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Title: Anthocyanin Composition of Red Raspberry Juice: Influences of Variety, Processing, and Environmental Factors

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Two HPLC (High Pressure Liquid Chromatography) methods were evaluated for separating red raspberry anthocyanins. A system using a silica based reverse phase (C_{18}) column and an acetic acid/acetonitrile gradient elution program was found to provide the best results.

Forty six red raspberry juice samples from the United States, Canada, and Poland were analysed for total anthocyanin content, percent polymeric color, anthocyanin profile, and anthocyanidin profile. These samples were comprised of ten cultivars (Willamette, Meeker, Heritage, Malling Promise, Malling Seedling, Norna, Veten, Skeena, Chilcotin, and Golden), three juice making processes (standard, enzymic liquefaction/centrifugation, diffusion extraction), and two juice concentrating processes (vacuum and osmotic). The samples were of three maturity levels (underripe, ripe, overripe) and one sample of juice made from moldy fruit were included. The results of these analyses were used as a database on authentic red raspberry juice anthocyanins and the effects of variety, processing, maturity level, and geographic origin on them.

The pigments in fourteen samples of commercial red raspberry juice concentrates were analysed and compared to the database sample results. Two were found to contain delphinidin pigments, indicating adulteration with other fruits or fruit pigments. Three samples contained high percentages of polymerized pigments, indicating poor quality due to processing or storage. The remaining samples were of better quality, authentic red raspberry juice.

Anthocyanin Composition of Red Raspberry Juice: Influences of Variety, Processing, and Environmental Factors

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LIST OF ABBREVIATIONS

1. HPLC	High Pressure Liquid Chromatography
2. TLC	Thin Layer Chromatography
3. HTST	High Temperature Short Time (pasteurization method)
4. CYD-3-SOP	Cyanidin-3-sophoroside
5. CYD-3-GLURUT	Cyanidin-3-glucosylrutinoside
6. CYD-3-RUT	Cyanidin-3-rutinoside
7. CYD-3-GLU	Cyanidin-3-glucoside
8. CYD-3,5-DIGLU	Cyanidin-3,5-diglucoside
9. CYD-3-SAM	Cyanidin-3-sambubioside
10. CYD-3-XYLRUT	Cyanidin-3-xylosylrutinoside
11. PGD-3-SOP	Pelargonidin-3-sophoroside
12. PGD-3-GLU	Pelargonidin-3-glucoside
13. PGD-3-GLURUT	Pelargonidin-3-glucosylrutinoside

ANTHOCYANIN COMPOSITION OF RED RASPBERRY JUICE: INFLUENCES OF VARIETY, PROCESSING, AND ENVIRONMENTAL FACTORS

INTRODUCTION

Anthocyanins are the pigments responsible for the bright colors of most fruits and flowers. There are hundreds of anthocyanins occurring in nature, but the anthocyanins occuring in any particular species are distinctive enough to be used to identify the species (chemotaxonomy). Differences in anthocyanins between different cultivars within a species are generally quantatative rather than qualitative. The anthocyanins occuring in red raspberries (*Rubus ideaus*) have been identified, and the anthocyanin distributions in many different cultivars have been reported. Very little work has been done on the effects of processing on red raspberry anthocyanins.

The purpose of this project was to generate a database on anthocyanin pigments in red raspberry juices, and the effects of variety, processing methods, and environmental factors on the pigments. This information can then be used for comparison with commercially produced red raspberry juices to determine authenticity and as an aid in quality assessment. Also, the total anthocyanin content of different varieties of red raspberries can determine their suitability for use in certain products. For a juice or wine, a high level of pigment is desirable. In the production of red raspberry jam or jelly, too high of a pigment content produces too dark of a product.

The anthocyanin and anthocyanidin profiles, total anthocyanin content, and percent polymeric color were determined for forty six samples of red raspberry juice. The juices were prepared in our lab for use in this and other related studies. Samples of ten different red raspberry cultivars from the United States, Canada, and Poland were obtained from commercial juice processors and agricultural research stations. Three juice making processes and two juice concentrating methods were used in preparing the juices. Also, a sample of juice made from moldy fruit was included.

The results of these analyses were used to evaluate fourteen commercial red raspberry juice concentrates. This project was supported by contributions from commercial juice producers and fruit samples were provided by the Oregon Caneberry Commission.

LITERATURE REVIEW

IDENTIFICATION

Anthocyanins are flavanoid glycosides that give most fruits their red, blue, and purple colors. The anthocyanins in many varieties of red raspberries (*Rubus ideaus*) have been reported. Early research on red raspberry anthocyanins used Thin Layer Chromatography (TLC) to separate the pigments (Barritt and Torre, 1973; Misic, 1973). Identification of the pigments was originally done by controlled hydrolysis experiments on purified pigments (Francis, 1972) and later by retardation factors (Rf) (Misic, 1973), and co-chromatography with known pigments (Barritt and Torre, 1973). Quantitation was generally done by densitometry on the TLC plates (Sagi et al, 1974; Torre and Barritt, 1977). Some older studies identified the pigments, and gave the relative amounts based on arbitrary methods such as relative size of spots on the TLC plates (Francis, 1972; Misic, 1973; Barritt and Torre, 1973). These methods gave only rough estimates of the pigment distributions.

