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Abstract approved _____ Signature redacted for privacy.
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Seven red raspberry (Rubus Ideaus L.) samples from Oregon, Washington, British Columbia and New Zealand were processed into juice. These, along with three juice samples supplied from West Germany were analyzed by high performance liquid chromatography, (HPLC), for nonvolatile acid, sugar, anthocyanin and anthocyanidin composition. In order to quantitate the compounds of interest, particular attention was given to selection and improvement of HPLC analytical procedures. Adequate resolution of malic acid from isocitric by reverse phase C-18 chromatographic media was achieved using a two column system. The more uniform response of analytes to refractive index detection allowed for quantitation of isocitric acid. Sugar analysis based on a crosslinked cation exchange resin required removal of the acids from the juice via anion exchange. Resolution of anthocyanin pigments was achieved using as organic modifier

15% acetic acid. Acetic acid (15%) demonstrated high selectivity in resolving pigments with similar polarity.

Ultraviolet-visible spectral techniques were employed to determine anthocyanin concentration, color density, polymeric color and browning index. Other data presented include ^{o}Brix, pH, titratable acidity, Stable Isotopic Carbon Ratios and Hunter color parameters. Data compiled are intended to create a reference data base for authentic red raspberry juice.

Anthocyanin Pigment, Nonvolatile Acid and Sugar Composition of Red Raspberry Juice

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ANTHOCYANIN PIGMENT NONVOLATILE ACID AND SUGAR COMPOSITION OF RED RASPBERRY JUICE

INTRODUCTION

The attractive color and flavor of red raspberries (Rubus Ideaus L.) have led to increased demand for red raspberry juice and juice concentrates. Red raspberry juice is increasingly used in blended fruit drinks and in other formulated food products of high quality. The relatively high cost of red raspberry fruit makes red raspberry juice concentrates products of high economic value. Price in 1985 ranged from $70-75 per gallon for 68° Brix concentrate. The increased demand along with its high value make red raspberry juice a likely target for adulteration. Much attention has been given in establishing methodology and criteria for detecting adulteration in more widely consumed fruit juices such as apple (1,2,3) and orange juice (3,4) and, recently, in the popular cranberry juice cocktails (5,6,7). Methodology and data base for red raspberry juice, however, are limited.

The purpose of this work was to develop the methodology for analyzing red raspberry juice for sugar, nonvolatile acid and pigment composition and to create a

reference data base for authentic red raspberry juice.

LITERATURE REVIEW

COMPOSITION OF RED RASPBERRIES

General Properties

Table I.1 lists °Brix, pH and titratable acidity data as compiled from the literature. °Brix exhibits a wide overall range between 7.2 and 15.0, with means ranging from 8.9-11.3. Titratable acidity shows that red raspberries are reasonably high in acid with means ranging from 1.23-1.88 g of citric / 100 mL. The pH values (11) exhibit a narrow range.

Nonvolatile Acids

Citric acid is the major acid of red raspberries (10,12,13,14). Low levels of malic (10,12,13) and trace amounts of isoctric acid (10) are also present. The German standard values for a number of compositional parameters, "Richtwerte und Schwankungsbreiten bestimmter Kennzahlen", (RSK values), which were published in 1981 (10) were based on 260 red raspberry juice samples as well as information from the scientific literature. The samples were

predominantly industrially produced during the years 1972-1979. The mean value for citric acid reported was 1.5 g/100 mL; levels of malic acid ranged from 200-1200 mg/L with a mean value of 500 mg/L; the content of isocitric ranged from 60-200 mg/L with a mean value of 100 mg/L. Sweeney et al. (12) reported a change from 1.60 to 1.27 g/100 mL of citric and from 1090 to 480 mg/L of malic for one variety in two successive years. Whiting (13) in the analysis of one sample found 1.89 g of citric / 100 g and 80 mg of malic acid / 100 g of fruit. Ryan and Dupont (14) using Gas-Liquid Chromatography found 2.48 g/100 mL of citric for one sample. These workers also measured concentrations of succinic and lactic as low as 550 and 120 mg/L, respectively.

Red raspberries contain significant amounts of ascorbic acid. Fejer et al. (15) reported a range of 21.0 to 35.3 mg/100 g of fruit for 30 red raspberry clones. They also found that the ascorbic acid content of any clone was similar in the same season in two consecutive years.

Sugars

The sugar composition of red raspberries, as compiled from the literature by Wrolstad and Shallenberger (16), is shown in the table I.2. The RSK values (10) show a narrower range of glucose to fructose ratio from that of table I.2. The RSK glucose to fructose ratio has a mean of

0.8 and range between 0.6 and 0.93. These values of glucose to fructose indicate an invert sugar pattern with slightly more fructose accumulating than glucose. The sucrose as percent of total sugars (table I.2) ranges from 8.3-51.8%. The levels of sucrose appear high in the fruit but low in the juice. According to RSK values (10), no sucrose or trace levels of sucrose are present in the processed juice. Similar findings have been reported by Fitelson (21) who found no sucrose in authentic red raspberry juice concentrates. Native invertase activity may account for the sucrose hydrolysis.

Red raspberries, from a practical standpoint contain no sorbitol (16). Makinen and Soderling (22) reported 85 µg of sorbitol per g of fruit in ripe red raspberries.

Anthocyanins

The anthocyanins of red raspberries have been thoroughly investigated (23-28). Four major cyanidin pigments have been identified: cyanidin-3-sophoroside (cyd-3-soph), cyanidin-3-glucoside (cyd-3-glu), cyanidin-3-rutinoside (cyd-3-rut) and cyanidin-3-glucorutinoside (cyd-3-glurut), although all four do not occur in all varieties and collections. Related pelargondin (pgd) pigments and cyanidin-3,5-diglucoside (cyd-3,5-diglu) have also been reported as minor pigments in some varieties (26).

Barritt and Torre (26) studied the distribution of the anthocyanin pigments in 37 red raspberry cultivars and selections. Two distinct cultivar groups were identified on the basis of the anthocyanin composition: firstly, 27 cultivars whose fruits contained four anthocyanins, cyd-3-soph, cyd-3-glu, cyd-3-rut and cyd-3-glurut and secondly, nine cultivars that contained only two major pigments, cyd-3-soph and cyd-3-glu. One unique selection, contained these four cyanidin glycosides plus two other major pigments, cyanidin-3-sambubiose (cyd-3-sam) and cyanidin-3-xyloserutinoside (cyd-3-xylrut). In cultivars where both cyd-3-soph and cyd-3-rut were detected there was an inverse relationship of the percent composition of these two pigments, in that the more cyd-3-soph the less cyd-3-rut. Total pigment content was not associated with the percent proportion of any of the major pigments. Cyd-3,5-diglu was present in 13 cultivars and selections as a minor pigment. Pelargonidin pigments occured in trace amounts in most cultivars. Pgd-3-soph was the most common pelargonidin glycoside while pgd-3-rut was the least common. The influence of maturity on the anthocyanin composition is shown in table I.3. The relative proportion of major pigments did not change appreciably with increasing fruit maturity. As the anthocyanin concentration increased with fruit maturity, minor pigments reached detectable levels.

The total anthocyanin concentration of red raspberries varies considerable with variety. Barritt and Torre (26) found the total anthocyanin concentration to range between 20-60 mg / 100 g of fruit.

Stable Isotopic Carbon Ratio

There are three isotopic forms of carbon in nature: ^{12}C, ^{13}C and ^{14}C. The first two are stable, whereas ^{14}C disintegrates with time. The three isotopes react in the same way chemically, but because of their different sizes they react at different rates. Thus the different chemical and metabolic pathways change the ratios between the isotopes in a characteristic way. There are three major photosynthetic pathways in plants, the C_3, C_4 and CAM. These show different kinetic isotope effects when fixing carbon dioxide resulting in different $^{13}C/^{12}C$ ratios. The carbon isotope ratios are expressed as $\delta^{13}C$ values:

$$\delta^{13}C = \left[\frac{^{13}C/^{12}C \text{ (sample)}}{^{13}C/^{12}C \text{ (std.)}} - 1\right] \times 1000$$

the denominator is the isotopic carbon ratio of the carbon dioxide prepared from the Pee Dee Belemnite (PDB) limestone.

Typical $\delta\ ^{13}C/^{12}C$ values for Calvin cycle (C_3) plants are -24 to -34; for Hatch-Slack (C_4) plants -7 to -19, and for CAM plants, -14 to -31, (29-31).

Krueger et al. (32) reported $\delta^{13}C$ of -26.1 and -24.6 for two red raspberry juice samples.

Proteins and Amino Acids

Boland et al. (9) have determined the protein and amino acid content of 16 red raspberry samples from Oregon and Washington. The percent of protein in whole red raspberries ranged from 0.27-0.52% with a mean of 0.42% In the same study, these workers found amino acid concentrations ranging from 1.35-2.75 meq./100 g with a mean of 2.14 meq./100 g. Regarding the nature of the individual amino acids, Burroughs (33) reported that α-alanine and serine were the dominant amino acids with intermediate levels of aspartic acid, asparagine, glutamic acid, glutamine, threonine, valine and leucine and trace levels of proline, arginine, lysine and tyrosine. The RSK proline content (10) in red raspberries has a mean value of 50 mg/L.

Ash Content

Boland et al. (9) in the analysis of 16 samples of red raspberry fruit found the percent ash to range from 0.269-0.416% with a mean of 0.350%. K_2O and P_2O_5

content of these samples were between 147-215 mg/100 g of fruit (mean 185) and 23.4-36.9 mg/100 g (mean 30.4), respectively. Osborn (8) in a survey of 12 and 54 samples of red raspberries in two seasons reported: ash between 0.326-0.455% (mean 0.395), K_2O between 165-240 mg/100 g (mean 200) and P_2O_5 between 23.2-51.2 mg/100 g (mean 41.5) for the 12 samples; ash between 0.327-0.535% (mean 0.397), K_2O and P_2O_5 between 149.4-286 mg/100 g (mean 194.3) and 22.2-70.1 mg/100 g (mean 43.6), respectively, for the 54 samples. The ash content reported in the RSK values (10) was between 3.0 and 6.0 g/L. Values for individual ions were: K 1300-2800 mg/L, mean 1700 mg/L (40-48%, mean 43% of total ash); Na maximum value 40 mg/L; Ca 110-230 mg/L, mean 140 mg/L; Mg 110-230 mg/L, mean 140 mg/L; phosphate 300-750 mg/L, mean 450 mg/L; sulfate 50-200 mg/L, mean 130 mg/L; Cl^- 40-200 mg/L, mean 110 mg/L; NO_3 max. 10 mg/L.

METHODS OF ANALYSIS

Analysis of Nonvolatile Acids

Analysis of nonvolatile acids can be achieved by physical, chemical, and enzymatic procedures (34). Of the different procedures available, chromatographic and enzymatic are the most commonly used. Chemical methods are group specific rather than substance specific (34). This lack of specificity makes chemical methods little suited for nonvolatile acid determination. The official chemical methods of analysis of organic acids in foods (35) are quite lengthy and usually exist only for a few acids (5,36).

A number of chromatographic techniques have been developed for organic acid analysis in fruit samples. Earlier work (37,38) indicated the usefulness of anion exchange resin for separation of a wide a variety of acids. Acids are eluted from the column in the order of increasing pK_a values. However, if acids with similar pK_a values are present in high concentration their separation is not sufficient to allow for quantitation (39). In such cases, mixed fractions can be further analyzed with partition chromatography using silica gel column (40). Gradient techniques also have been applied for quantitation of nonvolatile acids (39).

Gas-Liquid Chromatography, (GLC), has been used by many investigators (41-44), for analysis of nonvolatile acids by first converting them to volatile trimethylsily derivatives (TMS). For additional information GLC can be coupled to mass spectroscopy (14). In most cases, it is necessary to concentrate and purify the acids before GLC analysis. A common method of acid purification is their precipitation as lead salts (42,45). However, washing of sugars from the lead salts presents some difficulties (46). GLC is a sensitive and quantitative method of analysis of nonvolatile acids but it requires time consuming steps in the preparation of TMS derivatives.

High Performance Liquid Chromatography (HPLC), has been succesfully used as a rapid and specific method of analysis of nonvolatile acids in fruit juices (47). Separation is based on either ion exchange or reverse phase columns. Parker and List (36) used Aminex A-25 anion exchange resin. Aminex HPX-87, a strong cation exchange column has been effectively used in analysis of organic acids in fruit juices (48-50). Column selectivity is controled by changing the column temperature, the pH of the eluant, or by adding to the eluant an organic modifier such as acetonitrile.

Coppola et al. (5), Hong and Wrolstad (7) and Lee (51) have recently used reverse phase liquid chromatography at ambient temperature to quantitate major acids in cranberry

and apple juice. The method allows for rapid quantitation of quinic, tartaric, malic, citric and fumaric acid.

Enzymatic analysis is rapid and highly specific. An important type of specifity in enzymatic analysis is stereospecificity; enzymes utilize only one of the two stereoisomeric forms (34). Synthetic malic for example can be distinguished from natural malic as synthetic malic is racemic (DL) and natural malic has the L- configuration. When L-malic acid determined by enzymatic procedures is considerably lower than total malic as determined by GLC or HPLC, it is likely that synthetic malic is present (52). Because of their specificity enzymes are a valuable complement to chromatographic techniques. Enzymatic kits are commercially available for determining L-malic, citric and D-isocitric acid.

Analysis of Sugars

The analyst can choose between GLC, HPLC and enzymatic methods for determining the individual sugars (52). Reyes et al. (53) compared these methods in determining the sugars in strawberries and they found very good agreement between methods.

Gas chromatographic methods have been extensively used in the past for sugar analysis (21,46). Sugars can be converted into volatile derivatives such as trimethylsilyl ethers (TMS). The main difficulty in the separation of the

TMS sugar derivatives by GLC was the resolution of the anomers in multiple peaks (21,46). The lengthy preparation of TMS sugar derivatives limits the use of GLC in the analysis of free sugars.

