processing and enviromental factors. *J. Food Sci.* 58, 1135-1141.
- Brooks, R.M., and Olmo, H.O. 1997. The Brooks and Olmo register of fruit & nut varieties. 3rd ed. ASHS Press, Alexandria, Virginia.
- Code of Federal Regulation. 1999. 21 CFR parts 101.30. The office of the federal register national archives and records administration, Washington, DC.
- Darrow, G.M. 1967. The cultivated raspberry and blackberry in North America: Breeding and improvement. *Am. Hortic. Mag.* 46, 203-218.
- DeFrancesco, J.T., and Martin, L.W. 1990. Characteristics and management of Kotata and Waldo blackberries. *Ore. Hort. Soc, Proc.* 81, 183-186.
- Durst, R.W., Wrolstad, R.E., and Krueger, D.A. 1995. Sugar, nonvolatile acid, ¹³C/¹²C ratio, and mineral analysis for determination of the authenticity and quality of red raspberry juice composition. *Journal of AOAC International.* 78: 1195-1204.
- Fitelson, J. 1969. Paper Chromatographic Detection of major organic acid in fruit juices. *J. Assoc. Off. Anal. Chem.* 52: 646.
- Fitelson, J. 1970. Detection of adulteration in fruit juices by qualitative Determination of carbohydrates by gas-liquid chromatography. *J. Assoc. Off. Anal. Chem.* 53: 1193-1197.

- Fouassin, A. 1956. Identification par chromatographie des pigments anthocyanins des fruits et des legumes. *Rev. Ferment. Ind. Aliment.* 11, 173.
- Francis, F.J. 1982. Analysis of anthocyanins. *In Anthocyanins as food colors*, P. Markakis (Editor). Academic Press, New York.
- García-Viguera, C., Zafrilla, P., and Tomás-Barberán. 1997. Determination of authenticity of fruit jams by HPLC analysis of anthocyanins. *J. Sci. Food Agric.* 73: 207-213.
- Haborne, J.B. and Hall, E. 1964. Plant Polyphenols. XIII. The systematic distribution and origin of anthocyanins containing branched trisaccharides, *phytochemistry*, 3, 453.
- Harborne, J.B and Turner, B.L. 1984. *Plant chemosystematics*. Academic Press, New York.
- Hong, V. and Wrolstad, R.E. 1990a. Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection. *J. Agric. Food Chem.* 38(3): 698.
- Jennings, D. L., and Carmichael, E. 1979. Colour changes in frozen blackberry. *HortScience* 9:246.
- Jennings, D. L. and Carmichael, E. 1980. Anthocyanin variation in the genus *Rubus*. *New. Phytol.* 84: 505.
- Kline, D.A., Fernanadez-Flores, E., and Johnson, A.R. 1970. Quantitative determination of sugars in fruits by GLC separation of TMS derivatives. *J. Assoc. Off. Anal. Chem.* 53: 1198-1202.
- Lee, C.Y., Shallenberger, R.S., and Vittum, M.T. 1970. Free sugar in fruits and vegetables. *New York Food and Life Science Bulletin No. 1*. N.Y. State Agriculture Experiment Station, Geneva, NY.
- Mazza, G. and Miniati, E. (Ed.). 1993. *Anthocyanins in fruits, vegetables and grains*. CRC Press, Inc., Boca Raton, Florida.
- Moore, J.N., and Skirvin, R.M. 1989. Blackberry management *In Small fruit crop management*. Galletta, G.J and Himelrick, D.G. (Editor). Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

- Naumann, W.D. and Wittenburg, U. 1980. Anthocyanins, soluble solids, and titratable acidity in blackberries as influenced by preharvest temperatures. *Acta. Hort.* 112: 183-190.
- Nelson, E.K. 1925. The non-volatile acids of the blackberry. *J. Am. Chem. Soc.* 47, 568-572.
- Official Methods of Analysis (1990) 15th Ed., AOAC, Arlington, VA, Vol. II
- Robards, K., Haddad, P.R., and Jackson, P.E. 1994. Principles and practice of modern chromatographic methods. Academic Press Inc. San Diego, California.
- Rommel, A., Wrolstad, R.E. and Heatherbell, D.A. 1992. Blackberry juice and wine: processing and storage effects on anthocyanin composition, color and appearance. *J. Food Sci.* 57(2): 385-391.
- Sapers, G. M., Burgher, A.M., Phillips, J.G., and Galletta, G.J. 1985. Composition and color of fruits and juice of thornless blackberry cultivars. *J. Amer. Soc. Hort. Sci.* 110:243-248.
- Sapers, G.M., Hicks, K.B., Burgher, A.M., Hargrave, D.L., Sondey, S.M. and Bilyk, A. 1986. Anthocyanin patterns in ripening thornless blackberries. *J. Am. Soc. Hortic. Sci.* 11: 945-950.
- Spanos, G.A., and Wrolstad, R. E. 1987. Anthocyanin pigment, nonvolatile acid, and sugar composition of red raspberry juice. *J. Assoc. Off. Anal. Chem.* 70: 1036-1046.
- Strik, B.C. 1992. Blackberry cultivars and production trends in the Pacific Northwest. *Fruit Varieties Journal.* 46(4): 202-206.
- Torre, L.C. and Barritt, B.H. 1977. Quantitative evaluation of *Rubus* fruit anthocyanin pigments. *J. Food Sci.* 42, 488-490.
- Whiting, G.C. 1958. The non-volatile organic acids of some berry fruits. *J. Sci. Food Agric.* 9, 244.
- Widdowson, E.M., McCance, R.A. 1935. Determination of glucose, fructose, sucrose and starch. *Biochem. J.* 29, 151-156.
- Wrolstad, R.E. 1976. Color and pigment analyses in fruit products. Oregon St. Univ. Agric. Exp. Stn. Bull. No. 624.

- Wrolstad, R. E. 1998. 'Pigments and color evaluation' FST 561 class handout. Food Sceince and Technology Department, Oregon State University. Corvallis, Oregon.
- Wrolstad, R.E., Culbertson, J.D., Cornwell, C.J., and Mattick, L.R. 1982. Detection of adulteration in blackberry juice concentrates and wines. *J. Assoc. Off. Anal. Chem.* V. 65(6): 1417-1423.
- Wrolstad, R.E., Culbertson, J.D., Nagaki, D.A., and Madero, C.F. 1980. Sugars and nonvolatile acids of blackberries. *J. Agric. Food Chem.* 28: 553-558.
- Wrolstad, R.E., Hong, V., Boyles, M.J., and Durst, R.W. 1995. Use of anthocyanin pigment analyses for detecting adulteration in fruit juices *In Methods to detect adulteration of fruit juice beverages*. Volume I. Nagy, S. and Wade, R.L.(Editor). AGSCIENCE, INC., Auburndale, Florida.
- Wrolstad, R.E., and Shallenberger, R.S. 1981. Free sugar and sorbitol in fruits- A complation from the literature. *J. Assoc. Off. Anal. Chem.* 64(1): 91-103.