HPLC is particularly suitable for analyzing individual sugars in a fast and specific way (47). It retains the specificity of GLC while sample preparation and time of analysis are greatly reduced. Good resolution has been achieved with μBondapak column and elution with mixture of acetonitrile and water (54,55). However, sorbitol is not resolved from glucose. Dual column system (55,56) shows better resolution. The accurate quantitation of sorbitol is of extreme importance in fruit analysis since sorbitol is a very useful index for determining adulteration (52). Resolution of carbohydrates such as glucose, fructose, sucrose and sorbitol can also be achieved on a simple cation exchange columm (57). The mechanism of separation includes ion exclusion, ion exchange, ligand exchange, size exclusion, reverse phase and normal phase partitioning. These interactions result in an excellent resolution of sucrose, glucose, fructose and sorbitol using as eluant pure water.

HPLC provides a satisfactory resolution and speed of analysis and since sensitivity of detection does not present a problem in fruit juice analysis, it appears to be the method of choice (52).

Enzymatic methods are commercially available for determining glucose, fructose, sucrose and sorbitol (34). The easy analysis and high specificity are the main advantages of the enzymatic procedures.

Analysis of Anthocyanins

Paper chromatography, (PC), has been the most frequently used technique for separation and identification of anthocyanins ever since its introduction (58). The development of thin layer chromatography, (TLC), accelarated the analysis of pigments. This method was convenient to adapt because by using cellulose plates one could use solvent systems developed for paper chromatography. A number of papers have appeared on the anthocyanin separation on cellulose thin layer plates (24-29,59,60). Advantages of TLC over PC are the relatively short development time and the smaller pigment quantity required for analysis. TLC R_f values, however, are not as reproducible as those of PC from laboratory to laboratory (61); because of the difference in layer thickness the use of reference compound is required.

While some investigators were improving the solvent systems for paper and thin layer chromatography, others looked for alternative methods for faster separation. A few reports on gas chromatographic techniques appeared (62,63) in the literature but the anthocyanin chemistry has

benefited very little from these techniques (58). The general instability and the nonvolatility of the anthocyanin compounds, even as TMS derivatives, are the main problems associated with gas chromatography of anthocyanins (58). Derivatization of anthocyanins for GC or GC/MS is strongly hampered by the strucural transformation of these pigments. These reactions are pH dependent and make it difficult in practice to obtain homogenous derivatives (64).

With the recent developments in column material such as Lichosorb, Zorbax, Bondapack and μBondapack treated with octadecyltrichlorosilane, HPLC has opened up new possibilties in anthocyanin chemistry. Kingston (65) reviewed the range of packing and solvent combinations that have been reported in the literature for analysis of flavonoids. It is clear that in most cases reverse phase C-18 chromatographic media and solvents such as H_2O/methanol/acetic acid and water/acetonitrile have been succesfully used to chromatograph anthocyanins and other flavonoids.

Many investigators (66,67) have reported that key factors in anthocyanin separation by reverse phase are the overall polarity of the molecule, the substitution of the B ring and the nature of the glycosidic substituents. Anthocyanins molecules elute in order of decreasing polarity.

Williams et al. (67) reported that the reproducibility of anthocyanin analysis based on a μBondapak column and elution with acetic acid solution for the non-acylated glycosides and methanolic acetic acid for the acylated glycosides depends on the pH of the eluting system, the operating pressure and the temperature at which the chromatography is performed. A slightly differnt temperature and a very slight variation in the pH of the eluting solvent result in minor differences in retention times.

An HPLC profile of anthocyanins can be achieved by monitoring absorbance in the region of 500-530 nm. Since few other compounds absorb in this region, anthocyanins can be selectively detected without requiring extensive preliminary treatments of samples. The recent availability of the photodiode array detectors enables the recording of complete UV-visible spectra of each peak leaving the column and opens up new possibillities in anthocyanin identification (68,69).

Quantitative determination of individual anthocyanins is difficult because of lack of pure reference standards. Recently, an automated preparative HPLC procedure has been described for the isolation of purified anthocyanins from blackberry and cranberry fruits (70). The availability of such pigment will allow for quantitation of anthocyanins in natural products and will be useful in food regulatory,

plant pattern protection and plant biochemical applications (70).

Table I.1 Compilation of General Properties of Red Raspberry Juice

Ref.	Number of Samples	Degree Brix			pH		Titratable Acidity citrate g/100 mL		
		Min	Max	Mean	Min	Max	Min	Max	Mean
8	12	9.0	15.0	11.3			1.01 [a]	1.82 [a]	1.23 [a]
8	54	7.2	14.4	10.8					
9	16	8.2	10.9	8.9			1.38	2.01	1.88
10	260						1.15 *	1.70 *	1.45 *
11	49				2.9	3.4			

[a] original data were expressed as mL of 0.1 NaOH / 100 mL

* titrated to pH 7.0
original data expressed as tartaric

Table I.2 Sugar composition of Red Raspberry as compiled from the literature by Wrolstad and Shallenberger (16)

Origin	Ref.	n[a]	Sugar concentration, g/100g fruit				Percentage of total sugars			
			Sucrose	Glucose	Fructose	Total	Sucrose	Glucose	Fructose	Glucose/ Fructose
Swiss	17	9	1.51	1.80	2.04	5.38	28.1	33.4	37.9	0.88
USA (1st season)	12	1	2.01	0.77	1.10	3.88	51.8	19.8	28.3	0.70
USA (2nd season)	12	1	2.32	0.94	1.43	4.69	49.5	20.0	30.5	0.66
Germany (juice)	18	5	0.63	3.28	3.65	7.56	8.3	43.4	48.3	0.90
USA	19		3.68	2.40	1.58	7.82	47.0	30.7	20.2	1.52
England	20		0.96	2.26	2.39	5.61	17.1	40.3	42.6	0.95
Min:			0.66	0.77	1.10	3.88	8.3	19.8	20.2	0.66
Max:			3.68	3.28	3.65	7.82	51.8	43.4	48.3	1.52
Mean:			1.85	1.91	2.03	5.82	33.6	31.3	34.6	0.935
Std deviation:			1.09	0.948	0.914	1.57	18.5	9.92	10.3	0.309
% CV:			58.9	49.6	45.0	27.0	55.0	31.7	29.8	33.0

[a] n : sample size

Table I.3 Influence of fruit ripeness on the percent composition of anthocyanin pigments of red raspberries as studied by Barritt and Torre (26)

	Juice Absorbance (dil. 1:20 513 mu)	pH	Cyanidin					Pelargonidin		
			3-glu	3-rut	3-soph	3-glurut	3,5-diglu	3-glu	3-soph	3-glurut
Willamette										
incipient	0.208	2.90	26		74		T *		T	
pink	0.420	2.92	28		69		3		T	
prime ripe	1.080	3.00	26		71		3		T	
overripe	1.360	3.16	35		58		7	T	T	
Meeker										
incipient	0.130	3.07	15	8	62	15				
pink	0.321	3.07	19	7	56	18			T	
prime ripe	0.503	3.24	18	7	52	23	T		T	
overripe	0.653	3.47	23	7	50	20	T		T	T

* T = less than 2%

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ANTHOCYANIN PIGMENT NONVOLATILE ACID AND SUGAR COMPOSITION OF RED RASPBERRY JUICE

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ABSTRACT

Seven red raspberry (Rubus Ideaus L.) samples from Oregon, Washington, British Columbia and New Zealand were processed into juice. These, along with three juice samples supplied from West Germany were analyzed by high performance liquid chromatography, (HPLC), for nonvolatile acid, sugar, anthocyanin and anthocyanidin composition. The mean content of citric, malic and isocitric acid was 21.0 g/L (std dev. 4.4), 872 mg/L (std dev. 476) and 158 mg/L (std dev. 51), respectively; the mean content of sucrose, glucose and fructose was 0.17 g/100 mL (std dev. 0.31), 2.59 g/100 mL (std dev. 0.51) and 2.97 g/100 mL (std dev. 0.46), respectively; mean values for individual anthocyanins were cyanidin-3-sophoroside 74.2% (std dev. 13.7), cyanidin-3-glucoside 12.2% (std dev. 5.0), cyanidin-3-glucorutinoside 8.6% (std dev. 12.7), cyanidin-3-rutinoside 1.7% (std dev. 2.3) pelargonidin-3-sophoroside 2.9% (std dev. 1.6) and trace levels of pelargonidin-3-glucorutinoside. Nonvolatile acids were also analyzed enzymatically. HPLC and enzymatic analysis of nonvolatile acids were in close agreement. Anthocyanin

pigment content ranged from 23.8 to 101.0 mg/100 mL. Additional spectral determinations include color density, polymeric color, browning index, UV-Visible spectra and derivative spectra. Additional data include, pH, ^{o}Brix, titratable acidity and Hunter parameters. Stable Isotopic Carbon Ratios ranged from -24.7 to -23.2 with a mean of -24.1. Data compiled are intended to create a reference data base for authentic red raspberry juice.

INTRODUCTION

The attractive color and flavor of red raspberries (Rubus Ideaus L.) have led to increased demand for red raspberry juice and juice concentrates. Red raspberry juice is increasingly used in blended fruit drinks and in other formulated food products of high quality. The relatively high cost of red raspberry fruit makes red raspberry juice concentrates products of high economic value. Price in 1985 ranged from $70-75 per gallon for 68° Brix concentrate. The increased demand along with its high value make red raspberry juice a likely target for adulteration. Much attention has been given in establishing methodology and criteria for detecting adulteration in more widely consumed fruit juices such as apple (1,2,3) and orange juice (3,4) and, recently, in the popular cranberry juice cocktails (5,6,7,8). Methodology and data base for red raspberry juice, however, are limited.

The purpose of this work was to develop the methodology for analyzing red raspberry juice for sugar, nonvolatile acid and pigment composition and to create a reference data base for authentic red raspberry juice.

METHODS

SAMPLES

Red raspberry fruit samples (10-15 pounds per sample) were obtained from Oregon North Willamette Station, Aurora, OR, (ORNWS), Agricultural Canada Research Station, Vancouver, British Columbia, (ACRS) and Western Washington Research and Extension Center, Puyallup, WA, (WWREC). These had the following varietal make up: Meeker (ORNWS, ACRS and WWREC), Willamette (ORNWS and ACRS), and Skeena (ACRS). Fruit samples were washed, individually quick frozen (IQF), and stored frozen at -12^{o}C until needed. A New Zealand sample, variety Marcy, supplied in freeze-dried form by the Department of Scientific and Industrial Research, Auckland, New Zealand, (DSIRNZ) was reconstituted to initial moisture with water before pressing.

Three juice samples of European origin certified to be authentic by the supplier, were obtained from Bayernwald Fruchteverwertung GmbH Hengersberg, W. Germany. These had been pressed from Bavarian wild raspberries (picked from Buchelstein), Romanian wild raspberries (picked from central Cluj.) and Hungarian cultivated raspberries (northen Hungary, no name variety). The European samples had been pasteurized but not depectinized. After arrival, samples were stored frozen at -12^{o}C. Before analysis

they were thawed and centrifuged.

APPARATUS

(a). High Performance Liquid Chromatograph (HPLC).- Perkin-Elmer Series 400 (Perkin-Elmer Corp., Analytical Instruments, Norwalk, CT); Varian Model LC 5000 equipped with a column heater (Varian Instrument Group, Walnut Creek, CA).

(b). Detectors.- Varian UV 50 Variable Wavelength Detector; Varian Refractive Index Detector.

(c). Integrators.- Perkin-Elmer LCI-100; Hewlett-Packard HP 3380A (Hewlett-Packard Corp., Avondale, PA).

(d). HPLC Columns.- Bio-Rad Aminex HPX-87C column, 300 x 7.8 mm id fitted with a Bio-Rad Carbo C 4cm x 4.6mm Micro-Guard column (Bio-Rad Laboratories, Richmond, CA); Supelcosil LC-18 (5 micron), 250 x 4.6mm id column, (Supelco, Inc., PN); Nucleosil C-18 (10 micron), 250 x 4.6mm id column (Alltech Associates, Inc. Deerfield, IL); ODS-10 4cm x 4.6mm id Micro-Guard column (Bio-Rad Laboratories).

(e). Bath Circulator.- Lauda Model RMT-6 Refrigerated Circulator.

(f). UV-Visible Spectrophotometer.- Varian DMS 100 interfaced with the Varian DS-15 data station.

(g). Color Difference Meter.- Hunter DP-25P-2, (Hunter Instruments, Reston, VA).

(g). C-18 Mini Column.- C-18 SEP-PAK (Waters Associates, Milford, MA). To activate: pass 5 mL methanol through the cartridge followed by 5 mL of distilled water.

(h). Disposable Poly-Prep Chromatography Columns.- Graduated 0.8 x 4 cm columns holding up to 2 mL chromatographic media and including an integral 10 mL reservoir (Bio-Rad Laboratories).

REAGENTS

(a). Mobile phase for HPLC analysis of nonvolatile acids, (phosphate buffer pH 2.4).- Dissolve 27.2 g of KH_2PO_4 in 1000 mL of glass distilled deionized water and adjust pH to 2.4 with concentrated phosphoric acid. Filter through a 0.45 µm Millipore filter, type HA, (Millipore Corp., Bedford, MA) and degas.

(b). Mobile phase for HPLC analysis of sugars, (200 mg $Ca(NO_3)_2$ per 1000 mL of water).- Dissolve 200 mg of $Ca(NO_3)_2$ in 1000 mL of glass distilled deionized water, filter through a 0.45 µm Millipore filter, type HA, and degas.

(c). Mobile phase for HPLC analysis of anthocyanins: (solvent A, 15% Acetic Acid, solvent B, 15% Acetic Acid in methanol).- Add 150 mL of HPLC grade glacial acetic acid to 850 mL of distilled deionized water, mix, filter through a 0.45 µm Millipore filter, (HA), and degas; add 150 mL of HPLC grade glacial acetic acid to 850 mL of HPLC grade

methanol, mix, filter through a 0.45 µm Millipore filter, type HV, and degas. (Make solvent B fresh daily).

(d). Mobile phase for HPLC analysis of anthocyanidins, (solvent A, 10% Acetic Acid, solvent B, Acetonitrile : methanol, 2:1).- Add 100 mL of HPLC grade glacial acetic acid to 900 mL of glass distilled deionized water, mix, filter through a Millipore 0.45 µm, type HA, filter and degas; add 600 mL of HPLC grade acetonitrle to 300 mL of HPLC grade methanol, mix, filter through a 0.45 µm Millipore filter, type HV, and degas.

(e). Organic Acid Standards 1.- Add 1000 mg of reagent grade malic acid and 200 mg of reagent grade isocitric acid to a 1000 mL volumetric flask and dilute to volume with distilled water.

(f). Organic Acid Standards 2.- Add 1.5000 g of reagent grade citric acid to a 100 mL volumetric flask and dilute to volume with organic acid standard solution 1.

(g). Sugar Standard Solution 1 (Internal Standard).- Add 6.5000 g of reagent grade mannitol to a 250 mL volumetric flask and dilute to volume with sugar mobile phase.

(h). Sugar Standard Soluton 2.- Add 0.2500 g of reagent grade sucrose, 2.0000 g of reagent grade glucose and 2.4000 g of reagent grade fructose to a 100 mL volumetric flask and dilute to volume with sugar standard solution 1.

(i). Enzymatic analysis kits.- L-malic acid, D-isocitric acid and citric acid kits from Boehringer Mannheim

Biochemicals, Indianopolis, IN.

(j). Anion Exchange Resin.- An intermediate base anion exchange resin, BioRex 5 (Bio-Rad Laboratories), in the chloride form, 50-200 mesh size and 8.8 meq./dry g of resin or 2.8 meq./mL of resin bed ion exchange capacity. Slurry resin in distilled water. Make 1.2 mL and 1.4 mL resin bed volume to prepare samples for sugar analysis and for acid analysis, respectively. Rinse packed bed with 5 mL of distilled water. Do not let bed dry.

(k). 0.01% methanolic HCl.- Add 0.1 mL concentrated HCl to 1000 mL of reagent grade methanol.

(l). 0.01% HCl.- Add 0.1 mL concentrated HCl to 1000 mL of distilled water.

(m). 10% H_2SO_4.- Add 10 mL of concentrated H_2SO_4 to 90 mL of distilled water.

(n). 2N HCl.- Add 20 mL of concentrated HCl to 100 mL of distilled water.

PRODUCTION OF RED RASPBERRY JUICE

Allow frozen fruit to thaw at ambient temperature. Grind berries through a hammer mill (Model D Comminuting Machine, W. J. Fitzpatrick Co.) equipped with a 1/2" diameter circular pore mesh at a speed which produces about 1/5 berries. Weigh crushed fruit. Heat crushed fruit to 45^{o}C and depectinize by incubating with 0.1 mL rohapect D5L liquid pectic enzyme (Rohm & Haas, Co. Philadelphia,

PA) per lb of fruit. Add 1% cellulose as press aid and press in a Willmes bag press (60 type, Moffet Co., San Jose, CA) using the following program: 0 bar until no additional juice is expressed, increase pressure in steps of 0.2 bar waiting at each stage until no juice is expressed; reach 4.0 bar. At 1.0, 2.0 and 3.0 bar reduce the pressure by 1 bar and repeat process as described above. Weigh juice. Take 25-30 mL aliquot add 0.05% Diatomaceous Earth, (DE), filter through Whatman No. 1 filter paper and conduct alcholic test (2 mL juice plus 4 mL isopropyl alchol 50/50, mix gently). If precipitate forms, incubate juice at 42°C with 0.1 mL rohapect/1000 mL juice to complete depectinization. Pasteurize juice using a 0.44 cm inside tube diameter stainless steel coil consisting of 27 heating loops (ca. 11 cm diameter) and 16 cooling loops. Immerse heating loops in rapidly boiling water in a steam heated kettle and cooling loops in a tank filled with flowing tap water (temp. 12-14°C). Circulate juice through the coil with a peristaltic pump (Masterflex, Cole-Parmer Instrument Co. Chicago, IL). Measure coil volume when heating loops are immersed in the boiling water and cooling loops are immersed in the cold water. Calculate flow rates at different pump speeds from the time required to circulate through the coil liquid volume equal to the coil volume. Pasteurize juice under flow rate which allows the juice to remain in the heating loops for 17

seconds. This heat treatment should result a pasteurization at 98-100°C for 11-13 seconds. Add 0.05% DE and filter through Whatman No 1 filter paper. Store juice frozen.

CONCENTRATION OF SAMPLES

Concentrate ca. 250 mL of single strength juice on a rotary evaporator (water bath, 35°C) to 50° Brix. For analysis, redilute with distilled water to 10° Brix.

DETERMINATION OF TITRATABLE ACIDITY

Determine titratable acidity using the glass electrode method, AOAC 22.061 (9). Express results in terms of anhydrous citric acid per 100 mL of juice.

SPECTRAL ANALYSES

For visible spectra dilute single strength juice 1/6-1/14 (depending on the pigment concentration) with distilled water and scan from 700 to 400 nm. For UV spectra dilute juice 1/270 with distilled water and scan from 400 to 200 nm. Scanning conditions: 1.0 cm quartz cell, distilled water as blank, 1 nm slit width, 50 nm/min scan rate. Recompute first and second derivative spectra from the original zero order.

Measure anthocyanin concentration, color density and polymeric color as described by Wrolstad et al. (10).

Express anthocyanin concentration as mg of cyanidin-3-glucoside per 100 mL of juice using the extinction coefficient for cyanidin-3-glucoside, e=29,600, reported by Blundstone and Crean (11).

DETERMINATION OF HUNTER PARAMETERS

Set up the instrument to read transmitted color, spectral component included (arrangement III). Calibrate the instrument according to the manufacturer. Read L, a, b and X, Z, Y parameters of single strength juice using a 0.5 cm pathlength cell.

DETERMINATION OF $^{13}C/^{12}C$ STABLE ISOTOPE RATIO

Concentrated samples (ca. 50° Brix) were sent to Coastal Science Laboratory (5321 Industrial Oaks Blvd. Suite 103 Austin, Texas 78735).

SAMPLE PREPARATION FOR HPLC DETERMINATION OF NONVOLATILE ACIDS

Adjust pH of sample to 6-7 with concentrated ammonium hydroxide (NH_4OH). Prepare 1.4 mL BioRex 5 resin bed in the Poly-Pre column and rinse bed with 5 mL of distilled water. Apply carefully 3 mL of sample to the resin bed and wash bed with 3 mL of distilled water. Place a test tube under the column and elute acids with 3.5 mL of 10% sulfuric acid followed by 3.5 ml of distilled water. Mix

eluate well and pass it through an activated C-18 SEP-PAK. Discard first 3 mL, collect remaining eluate; filter through a 0.45 μm Millipore filter (type HA) and inject onto HPLC.

HPLC DETRMINATION OF NONVOLATILE ACIDS

Operate LC (Varian 5000) under following conditions: columns, Supelcosil LC-18 and Nucleocil C-18 fitted with ODS-10 Micro Guard column; mobile phase, 0.2 M KH_2PO_4 pH 2.4; flow rate, 0.7 mL/min; elution temp., 25°C; detection, refractive index, 1 x attenuation; detection temp., 20 ± 0.1°C (controlled with bath circulator); integrator, Perkin-Elmer LCI-100; injection volume, 50 μL.

Calculate organic acid composition with external standard method and graphical interpretation of the results. Take 1:0, 2:3, 1:1 and 1:3 dilution of the organic acid standard solution 2, plot peak area (peak height for isocitric acid) versus concentration and fit a curve with the linear regression model for each individual acid:

$$C = a + b \times A \quad (1)$$

where C is the concentration in mg/mL, A is the peak area (peak height for isocitric acid), a is the curve intercept and b is the curve slope. Calculate the concentration, (C'_s), of individual acids in the prepared sample from peak area (peak height for isocitric) and formula (1). The

acid concentrations, (C_s), in the original sample can be calculated by the formula:

$$C_s = C'_s \times DF / R \quad (2)$$

where R is the recovery and DF is the dilution factor. To determine recovery, (R), of each individual acid, subject the standard acid solution to anion exchange and SEP-PAK clean up procedure, calculate concentrations, (C'_{std}), of the individual acids in the treated mixture from peak area (peak height for isocitric) and equation (1) and compare to the concentrations, (C_{std}), in the original mixture. The percentage recovery is given by the formula:

$$\%R = C'_{std} \times DF \times 100 / C_{std} \quad (3)$$

ENZYMATIC DETERMINATION OF NONVOLATILE ORGANIC ACIDS

Determine the citric, D-isocitric and L-malic acid content following the procedure provided with the Boehringer test kits. Dilute samples with distilled water, 1:50 for citric acid and 1:0 for isocitric analysis. For malic analysis, add 1 mL of sample to a 10 mL volumetric flask, adjust the pH to 7-8 with 0.1 N NaOH, (estimate required volume of 0.1 NaOH from titratable acidity data), and dilute to volume with distilled water. Monitor absorbance at 340 nm.

SAMPLE PREPARATION FOR HPLC DETERMINATION OF SUGARS

Mix 5 mL of juice with 3 mL of sugar standard solution

1 (internal standard) and pass mixture through an activated C-18 SEP-PAK. Discard first 3 mL and collect remaining eluate. Apply 4 mL of eluate to 1.2 mL BioRex 5 resin bed. Discard first 2 mL, collect remaining eluate, mix, filter through a 0.45 μm Millipore fiter (type HA) and inject onto HPLC.

HPLC DETERMINATION OF SUGARS

Operate LC (Varian 5000) under following conditions: column, Bio-Rad Aminex HPX-87C; mobile phase, 200 mg $Ca(NO_3)_2$/1000 mL water; flow rate, 0.7 mL/min; elution temp., 85°C; detection, refractive index, 4 x attenuation; detection temp., 25°C; integrator, HP 3380A; injection volume, 25 μL.

Quantitate sugars via internal standard method and graphical interpretation of the results. Take 1:0 2:3, 1:1 and 1:3 dilution of the sugar standard solution 2, plot area versus concentration and fit a curve with the linear regression model for each individual sugar and for the internal standard:

$$C = a + b \times A \quad (4)$$

where C is the concentration in mg/mL, A is the peak area, a is the curve intercept and b is the curve slope. Calculate the concentration of each individual sugar and internal standard in the prepared sample from peak area and equation (4). The sugar concentration, (C_s), in the

original sample can be calculated by the formula:

$$C_s \text{ (in mg/mL)} = C'_s \times C_{is} \times DF / C'_{is} \quad (5)$$

where DF is the dilution factor, C'_s is the concentration of the sugar in question in the prepared sample, C'_{is} is the concentration of the internal standard after sample preparation and C_{is} is the concentration of the internal standard in the sample before sample preparation.

SAMPLE PREPARATION FOR HPLC DETERMINATION OF ANTHOCYANINS

Adsorb pigments contained in 3 to 7 mL of juice sample (depending on anthocyanin concentration) onto an activated C-18 SEP-PAK. Wash bed with 5 mL of 0.01% HCl and elute pigments with minimum required volume of acidified methanol (4 to 6 mL) in a 10 mL beaker. Evaporate methanol to ca. 2 mL under nitrogen stream and dilute pigments to ca. 5 mL with 0.01% HCl. Filter isolated pigments through a 0.45 μm Millipore filter (type HA) and inject onto HPLC immediately. Store sample in the dark in an ice bath between injections.

HPLC DETERMINATION OF ANTHOCYANINS

Operate LC (Perkin-Elmer 400) under the following conditions: column, Supelcosil LC-18 fitted with ODS-10 Micro-Guard column; mobile phase, solvent A 15% acetic acid, solvent B 15% acetic acid in methanol; flow rate, 1.2 mL/min; elution program, 15% A isocratic for 10 min

followed by a 0-5% linear gradient with B for 8 min and holding with 5% B for an additional 7 min (isocratic), equilibrate column to initial conditions for 5 min between injections; detection, visible at 515 nm, 0.2 Absorbance Units Full Scale (AUFS); integrator, Perkin-Elmer LCI-100; injection volume, 25 µL.

Calculate anthocyanin ratios from the area percentage of individual anthocyanins.

SAMPLE PREPARATION FOR HPLC DETERMINATION OF ANTHOCYANIDINS

Prepare samples for HPLC analysis of anthocyanidins following the procedure described by Hong and Wrolstad (7).

HPLC DETERMINATION OF ANTHOCYANIDINS

Operate LC (Perkin-Elmer Series 400) under the following conditions: column, Nucleosil C-18 fitted with ODS-10 Micro Guard column; mobile phase, solvent A 10% acetic acid, solvent B acetonitrile : methanol (2:1); elution, isocratic 84% A and 16% B; flow rate, 2.0 mL/min; elution temp., 25°C; detection, visible at 530 nm, 0.2 Absorbance Units Full Scale (AUFS); integrator, Perkin-Elmer LCI-100; injection volume, 25 µL.

Calculate anthocyanidin ratios from the area percentage of individual anthocyanidins.

RESULTS AND DISCUSSION

GENERAL PROPERTIES

Table II.1A shows the yield, degree Brix, pH, titratable acidity and $\delta^{13}C$ values for the juice samples prepared in our pilot plant. Table I.1 shows the ^{o}Brix, pH and titratable acidity data compiled from the literature.

There were two replications for Meeker (ORNWS) and Willamette (ORNWS) samples. These replications showed considerable variation in juice yield and ^{o}Brix. Factors contributing to this variation would have been the relative small sample size and the washing of the equipment between pressing. Normalization of the yield to 10^{o} Brix (table II.1A) shows close agreement between replications which implicates dilution as the cause of variation in yield.

Degree Brix values range from 5.6 to 10.7 with a mean of 7.8. These values are lower than those reported in the literature (table I.1) and well below the 10.5 degree Brix standard for red raspberries as specified by the USDA jelly standard. However, 10.0 degree Brix is the commonly accepted value for single strength juice by the fruit juice industry and all the analytical data were normalized to that value. Normalization facilitates compositional comparisons between samples and circumvents the problem of

yield variation and sample dilution.

pH values range from 2.94 to 3.23 and they are in agreement with those previously reported (table I.1).

Titratable acidity ranges from 1.40 to 1.86 g citrate per 100 mL juice which is similar to values reported in the literature (table I.1). There is a large difference in the titratable acidity between the replications of the Meeker (ORNWS). However, if these values are normalized to 10° Brix they show no difference, again suggesting that dilution with rinse water was the primary cause of the yield differences between replications.

The $\delta^{13}C$ values show a narrow range and low % CV. This data provides a reference value which can be used for estimating the content of Hatch-Slack sweeteners such as cane sugar or corn syrup in a red raspberry product. Krueger et al. (12) reported -26.1 and -24.6 as $\delta^{13}C$ values for two red raspberry juice samples.

The European samples (table II.1B) exhibit similar °Brix, pH and titratable acidity characteristics.

NONVOLATILE ACID ANALYSES

Red raspberries contain citric acid as the major acid, low levels of malic (13,14-17) and trace amounts of isocitric (13); trace amounts of lactic and succinic acid have also been reported (17).

Quantitation of isocitric acid by reverse phase HPLC

is difficult as it elutes very close to malic and it is present in very low levels. Adequate resolution of isocitric acid from malic can be achieved, however, by using two C-18 columns in series. Figure II.1 shows a chromatogram of red raspberry nonvolatile acids using UV detection at 227 nm and a two column-system. Sample preparation only involved clean up with a C-18 SEP-PAK cartridge. In addition to malic acid (peak 1), isocitric (peak 2) and citric (peak 5) the following acids were tentatively identified: α-ketoglutaric (peak 3), shikimic and ascorbic coeluting under peak 4, succinic (peak 6) and fumaric (peak 7). Identification of α-ketoglutaric, was based on its retention time and production of symmetrical peak in spiked samples. Presence of α-ketoglutaric and shikimic has not been previously reported. Quantitation of isocitric acid based on this procedure was about four times higher than the quantitation based on enzymatic analysis. This was probably due to coelution of an unidentified, highly UV absorbing compound with isocitric. Morever, late eluting compounds with high response in the UV made the total time of analysis very long, ca. 1 hour. Modification in the sample preparation using anion exchange resin resulted in no change in the measured concentration of isocitric acid. Thus the interferering compound is believed to be acidic and not a neutral compound.

A typical chromatogram of the red raspberry acids

obtained using refractive index detection and anion exchange clean up is shown in figure II.2. The more uniform response of analytes in the refractive index detection allowed for quantitation of isocitric acid and shortened the total time of analysis to less than 25 min. Table II.2 shows the % recovery of two acid standard solutions subjected to anion exchange and SEP-PAK clean up procedure. These solutions were formulated to be representative of the upper and the lower levels of the total acid content in the juice samples analyzed. They also represent concentrations of malic and isocitric higher and lower than the average concentrations of these acids in the juice samples. The degree of reproducibility between preparations as indicated by the coefficient of variation, (%CV), is high for citric and malic and lower for isocitric. The high variation between preparations for isocitric is expected because of its low concentration. It is worth mentioning that the correlation coefficient (r^2) of the standard curve for the isocitric acid, obtained by correlating peak height versus concentration, was 0.95. A lower correlation coefficient was obtained when peak area was measured instead of peak height. The correlation coefficient of the citric and malic standard curves were 0.99 and 0.98 respectively.

Tables II.3A and II.4A show the results of the HPLC and enzymatic analyses of the nonvolatile acids for the

juice samples prepared in our pilot plant. Table II.3B and II.4B list the results of the HPLC and enzymatic analyses of the European samples. There appears to be reasonably good agreement between the two analytical methods from visual inspection of the data. HPLC values for citric and malic are consistently lower than enzymatic results by 5 to 8 percent. HPLC determinations for isocitric showed a wider range than the enzymatic. Statistical analysis, however, with paired t-test shows that the concentrations of citric and malic acid as well as total acid content by HPLC are significantly different from those by enzymatic analysis at 0.05 level. Citric and malic concentrations expressed as percent of total acids (by summation) do not differ significantly by the two different methods.

The German standard values for a number of compositional parameters,"Richtwerte und Schwankungsbreiten bestimmter Kennzahlen" (RSK values) is the predominant data base available for red raspberries (13). The values reported in 1981 were based on 260 juice samples as well as information from the scientific literature. These were mainly industrially produced juices for the period from 1972-1979. The mean values of citric, malic and isocitric acid were 15 g/L, 500 mg/L and 100 mg/L, respectively. The range for malic and isocitric content was 200-1200 mg/L and 60-220 mg/L, respectively. Our data exhibit wider ranges and higher mean values. The RSK citric to isocitric ratio

had a mean of 150 and range between 80-200. Our range is narrower but our mean value is similar for this ratio. It is worth noting that one European sample (Romanian, tables II.3B and II.4B) exhibits atypical concentrations of malic and isocitric acid on the basis of RSK values. The non normalized malic content, however, is in agreement with the RSK values.

The acid profile of red raspberry is simple; citric acid accounts for up to 97% of the acids. Synthetic citric acid is the most likely acidulant to be used in adulteration of red raspberry juice and its detection would be difficult. The low levels of malic concentrations occuring limit the use of malic as potential adulderant. A very high citric to isocitric ratio would suggest adulteration with citric acid. Addition of isocitric acid is unlikely because of its relative high cost. Microbial activity has been reported to preferentially reduce the isocitric content (13) and could also account for a high citric to isocitric ratio.

SUGAR ANALYSES

A typical chromatogram of red rasbperry sugars is shown in figure II.3. Resolution is excellent and the total time of analysis is less than twenty minutes (retention time of sorbitol is ca. 18 minutes). Removal of acids from the juice via anion exchange mini column is

required, however, as interactions of acids with the calcium of the resin cause calcium leaching. The resulting protonated resin and the high elution temperature (85°C) catalyze on column sucrose hydrolysis. If calcium is added in the mobile phase and the acidic fraction is not removed with sample preparation sucrose hydrolysis is prevented and column life is extended; however, the increased Ca^{++} and COO^{-} interactions cause increase in acid retention and coelution of acids with sugars.

Table II.5 shows the % recovery of a standard solution containing sucrose, glucose, fructose and mannitol (internal standard) subjected to the sample preparation procedure. All analytes have essentially the same range and mean recovery with low %CV (ca. 2%). When recoveries of the individual analytes were normalized, they showed even lower standard deviation and % CV. Consequently, use of the internal standard to compensate for analyte loss during sample preparation reduces effort and increases reproducibility.

The results of the sugar analyses of juice samples prepared in our pilot plant are shown in table II.6A. There is considerable variation in the individual and total sugar content 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. All samples contained slightly more fructose than glucose

with the glucose to fructose ratio showing essentially an invert sugar pattern, hardly any sucrose and no sorbitol.

The red raspberry sugar profile as compiled from the literature by Wrolstad and Shallenberger (18), table I.2, shows much higher sucrose levels and lower glucose and fructose levels. The total sugar content and the glucose to fructose ratio are in agreement with our data. The sucrose levels reported in the literature are high in fruit but low in juice. The replicants of Meeker (ORNWS) and Willamette (ORNWS) (table II.6A) show different sucrose levels. Trace of sucrose were detected in one replication of Meeker (ORNWS) but not in the other; low levels of sucrose (0.08 g/100 mL) were measured only in one replication of Willamette (ORNWS). Analysis of 50° Brix concentrates prepared in our labarotary from Skeena (ACRS) and Willamette (ACRS) juice showed about 7 % decrease in the sucrose content. Similar findings have been reported by Fitelson (19) who found no sucrose in authentic red raspberry juice concentrates. While invertase activity has not been documented for red raspberry fruit, it is very likely that it is responsible for the low sucrose content of processed juice relatively to the fruit.

The sugar composition of the European samples (table II.6B) exhibits the same pattern with a trend for slightly higher total sugar content and glucose to fructose ratio.

The sugar profile has limited utility in checking

authenticity of red raspberry juices and juice concentrates. It is a simple invert profile and invert syrups or fruits with invert pattern could be difficult to detect. Presence of sorbitol, however, is a very usefull indicator of dilution with a sorbitol containing juice such as cherry, plum, apple or pear.

ANTHOCYANIN ANALYSES

The anthocyanins of red raspberries have been throrougly investigated with the use of TLC or paper chromatography. Four major anthocyanin pigments have been identified (20-25): cyanidin-3-sophoroside (cyd-3-soph), cyanidin-3-glucoside (cyd-3-glu), cyanidin-3-glucorutinoside (cyd-3-glurut) and cyanidin-3-rutinoside (cyd-3-rut), although all 4 do not occur in all varieties and selections. Related pelargonidin (pgd) anthocyanins and cyanidin-3,5-diglucoside (cyd-3,5-diglu) have also been reported as minor pigments (23).

The use of reverse phase HPLC for separation of anthocyanins and related substances have been reported for a number of commodities such as elderberries, cranberries and cowberries (26-28) but not for red raspberries. Most of the reported systems include gradient elution involving methanol or a combination of methanol and acetonitrile as organic modifiers. Initial investigations in our lab showed that such systems did not have enough selectivity to

resolve the anthocyanins of red raspberry. Figure II.4 shows red raspberry anthocyanins eluted with 10% acetic acid (solvent A) and methanol (solvent B). The elution program was isocratic with 7% solvent B and a flow rate of 1.5 mL/min. Cyd-3-glurut, cyd-3-glu and pgd-3-soph coelute under peak 2. We succeeded in completely resolving the anthocyanins of red raspberries using acetic acid as organic modifier. Isocratic elution with 15% acetic acid for 10 minutes followed by a 0-5% gradient with methanol resolved the coeluting pigments, (figure II.5), in less than 25 minutes. The solvent in which the isolated anthocyanin pigments were dissolved was found to strongly influence the separation. If the injected anthocyanins were dissolved in acidified methanol double peaks for the major anthocyanins and asymmetrical peaks with gradual up slope and sharp down slope for minor anthocyanins resulted. The methanol injected with the pigments moves faster than the mobile phase in the column creating a local methanolic gradient. This front of methanol causes faster pigment elution than the mobile phase and may account for the poor peak shape observed. Evaporation of methanol and redilution with 0.01% HCl improved peak shape giving a single peak for each compound. It is worth noting that there was no loss of resolution or peak shape deterioration over a long period of column use due to the acidity of mobile phase (pH of 15% acetic acid 1.9 to 2.0) throughout

this investigation.

Peak identification was based on: (a) the chemistry of separation which includes the overall polarity of the anthocyanin molecule, the nature of the attached sugar and the substitution of the B ring, (b) the relative magnitude of the peak and (c) the retention times of anthocyanins contained in sour cherry, blackberry, black currant and strawberry fruits. Extracts of these fruits were chromatographed under the same conditon as described for red raspberries. Chromatograms of these extracts are shown in figure II.6. Chromatograms of Meeker, Willamette and Marcy anthocyanins are shown in figure II.5.

Peak 1 is the major peak in all red raspberry samples analyzed (42.0-85.3%). Several investigators (20-25) have certified cyd-3-soph as the major pigment in red raspberries. Peak 2 was identified as cyd-3-glurut. Cyd-3-glurut is the major pigment of sour cherries (29). Barritt and Torre (23,24) reported that cyd-3-glurut is present in Meekers but not in Willamettes. Peak 2 is the major peak of sour cherry chromatogram and its presence distinguishes Meeker and Willamette chromatograms (fig. II.5A,B II.6A). Peak 3 was identified as cyd-3-glu. Cyd-3-glu is common to blackberries as the major pigment (21,24), has also been found in strawberries and black currant as a minor pigment (30) and in raspberries in intermediate levels (21-25). Peak 3 is the only peak

common to the chromatograms of these fruits and it is the major peak in blackberry, minor peak in strawberry and black currant and intermediate in red raspberry (fig. II.5A,B,C, II.6B,C,D). Peak 4 was not detected in varieties with trace amounts of pelargonidin and it was tentantively identified as pgd-3-soph. Of the pelargonidin pigments in red raspberries, pgd-3-soph is contained in largest quantities (23). Peak 5 was identified as cyd-3-rut. Cyd-3-rut is the major pigment in black currant (30), Marion blackberry (21), and sour cherry (29), minor pigment in Meeker red raspberry cultivar and not present in Willamette (23,24). Peak 5 fits this matrix. Peak 6 was tentatively identified as pgd-3-glurut (see below). Peak 7 was identified as pgd-3-glu because it is the major peak in the strawberry chromatogram (fig. II.6.D).

Summarizing, the elution order of the cyanidin pigments was as follows: cyd-3-soph, cyd-3-glurut, cyd-3-glu and cyd-3-rut. This elution order suggests that the hydrophobic CH_3 group of rhamnose (rutinose is rhamnose-α-1-6 glucose) causes increased retention of the rutinose glycosides and reverses the general rule that the elution order is tri, di, mono-saccharide of the same aglycone. Considering the effect of the rhamnose CH_3 group on anthocyanin retention, the elution order for the pelargonidin pigments should be the following: pgd-3-soph, pgd-3-glurut, pgd-3-glu and pgd-3-rut. This tentatively

identifies peak 6 as pgd-3-glurut. Combining the effect of sugar moiety, determined by the number of sugar units and the presence of the CH_3 group, with the effect of B ring substitution on the overall polarity of the anthocyanin molecule gives the resulting elution order: cyd-3-soph, cyd-3-glurut, cyd-3-glu, pgd-3-soph, cyd-3-rut, pgd-3-glurut, pgd-3-glu, and pgd-3-rut. The presence of the trace pelargonidin pigments was readily detected with injection of more concentrated anthocyanin preparations. In these cases cyd-3-soph exceeded the dynamic range of the detector which would not allow for accurate quantitation of all pigments.

The pigment assignment by HPLC retention certified previous identifications of red raspberry anthocyanins. No additional pigments were detected. Cyd-3,5-diglu which was tentatively reported on the base of TLC by Barritt and Torre (23) as a minor pigment in some varieties was not found in any of the samples we analyzed. From our analyses we could not conclusively determine whether this pigment was absent or coelutes, possibly with cyd-3-soph, because we do not have a standard source for determination of retention time. All other anthocyanin pigments that have been previously reported in red raspberry varieties were detected and separated. Similarly, the HPLC profiles for black currant, sour cherry, blackberry and strawberry confirmed the published anthocyanin composition of these fruits (30,29,21).

The results of the anthocyanin analyses of the samples pressed in our pilot plant are shown in table II.7A. Table II.8 shows the quantitation of cyanidin pigments by TLC densitometry as determined by Barritt and Torre (23) for 37 cultivars and collections. Related pelargonidin pigments were listed as trace (less than 2 %). Our data show higher mean values for cyd-3-soph and cyd-3-glurut and lower values for cyd-3-glu and cyd-3-rut. Partial hydrolysis or polymerization of anthocyanins during the more rigorous pigment isolation for TLC analysis as well as the quantitation by densitometry could account for these differences.

The results of the anthocyanin analyses of the European samples are shown in table II.7B. These samples were lower in monomeric pigment with higher percentage of polymeric color (table II.10C). The anthocyanin percentages, however, are in agreement with the profile of those processed in our pilot plant.

The anthocyanin profile of red raspberries is distinctive. Samples tested show two different cyanidin profiles. Varieties such as Willamette and Skeena, and the Bavarian and Hungarian samples show a simple profile consisting of two major cyanidin pigments, while varieties such as Meeker and Marcy and the Romanian sample show a complex profile consisting of four cyanidin pigments. Percentages of the cyanidin pigments show considerable

variation.

Most anthocyanin containing fruits have qualitatively and quantitatively different anthocyanin profiles from red raspberry and should be detected by this analysis.

ANTHOCYANIDIN ANALYSES

The results of the anthocyanidin analyses by HPLC for the seven juice samples pressed in our pilot plant are shown in table II.9A and a typical chromatogram in figure II.7. Whereas the anthocyanin profile is complex, the anthocyanidin profile is simple with cyanidin levels up to 100%. Anthocyanidin analysis would be particularly useful in detecting adulterations with colorants containing malvinidin, delpinidin, peonidin or petunidin. The European samples show a similar anthocyanidin profile (table II.9B).

Since there are many possible glycosidic patterns of an anthocyanidin the anthocyanin profiles show more complexity than the anthocyanidin profiles. A typical anthocyanidin profile may have been originated from an atypical anthocyanin composition. The easier sample preparation and the increased anthocyanin stability are additional advantages of the anthocyanin analysis relatively to anthocyanidin analysis.

SPECTRAL CHARACTERISTICS

The color characteristics of the samples pressed in our pilot plant are shown in table II.10A. Anthocyanin concentrations range between 23.8 and 101.0 mg/ 100 mL. Barritt and Torre (24) in their analysis of fruit found a lower range of anthocyanin concentration between 20 and 60 mg/100 g of fruit. Variety, origin and maturity may account for the differences in anthocyanin levels. Our data show that Willamette is about 50% higer in color than Meeker, given that both varieties come from the same region. Similarly, Barritt and Torre (24) found that Willamette fruit is about 50% higher in anthocyanin concentration from the Meeker fruit. The number of samples we analyzed, however, is too limited to indicate the primary source of variation in anthocyanin pigment accumulation. Color density, percent polymeric color and browning index show that juices are high in color and contain low level of polymerized anthocyanin pigments.

The replicates of Meeker (ORNWS) and Willamette (ORNWS) (table II.10A) show very little difference in color indices. Of the color characteristics browning index shows the higher difference between replications.

The effect of concentration (under laboratory conditions) on monomeric and polymeric color is shown in table II.10B. Monomeric color decreases by an average of 11% while color density increases by an average of 9%. Polymeric color, percent polymeric color and browning index

increase by even more than 100%.

Anthocyanin concentration and color density of the European samples (table II.10C) show that these samples are low in pigment. Polymeric color and percent polymeric show a high concentration of polymerized pigments. This suggests that pigment degradation with processing and storage would be responsible for the low monomeric pigment content, although varietal and geographic infuence could also be contributive.

The spectral characteristics of the analyzed samples are shown in table II.11. Typical absorbance, first and second derivative spectra are shown in figures II.8 and II.9. Derivative spectra enhance the fine structure of the zero order spectra not normally seen in the absorbance plots (31).

HUNTER PARAMETERS

Table II.12A shows the Hunter parameters for the seven samples pressed in our pilot plant. The European samples (table II.12B) show higher L values due to their lighter color.

SUMMARY

The methodology for sugar, nonvolatile acid and anthocyanin analysis by HPLC in red raspberry juice was developed. Adequate resolution of malic acid from isocitric by reverse phase C-18 chromatographic media can be achieved using two column system. The more uniform response of analytes to refractive index detection allows for quantitation of isocitric acid. Sugar analysis based on a crosslinked cation exchange resin in the calcium form requires removal of the acids from the juice via anion exchange. Resolution of anthocyanin pigments was achieved using as organic modifier 15% acetic acid. Acetic acid (15%) demonstrated high selectivity in resolving pigments with similar polarity.

Nonvolatile acid, sugar and anthocyanidin profiles are simple. This limits their use in detecting adulterations. However, presence of sorbitol or detection of anthocyanidins other than cyanidin and low levels of pelargonidin would be substantive evidence for adulteration. The anthocyanin profile is distinctive. Two different patterns were found. One consists of two cyanidin (3-soph and 3-glu) while the other consists of four cyanidin pigments (3-soph, 3-glurut, 3-glu and

3-rut). Anthocyanin analysis should prove useful in detecting adulteration of red raspberries with other anthocyanin containing fruits. Anthocyanin pigment concentration exhibited a very wide range. Three European samples analyzed showed very low pigment concentration. These findings indicate the dependence of the total pigment content on maturity, variety, geographic origin and processing.

Data compiled should be a useful reference data base. As future work, it is recommended extension of this data base and testing of the effectiveness of the developed methodology in analysis of commercial products.

Table II.1A General Properties of Red Raspberry Juice

Sample:	Yield (%)	Yield (%)[a]	Degree Brix	pH	Titratable Acidity[b]	Titratable Acidity[a,b]	$\delta^{13}C$ (PDB)
Meeker (ORNWS)							
(repl. 1)	(68.7)	(62.5)	(9.1)	(3.19)	(1.77)	(1.95)	
(" 2)	(81.9)	(61.4)	(7.5)	(3.19)	(1.45)	(1.93)	
Avg. of repl. 1,2	75.3	62.5	8.3	3.19	1.61	1.94	-24.7
Meeker (ACRS)	90.1	73.0	8.1	3.07	1.49	1.84	-23.3
Meeker (WWREC)	74.7	79.9	10.7	3.10	1.41	1.32	-24.4
Willamette (ORNWS)							
(repl. 1)	(77.8)	(56.8)	(7.3)	(3.15)	(1.85)	(2.53)	
(" 2)	(72.6)	(53.7)	(7.4)	(3.14)	(1.86)	(2.51)	
Avg. of repl. 1,2	75.2	55.6	7.4	3.15	1.86	2.51	-24.7
Willamette (ACRS)	91.5	59.5	6.5	3.02	1.80	2.78	-23.8
Skeena (ACRS)	86.4	69.1	8.0	2.94	1.68	2.10	-24.4
Marcy (DSIRNZ)	90.1	50.5	5.6	3.23	1.40	2.50	-23.2
Min:	74.7	50.5	5.6	2.94	1.40	1.32	-24.7
Max:	91.5	79.9	10.7	3.23	1.86	2.78	-23.2
Mean:	83.3	64.3	7.8	3.10	1.61	2.14	-24.1
Std Deviation:	7.3	9.5	1.5	0.09	0.17	0.46	0.6
%CV:	8.8%	14.8%	19.1%	3.0%	10.6%	21.5%	-2.4%

[a] normalized to 10 degree Brix

[b] expressed as g citrate / 100 mL

Table II.1B General Properties of European Red Raspberry Juice

Sample:	Degree Brix	pH	Titratable Acidity[a]	Titratable Acidity[a,b]
Bavarian	7.7	3.17	1.59	2.06
Romanian	8.4	3.23	1.23	1.46
Hungarian	8.9	3.22	1.58	1.77

[a] expressed as g of citrate / 100 mL

[b] normalized to 10 degree Brix

Table II.2 Recovery of acid standard solutions subjected to sample preparation procedure

	Percentage recoveries (%)[a]					
	[b] Malic	[c] Malic	[b] Isocitric	[c] Isocitric	[b] Citric	[c] Citric
Min:	81.7	80.2	74.2	73.1	86.2	85.9
Max:	87.5	87.8	94.8	96.8	93.8	93.5
Mean:	84.7	83.7	83.4	87.3	90.1	89.9
Std Dev:	2.5	2.7	8.1	9.4	2.8	2.8
% CV:	2.9	3.3	9.7	10.8	3.1	3.1

[a] four repeated preparations

[b] standard solution of 18 g citric, 1000 mg malic and 200 mg isocitric / L

[c] standard solution of 12 g citric, 500 mg malic and 100 mg isocitric /L

Table II.3A Nonvolatile Acid Composition[a] of Red Raspberry Juice as determined by HPLC

Sample:	Citric (g/L)	Malic (mg/L)	Isocitric (mg/L)	Total (g/L)	Percentage of total Citric	Malic	Isocitric	Citric/ Malic	Citric/ Isocitric	Malic/ Isocitric
Meeker (ORNWS)										
(repl. 1)	(19.1)	(496)	(186)							
(" 2)	(18.4)	(480)	(172)							
Avg. of repl. 1,2	18.8	488	179	19.4	96.6%	2.5%	0.9%	38.4	104.7	2.7
Meeker (ACRS)	18.7	884	119	19.7	94.9%	4.5%	0.6%	21.1	156.7	7.4
Meeker (WWREC)	13.1	393	75	13.5	96.5%	2.9%	0.6%	33.3	174.3	5.2
Willamette (ORNWS)										
(repl. 1)	(23.3)	(561)	(181)							
(" 2)	(24.1)	(539)	(169)							
Avg. of repl. 1,2	23.7	550	175	24.4	97.0%	2.3%	0.7%	43.0	135.2	3.1
Willamette (ACRS)	27.1	1903	231	29.2	92.7%	6.5%	0.8%	14.2	117.4	8.2
Skeena (ACRS)	21.1	812	123	22.1	95.8%	3.7%	0.6%	26.0	171.8	6.6
Marcy (DSIRNZ)	24.8	1075	205	26.1	95.1%	4.1%	0.8%	23.1	121.1	5.2
Min:	13.1	393	75	13.5	92.7%	2.3%	0.6%	14.2	104.7	2.7
Max:	27.1	1903	231	29.2	97.0%	6.5%	0.9%	43.0	174.3	8.2
Mean:	21.0	872	158	22.1	95.5%	3.8%	0.7%	28.5	140.2	5.5
Std Deviation:	4.4	476	51	4.8	1.4%	1.4%	0.1%	9.5	25.6	1.9
% CV:	20.7%	54.6%	32.0%	21.6%	1.4%	35.7%	18.2%	33.2%	18.3%	34.8%

[a] results normalized to 10 degree Brix

Table II.3B Nonvolatile Acid Composition of European Red Raspberry Juice as determined by HPLC[a]

Sample:	Citric (g/L)	Malic (mg/L)	Isocitric (mg/L)	Total (g/L)	Percentage of total			Citric/ Malic	Citric/ Isocitric	Malic/ Isocitric
					Citric	Malic	Isocitric			
Bavarian	21.9	742	119	22.7	96.2%	3.3%	0.5%	29.5	183.9	6.2
Romanian	14.8	1326	trace *	16.1	91.8%	8.2%	0.0%	11.1	--	--
Hungarian	19.1	483	91	19.6	97.1%	2.5%	0.5%	39.5	209.6	5.3

* less than 50 mg/L

[a] results normalized to 10 degree Brix

Table II.4A Nonvolatile Acid Composition of Red Raspberry Juice as determined by Enzymatic Analysis[a]

Sample:	Citric (g/L)	Malic (mg/L)	Isocitric (mg/L)	Total (g/L)	Percentage of total: Citric	Malic	Isocitric	Citric/ Malic	Citric/ Isocitric	Malic/ Isocitric
Meeker (ORNWS)										
(repl. 1)	(20.4)	(529)	(159)							
(" 2)	(19.8)	(511)	(165)							
Avg. of repl. 1,2	20.1	520	162	20.7	96.7%	2.5%	0.8%	38.6	123.8	3.2
Meeker (ACRS)	20.1	887	128	21.1	95.2%	4.2%	0.6%	22.6	156.7	6.9
Meeker (WWREC)	13.8	411	83	14.3	96.5%	2.9%	0.6%	33.6	166.1	5.0
Willamette (ORNWS)										
(repl. 1)	(24.7)	(638)	(182)							
(" 2)	(25.5)	(614)	(174)							
Avg. of repl. 1,2	25.1	626	178	25.9	96.9%	2.4%	0.7%	40.1	141.1	3.5
Willamette (ACRS)	29.0	1928	179	31.1	93.2%	6.2%	0.6%	15.0	161.7	10.8
Skeena (ACRS)	22.4	856	119	23.4	95.8%	3.7%	0.5%	26.1	188.1	7.2
Marcy (DSIRNZ)	26.7	1062	184	28.0	95.5%	3.8%	0.7%	25.2	145.2	5.8
Min:	13.8	411	83	14.3	93.2%	2.4%	0.5%	15.0	123.8	3.2
Max:	29.0	1928	184	31.1	96.9%	6.2%	0.8%	40.1	188.1	10.8
Mean:	22.4	899	148	23.5	95.7%	3.7%	0.6%	28.7	154.7	6.0
Std Deviation:	4.7	470	35	5.1	1.2%	1.2%	0.1%	8.4.	19.0	2.4
% CV:	20.9%	52.3%	24.1%	21.7%	1.2%	33.1%	13.1%	29.3%	12.3%	39.6%

[a] results normalized to 10 degree Brix

Table II.4B Nonvolatile Acid Composition[a] of European Red Raspberry Juice as determined by Enzymatic Analysis

Sample:	Citric (g/L)	Malic (mg/L)	Isocitric (mg/L)	Total (g/L)	Percentage of total			Citric/ Malic	Citric/ Isocitric	Malic/ Isocitric
					Citric	Malic	Isocitric			
Bavarian	22.86	786	111	23.76	96.22%	3.31%	0.47%	29.1	205.9	7.1
Romanian	15.42	1445	45	16.91	91.19%	8.55%	0.27%	10.7	342.7	32.1
Hungarian	19.81	532	103	20.45	96.89%	2.60%	0.50%	37.2	192.3	5.2

[a] results normalized to 10 degree Brix

Table II.5 Recovery of sugar standard solution subjected to sample preparation procedure

	Percentage recoveries (%)[a,b]				Normalized recoveries[c]		
	Sucrose	Glucose	Fructose	Mannitol	Sucrose	Glucose	Fructose
Min:	93.1	92.9	91.7	92.1	99.6	98.8	98.5
Max:	98.1	96.5	96.1	97.0	102.7	100.9	99.9
Mean:	95.9	94.8	94.2	94.9	101.0	99.9	99.3
Std Dev:	1.9	1.7	1.7	1.8	1.0	0.6	0.4
% CV:	2.0	1.8	1.8	1.9	0.9	0.6	0.4

[a] standard solution 0.4 g sucrose and 1.2 g each of glucose, fructose and mannitol / 100 mL

[b] four repeated preparations

[c] normalized to 100 % mannitol recovery

Table II.6A Sugar Composition of Red Raspberry Juice

Samples:	sugar concentration, g / 100 mL[a] Sucrose	Glucose	Fructose	Total	percentage of total sugars Sucrose	Glucose	Fructose	Glucose/ Fructose
Meeker (ORNWS)								
(repl. 1)	(0.00)*	(2.74)	(3.01)					
(" 2)	(trace)*	(2.78)	(3.09)					
Avg. of repl. 1,2	0.00	2.76	3.05	5.81	0.0%	47.5%	52.5%	0.90
Meeker (ACRS)	0.10	2.99	3.37	6.46	1.5%	46.3%	52.2%	0.89
Meeker (WWREC)	0.00	3.44	3.79	7.23	0.0%	47.6%	52.4%	0.91
Willamette (ORNWS)								
(repl. 1)	(0.00)	(2.27)	(2.56)					
(" 2)	(0.08)	(2.23)	(2.50)					
Avg. of repl. 1,2	0.04	2.25	2.53	4.82	0.8%	46.7%	52.5%	0.89
Willamette (ACRS)	0.17	2.23	2.62	5.02	3.4%	44.4%	52.2%	0.85
Skeena (ACRS)	0.91	2.69	3.04	6.64	13.7%	40.5%	45.8%	0.88
Marcy (DSIRNZ)	0.00	1.80	2.41	4.21	0.0%	42.8%	57.2%	0.75
Min:	0.00	1.80	2.41	4.21	0.0%	40.5%	45.8%	0.75
Max:	0.91	3.44	3.79	7.23	13.7%	47.6%	57.2%	0.91
Mean:	0.17	2.59	2.97	5.74	2.8%	45.1%	52.1%	0.87
Std Deviation:	0.31	0.51	0.46	1.02	4.6%	2.5%	3.1%	0.05
%CV:	175.6%	19.5%	15.4%	17.7%	165.5%	5.5%	5.9%	6.0%

[a] expressed as single strength juice normilized to 10.0 degree Brix

* less than 0.02 g / 100 mL

Table II.6B Sugar Composition of European Red Raspbery Juice

	sugar concentration[a], g / 100 mL				percentage of total sugars			
Samples:	Sucrose	Glucose	Fructose	Total	Sucrose	Glucose	Fructose	Glucose/ Fructose
Bavarian	0.36	2.76	3.21	6.32	5.7%	43.6%	50.8%	0.86
Romanian	0.00	3.51	3.69	7.19	0.0%	48.8%	51.2%	0.95
Hungarian	0.16	3.11	3.69	6.96	2.3%	44.7%	53.1%	0.84

[a] expressed as single strength juice normilized to 10.0 degree Brix

Table II.7A Anthocyanin Composition (%) of Red Raspberry Juice

Sample:	Cyanidin				Pelargonidin	
	3-soph	3-glu	3-glurut	3-rut	3-soph	3-glurut
Meeker (ORNWS)						
(repl. 1)	(79.1)	(10.9)	(3.7)	(1.1)	(5.2)	(trace)*
(" 2)	(78.7)	(11.5)	(3.5)	(1.1)	(5.2)	(trace)
Avg. of repl. 1,2	78.9	11.2	3.6	1.1	5.2	0.0
Meeker (ACRS)	75.7	10.8	8.6	1.9	2.9	0.0
Meeker (WWREC)	75.8	8.9	9.3	1.7	4.2	0.0
Willamette (ORNWS)						
(repl. 1)	(85.5)	(10.7)	(0.0)	(0.0)	(3.8)	(0.0)
(" 2)	(85.2)	(10.5)	(0.0)	(0.0)	(4.2)	(0.0)
Avg. of repl. 1,2	85.3	10.6	0.0	0.0	4.0	0.0
Willamette (ACRS)	85.2	12.6	0.0	0.0	2.2	0.0
Skeena (ACRS)	76.1	23.9	0.0	0.0	0.0	0.0
Marcy (DSIRNZ)	42.0	7.6	38.3	7.0	2.0	3.2
Min:	42.0	7.6	0.0	0.0	0.0	0.0
Max:	85.3	23.9	38.3	7.0	5.2	3.2
Mean:	74.2	12.2	8.6	1.7	2.9	0.5
Std Deviation:	13.7	5.0	12.7	2.3	1.6	1.1
%CV:	18.4%	40.9%	148.5%	136.8%	54.9%	244.9%

Table II.7B Anthocyanin Composition (%) of European Red Raspberry Juice

Sample:	Cyanidin				Pelargonidin	
	3-soph	3-glu	3-glurut	3-rut	3-soph	3-glurut
Bavarian	85.1	14.9	0.0	0.0	0.0	0.0
Romanian	53.1	13.0	21.5	9.5	1.5	1.4
Hungarian	80.1	14.4	0.0	0.0	5.5	0.0

* less than 1 %

Table II.8 Red Raspberry Anthocyanin Composition (%) as determined by Barritt and Torre (23)

	Cyanidin				
	3-soph	3-glu	3-glurut	3-rut	3-diglu
Min:	20	11	0	0	0
Max:	72	45	43	32	6
Mean:	47.2	22.4	18.9	10.9	0.6
Std Dev:	15.2	8.2	13.4	9.4	1.4
% CV:	32%	37%	71%	86%	260%

Table II.9A Anthocyanidin Composition (%) of Red Raspberry Juice

Sample:	% Cyanidin	% Pelargonidin
Meeker (ORNWS)		
(repl. 1)	(96.0)	(4.0)
(" 2)	(96.4)	(3.6)
Avg. of repl. 1,2	96.2	3.8
Meeker (ACRS)	98.1	1.9
Meeker (WWREC)	97.2	2.8
Willamette (ORNWS)		
(repl. 1)	(97.1)	(2.9)
(" 2)	(96.9)	(3.1)
Avg. of repl. 1,2	97.0	3.0
Willamette (ACRS)	98.5	1.5
Skeena (ACRS)	100.0	trace*
Marcy (DSIRNZ)	95.1	4.9
Min:	95.1	0.0
Max:	100.0	4.9
Mean:	97.4	2.6
Std Deviation:	1.5	1.5
% CV:	1.5%	58.0%

Table II.9B Anthocyanidin Composition (%) of European Red Raspberry Juice

Sample:	% Cyanidin	% Pelargonidin
Bavarian	100.0	trace
Romanian	98.4	1.6
Hungarian	97.6	2.4

* less than 1%

Table II.10A Color Analyses of Red Raspberry Juice

Sample:	Anthocyanin Concentration mg / 100 mL [a,b]	Color Density [b]	Polymeric Color [b]	Percent Polymeric Color [b]	Browning Index [b]
Meeker (ORNWS)					
(repl. 1)	(68.2)	(22.6)	(1.0)	(4.6)	(0.64)
(" 2)	(68.9)	(24.5)	(1.5)	(5.9)	(0.89)
Avg. of repl. 1,2	68.6	23.6	1.3	5.3%	0.77
Meeker (ACRS)	29.0	13.5	1.4	10.1%	0.86
Meeker (WWREC)	31.6	13.1	0.8	6.0%	0.45
Willamette (ORNWS)					
(repl. 1)	(98.6)	(32.9)	(1.4)	(4.3)	(0.91)
(" 2)	(103.5)	(34.2)	(1.6)	(4.7)	(1.01)
Avg. of repl. 1,2	101.0	33.6	1.5	4.6%	0.96
Willamette (ACRS)	53.0	24.6	1.7	7.1%	1.05
Skeena (ACRS)	23.8	11.3	0.7	6.6%	0.45
Marcy (DSIRNZ)	57.1	19.9	2.1	10.7%	1.26
Min:	23.8	11.3	0.7	4.6%	0.5
Max:	101.0	33.6	2.1	10.7%	1.3
Mean:	52.0	19.9	1.4	7.2%	0.8
Std Deviation:	25.2	7.4	0.5	2.2%	0.3
%CV:	48.4%	37.2%	34.0%	30.3%	33.6%

[a] expressed as cyd-3-glu (e=29,600)

[b] results normalized to 10 degree Brix

Table II.10B Color Analyses of Authentic Red Raspberry Juice concentrated to 50 degree Brix

Sample	[a,b] Anthocyanin Concentration mg / 100 mL	[b] Color Density	[b] Polymeric Color	Percent Polymeric Color	[b] Browning Index
Meeker (ORNWS repl. 1)	63.1	26.8	2.5	9.3%	1.56
Willamette (ACRS)	45.9	24.2	3.4	14.0%	2.09
Skeena (ACRS)	19.9	12.2	1.4	11.9%	0.93
Marcy (DSIRNZ)	53.2	22.2	3.2	14.5%	1.98

Table II.10C Color Analyses of European Red Raspberry Juice

Sample:	[a,b] Anthocyanin Concentration mg /100 mL	[b] Color Density	[b] Polymeric Color	Percent Polymeric Color	[b] Browning Index
Bavarian:	9.2	8.0	2.9	36.3%	1.71
Romanian:	9.6	4.7	1.0	20.2%	0.65
Hungarian:	10.0	6.2	2.0	32.2%	1.31

[a] expressed as cyd-3-glu (e=29,600)

[b] results normalized to 10 degree Brix

Table II.11 Spectral Characteristics of Red Raspberry Juice

Mode	Wavelength Range (nm)	
	Maxima	Minima
Absorbance:	513-515	
	273-278	
First Derivative:	664-665	537-541
	477-479	282-283
	391-392	224-230
	258-260	207-209
		202-204
Second Derivative:	550-551	503-504
	284-285	264-274
	226-232	210-211
	215-216	
	204-209	

Table II.12A Hunter and CIE Parameters of Red Raspberry Juice

Sample:	Hunter Parameters L	a	b	CIE Parameters Y	X	Z	Hue	SI	Hue/ SI
Meeker (ORNWS)									
(repl. 1)	(22.3)	(48.5)	(15.3)	(5.0)	(10.9)	(0.1)	(72.5)	(50.9)	(1.4)
(" 2)	(24.2)	(51.4)	(16.6)	(5.8)	(12.6)	(0.3)	(72.1)	(54.0)	(1.3)
Avg. of repl. 1,2	23.3	50.0	16.0	5.4	11.8	0.2	72.3	52.5	1.4
Meeker (ACRS)	35.2	61.3	24.1	12.4	24.1	0.3	68.6	65.9	1.0
Meeker (WWREC)	30.0	58.3	20.7	9.0	18.5	0.1	70.5	61.9	1.1
Willamette (ORNWS)									
(repl. 1)	(20.2)	(44.8)	(13.7)	(4.1)	(9.0)	(0.1)	(73.0)	(46.8)	(1.6)
(" 2)	(20.0)	(44.5)	(13.6)	(4.0)	(8.8)	(0.1)	(73.0)	(46.5)	(1.6)
Avg. of repl. 1,2	20.1	44.7	13.7	4.1	8.9	0.1	73.0	46.8	1.6
Willamette (ACRS)	30.3	58.2	20.9	9.2	18.8	0.1	70.3	61.8	1.1
Skeena (ACRS)	37.7	63.0	25.2	14.2	27.1	0.8	68.2	67.9	1.0
Marcy (DSIRNZ)	32.5	59.1	22.2	10.6	21.0	0.3	69.4	63.1	1.1
Min:	20.1	44.7	13.7	4.1	8.9	0.1	68.2	46.8	1.0
Max:	37.7	63.0	25.2	14.2	27.1	0.8	73.0	67.9	1.6
Mean:	29.9	56.4	20.4	9.3	18.6	0.3	70.3	60.0	1.2
Std Deviation:	5.8	6.1	3.9	3.3	6.0	0.2	1.7	7.0	0.2
%CV:	19.4%	10.8%	18.9%	35.9%	32.0%	85.2%	2.4%	11.7%	15.6%

S.I.=Saturation Index

Table II.12B Hunter and CIE Parameters of European Red Raspberry Juice

Sample:	Hunter Parameters			CIE Parameters			Hue	SI	Hue/ SI
	L	a	b	Y	X	Z			
Bavarian	36.2	53.5	19.9	13.1	23.6	3.3	69.6	57.1	1.2
Romanian	47.5	56.3	22.4	22.5	36.9	8.7	68.3	60.6	1.1
Hungarian	40.6	54.8	22.1	16.5	28.5	4.3	68.1	59.1	1.2

S.I.=Saturation Index

Figure II.1 HPLC Separation of Red Raspberry Nonvolatile Acids. Detection UV at 227 nm

Peak Identification: 1. malic, 2. isocitric, 3. a-ketoglutaric, 4. shikimic and ascorbic, 5. citric, 6. succinic, 7. fumaric

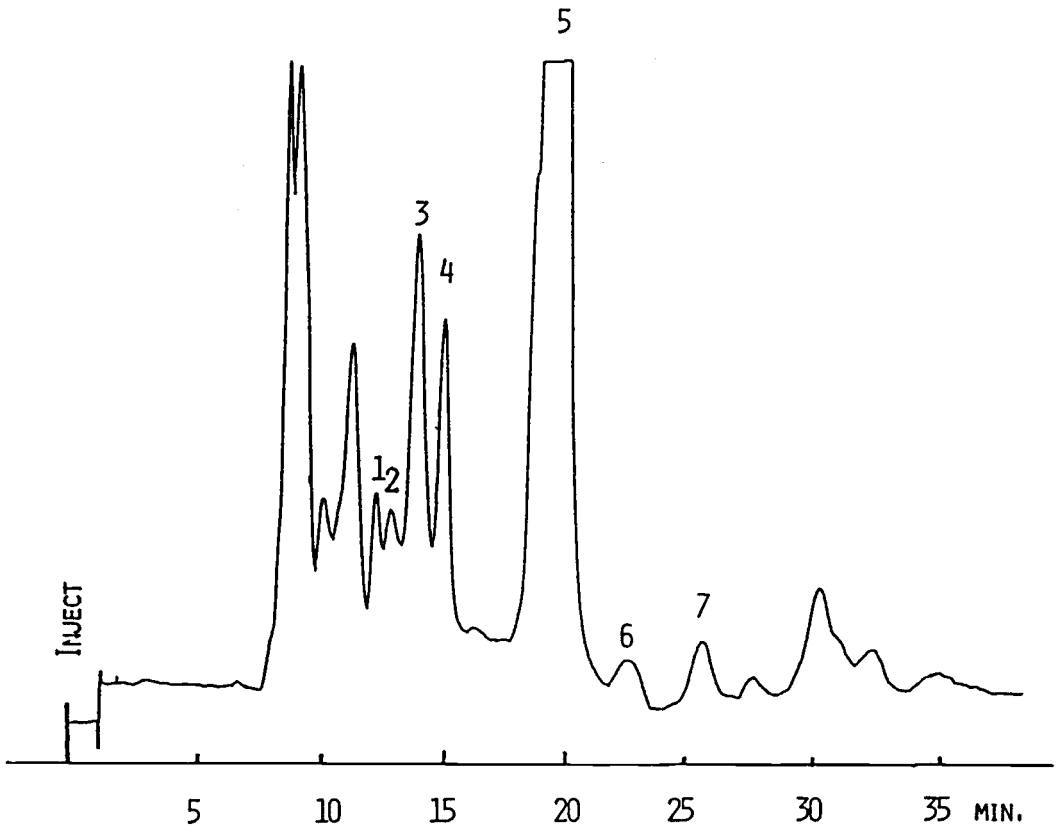


Figure II.2 HPLC Separation of Red Raspberry Nonvolatile Acids. Detection Refractive Index

Peak Identification: 1. malic, 2. isocitric, 3. citric

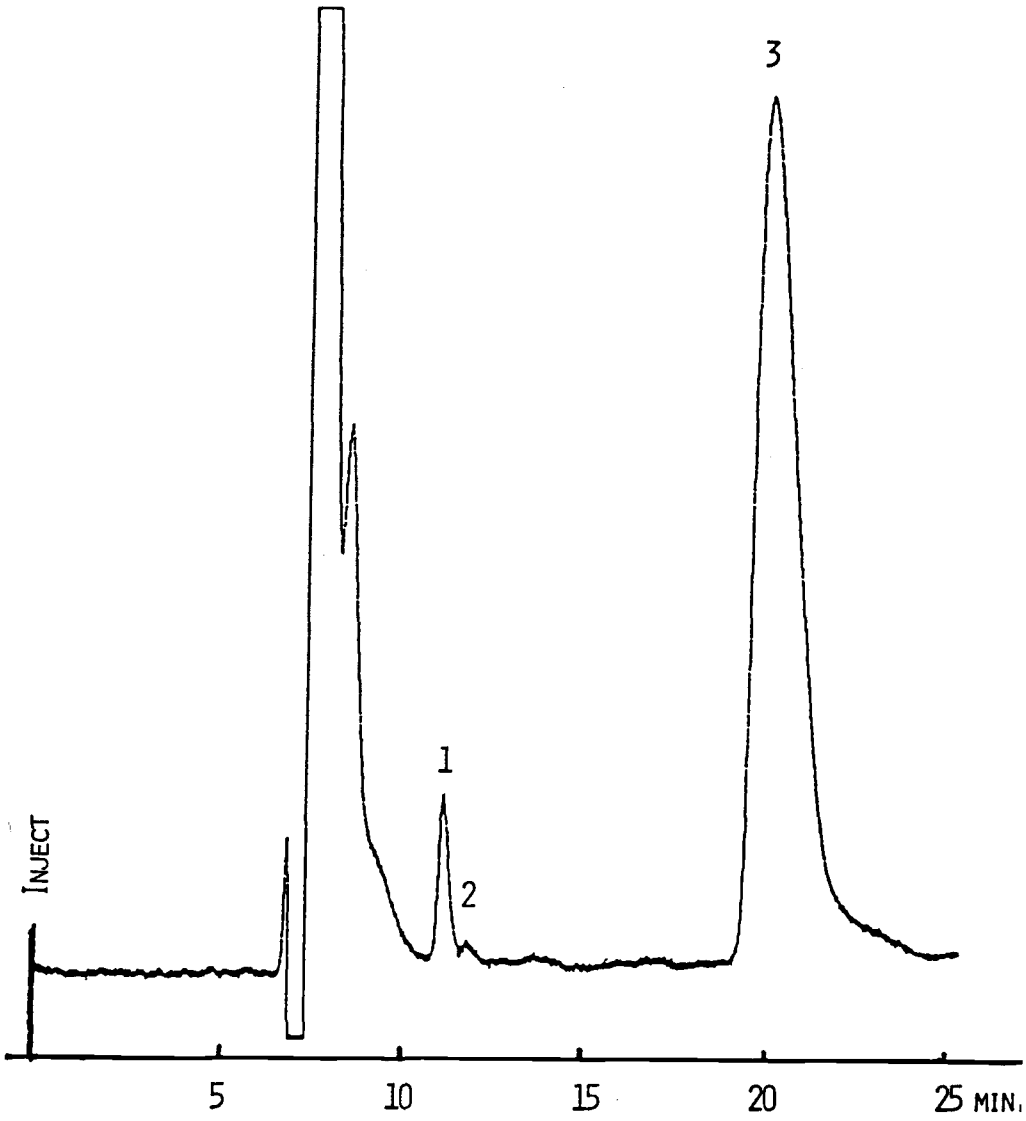


Figure II.3 HPLC Separation of Red Raspberry Sugars

Peak Identification: 1. sucrose, 2. glucose, 3. fructose, 4. mannitol (internal standard)

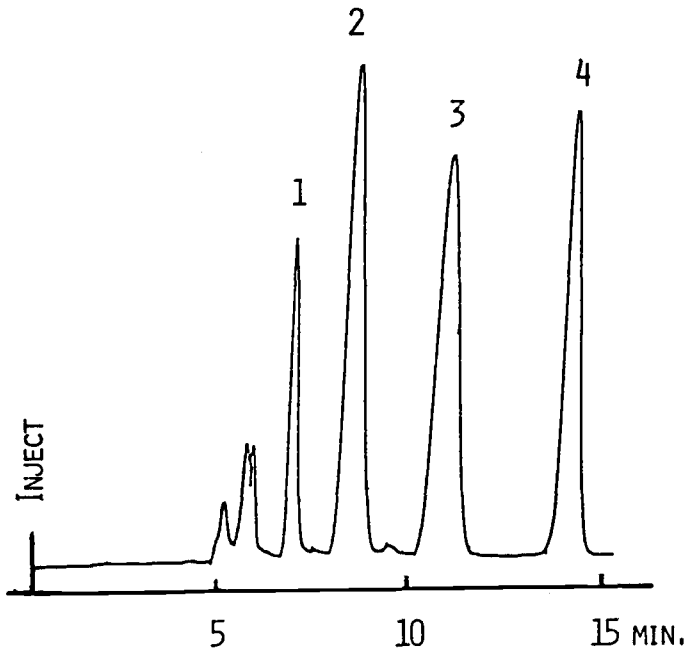


Figure II.4 HPLC Separation of Red Raspberry Anthocyanins. Elution: solvent A, 10% acetic acid in water, solvent B 10% acetic acid in methanol.

Peak Identification: 1. cyd-3-soph, 2. cyd-3-glurut, cyd-3-glurut and pgd-3-soph

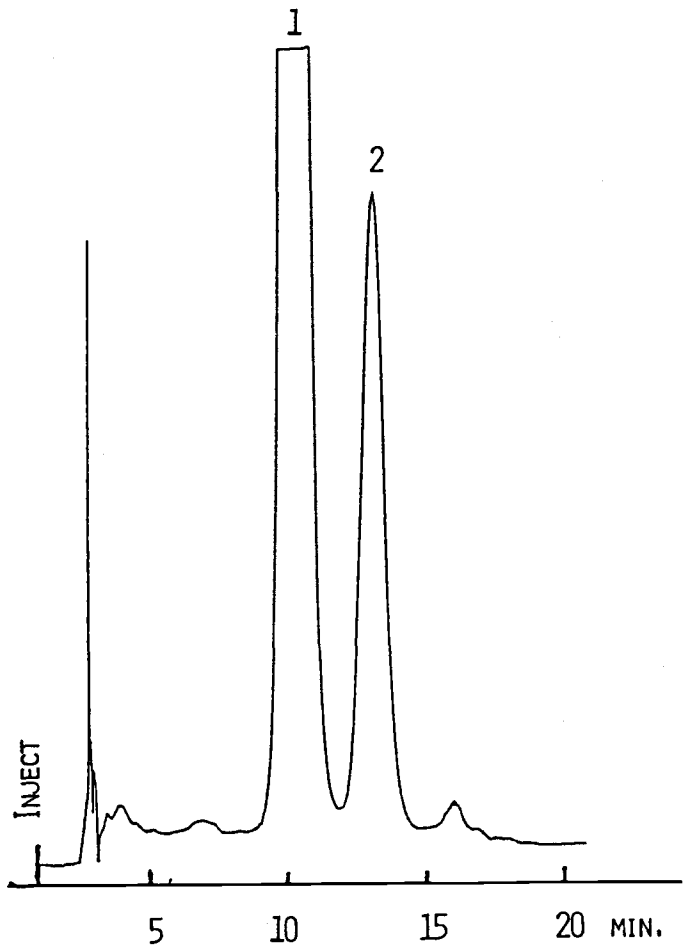


Figure II.5 HPLC Separation of Anthocyanins from Red Raspberries: A-Meeker (ORNWS), B-Willamette (ORNWS), C-Marcy (DSIRNZ)

Peak Identification: 1. cyd-3-soph, 2. cyd-3-glurut, 3. cyd-3-glu, 4. pgd-3-soph, 5. cyd-3-rut, 6. pgd-3-glurut, 7.pgd-3-glu

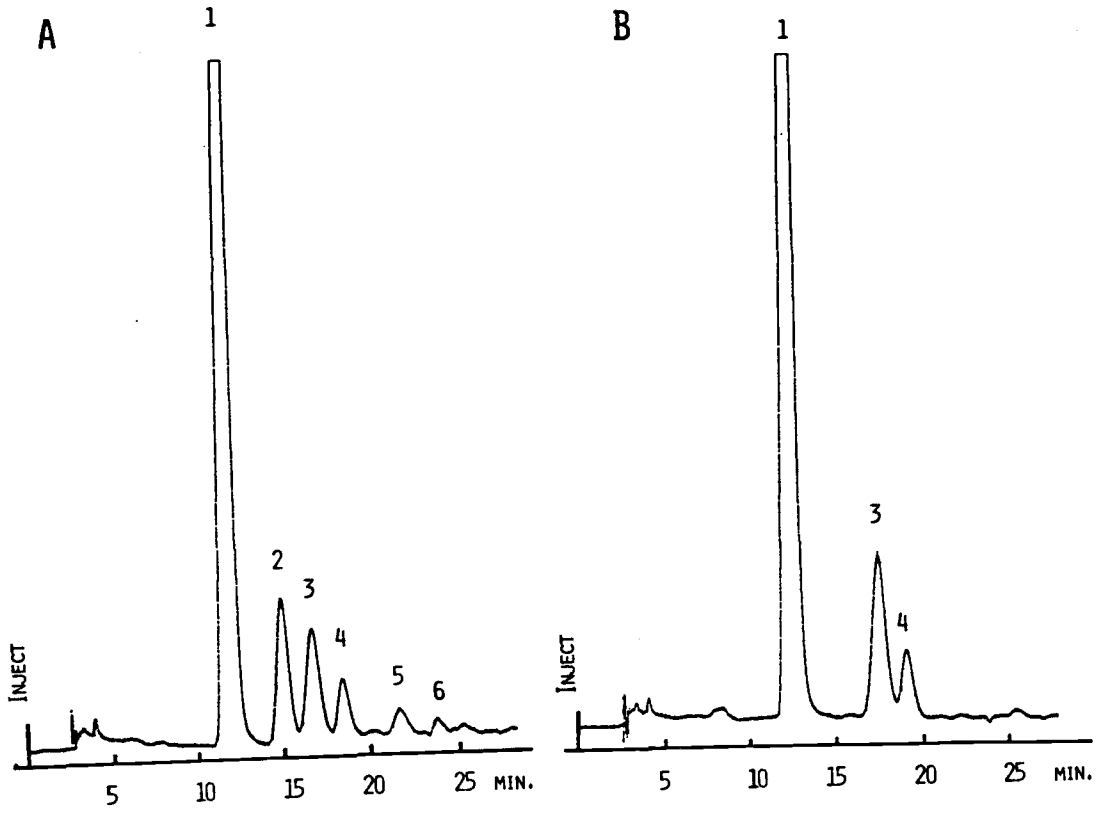


Figure II.5 (continued)

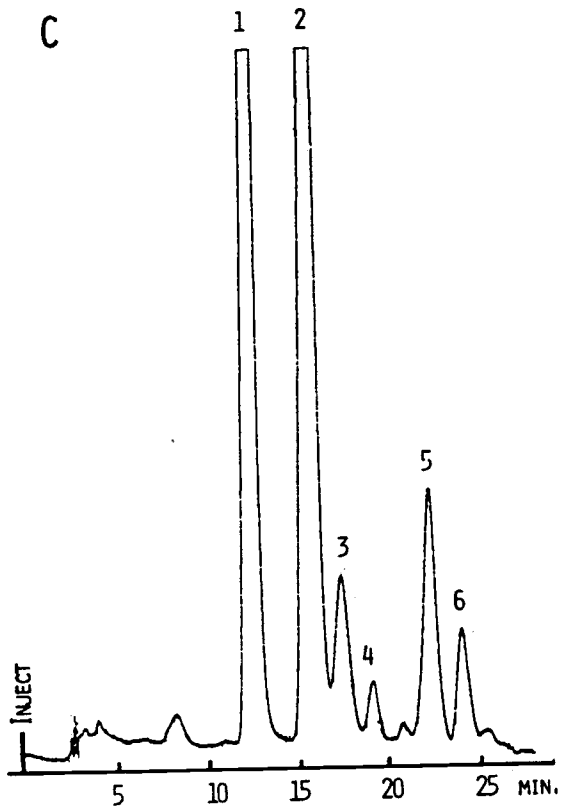


Figure II.6 HPLC Separation of Anthocyanins from: A-Sour Cherry, B-Blackberry, C-Black Currant, D-Strawberry

Peak Identification: 1. Cyd-3-soph, 2. cyd-3-glurut, 3. cyd-3-glu, 5. cyd-3-rut, 7. pgd-3-glu

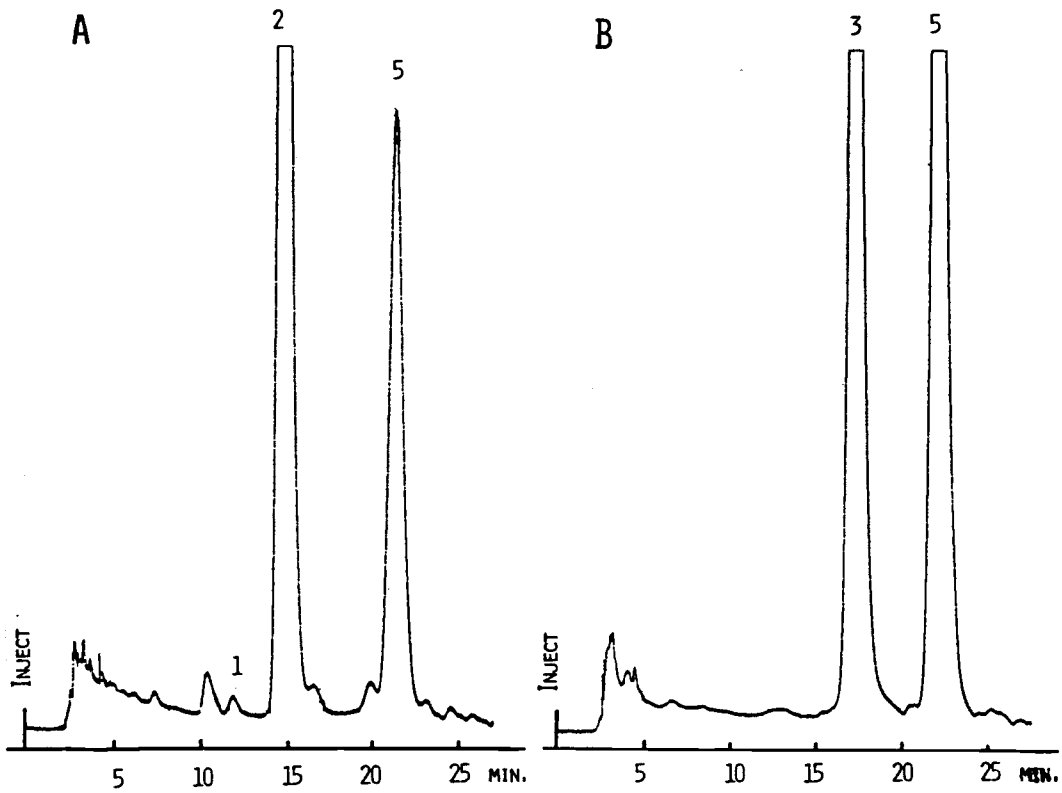


Figure II.6 (continued)

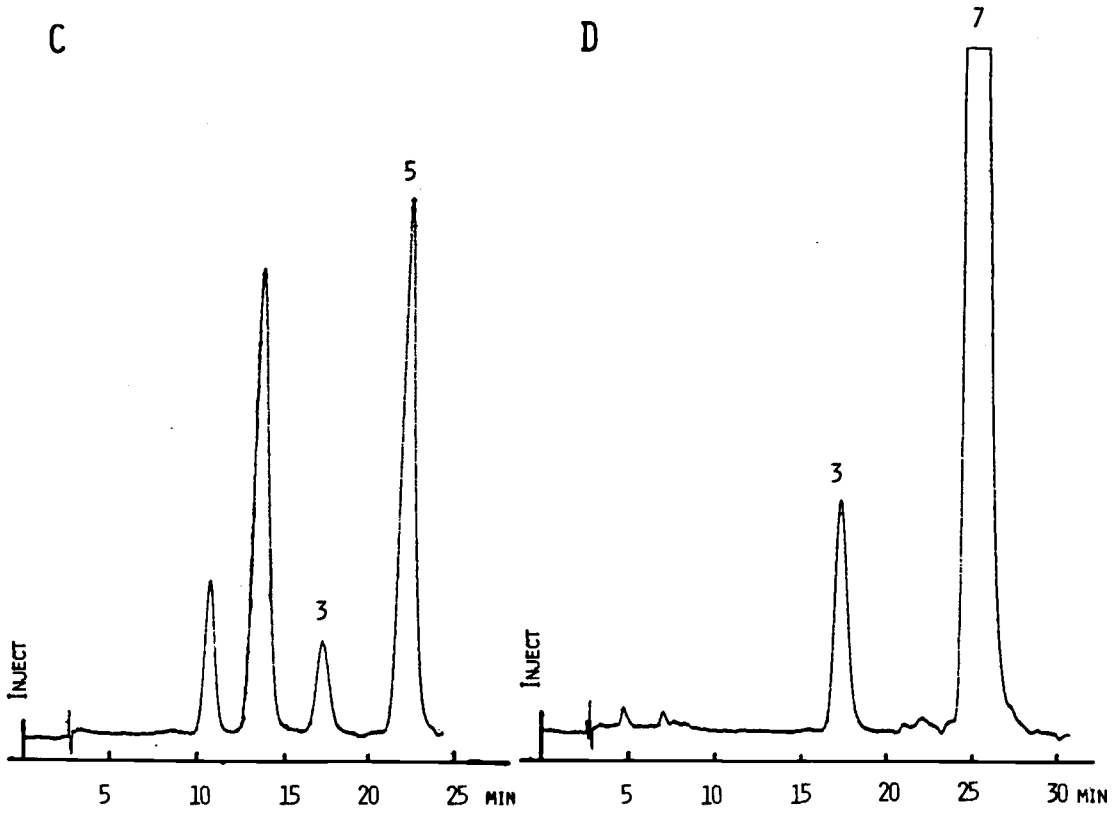


Figure II.7 HPLC Separation of Red Raspberry Anthocyanidins

Peak Identification: 1. cyanidin, 2. pelargonidin

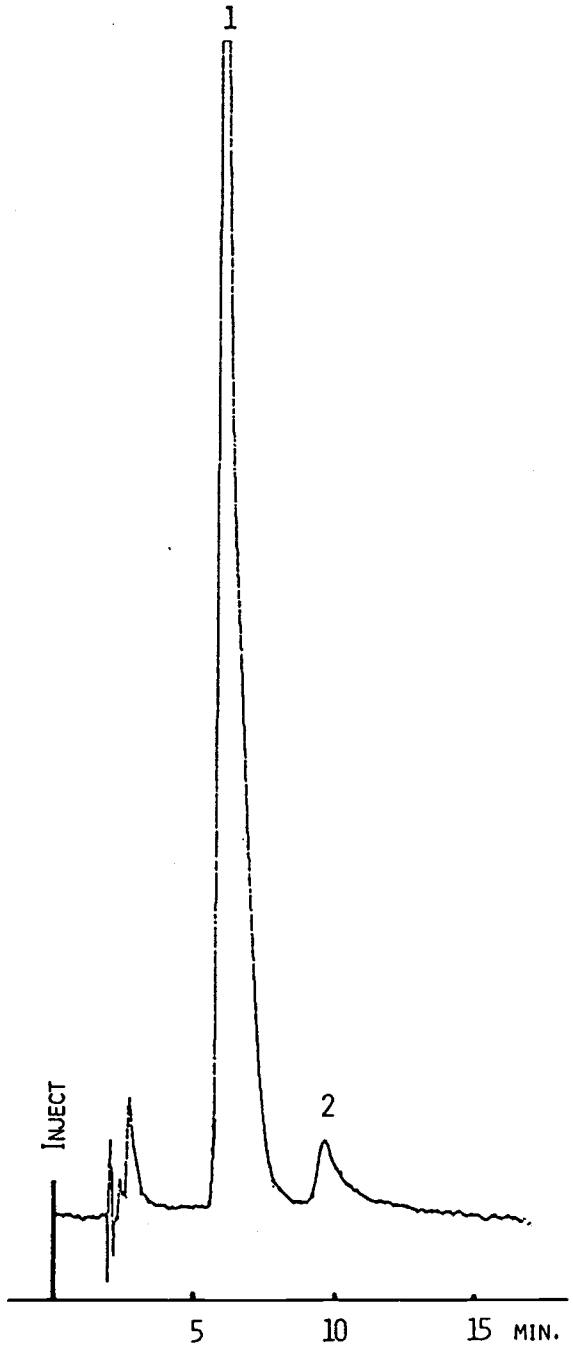


Figure II.8 Typical Absorbance Spectra of Red Raspberry Juice

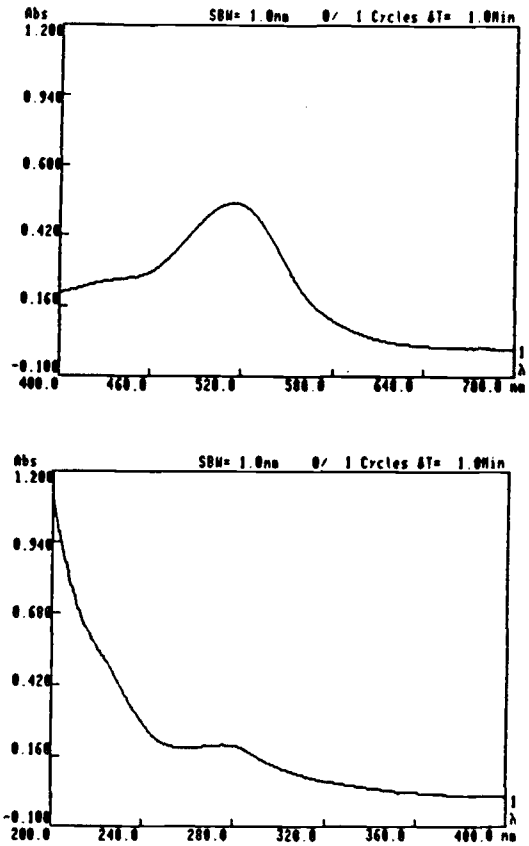
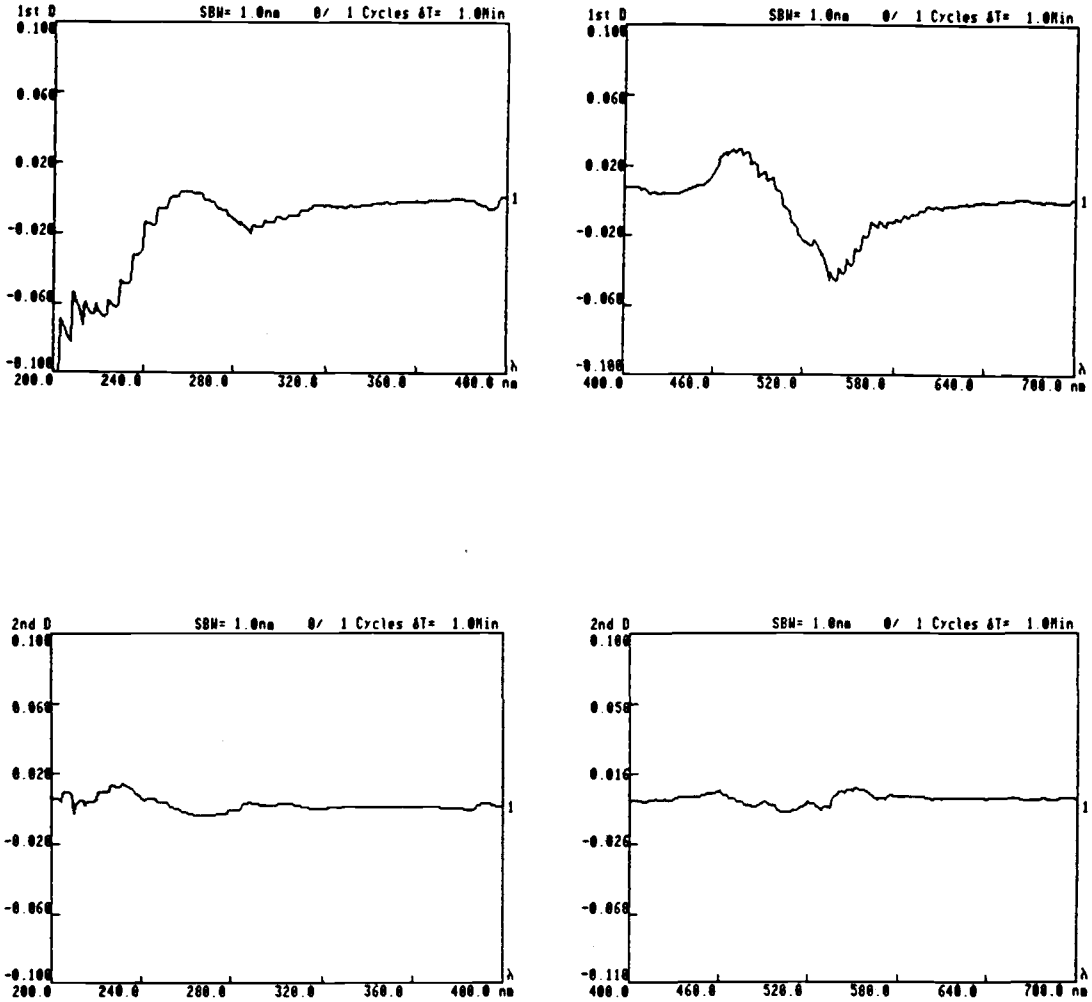


Figure II.9 Typical First and Second Derivative Spectra of Red Raspberry Juice



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