Francis(1972) and Misic (1973) reported four cyanidin pigments in various red raspberry cultivars: cyanidin-3-sophoroside (CYD-3-SOP), cyanidin-3-glucosylrutinoside (CYD-3-GLURUT), cyanidin-3-rutinoside (CYD-3-RUT), and cyanidin-3-glucoside (CYD-3-GLU). Barritt and Torre (1973) reported these four as well as small amounts of cyanidin-3,5-diglucoside (CYD-3,5-DIGLU), and unidentified pelargonidin glycosides. The presence of CYD-3,5-DIGLU in red raspberries was not proven, and is believed not to occur in them. Further work by Barritt and Torre at the Western Washington Research and Extension Center, and others, identified pelargonidin pigments corresponding to the cyanidin pigments previously

reported (Barritt and Torre, 1975; Torre and Barritt, 1977, Spanos and Wrolstad, 1987). More recent work used High Pressure Liquid Chromatography (HPLC) to separate *Rubus* fruit anthocyanins (Spanos and Wrolstad, 1987; Sapers et al, 1986). This method, along with UV-Visible spectrophotometric detection provides much more accurate pigment quantitation than older methods.

CULTIVARS

Many different cultivars of red raspberries exist, and new ones are being developed by horticultural researchers around the world. A study by Misic (1973) reported pigment profiles for 29 varieties, including 10 cultivars native to or developed in Yugoslavia. Four cyanidin glycosides were separated and identified, CYD-3-GLU, CYD-3-SOP, CYD-3-RUT, and CYD-3-GLURUT. A study of anthocyanin pigments in 37 cultivars and selections, including 4 red raspberry/black raspberry hybrids was performed by Barritt and Torre (1975). Only one sample was found to contain anthocyanins normally associated with black raspberries (cyanidin-3-sambubioside and cyanidin-3-xylosylrutinoside) in addition to red raspberry pigments. Sagi et al (1973) reported the anthocyanin distributions for three Hungarian varieties. All had pigments typical for red raspberries.

RELATED SPECIES

Other *Rubus* species include blackberry and black raspberry. Blackberries are a group of closely related *Rubus* species (Macheix *et al*, 1990). Boysenberry and loganberry are red raspberry/blackberry hybrids. Both blackberries and black raspberries have very high total anthocyanin content. Blackberries have about four times as much pigments as red

raspberries, and black raspberries up to 10 times as much (Torre and Barritt, 1977). Black raspberries have the xylose glycosides cyanidin-3-sambubioside (CYD-3-SAM) and cyanidin-3-xylosylrutinoside (CYD-3-XYLRUT). Blackberries have CYD-3-GLU as the major pigment, and lesser amounts of CYD-3-RUT (Macheix *et al*, 1990; Torre and Barritt, 1977). Pelargonidin-3-glucoside was found in one species of blackberry, *R. caucasicus* (Torre and Barritt, 1977). This is the only reported occurance of pelargonidin based pigments in blackberries. Also reported in blackberries are a xylose derivative of cyanidin, and two unidentified dicarboxylic acid acylated derivatives of cyanidin (Sapers *et al*, 1986; Rommel *et al*, 1992). Boysenberries and loganberries have red raspberry pigments, but at levels similiar to blackberries (Torre and Barritt, 1977). No other *Rubus* species contain CYD-3-SOP.

QUANTITATIVE REPORTS

The total amount of anthocyanin pigments in fruits and fruit products has been done by various methods. Older studies used the absorbance at the visible wavelength absorbance maximum of a juice (Barritt and Torre, 1975), sometimes along with published extinction coefficients to quantify the pigments (Sagi *et al*, 1973; Sjulin and Robbins, 1987). Methods relying on separation of pigments by TLC and spectrophotometric determination of recovered pigments from TLC plates are prone to large errors. Methods using absorbance of juices or extracts do not differentiate between monomeric anthocyanins and polymerized pigments. A method using a pH differential/spectrophotometric determination of monomeric anthocyanins overcomes these problems (Wrolstad, 1976).

EFFECTS OF MATURITY

The effect of maturity of fruits on pigment content has been investigated. All have shown that the pigment content increases with ripeness (Sagi *et al*, 1973; Sjulin and Robbins, 1987). It was also shown that the pigment distributions in Rubus fruits change very little upon ripening; the effect is mainly quantitative (Barritt and Torre, 1975; Sapers *et al*, 1986).

PROCESSING AND STORAGE EFFECTS

The effects of processing and storage on the anthocyanins in red raspberries is somewhat limited. Red raspberry fruits were shown to continue to accumulate anthocyanins after picking, with an increase of up to 44% over 9 days of storage (Sjulin and Robbins, 1987). A study on the quantitative and qualitative changes occuring in red raspberry juice concentrate anthocyanins upon storage was performed by Withy *et al* (1991). They found that the relative proportions of CYD-3-SOP and CYD-3-GLURUT decreased, and the proportion of CYD-3-GLU increased. This was attributed to the stepwise hydrolysis of the di- and trisaccharides to form the monosaccharide, CYD-3-GLU. A study on the pigment changes in red raspberry juice and wines (Rommel *et al*, 1990) showed that CYD-3-GLU was the least stable pigment in red raspberries.

A study by Jiang *et al* (1990) showed that some commercial pectolytic enzyme preparations caused glycoside hydrolysis that produced CYD-3-GLU from CYD-3-SOP, and CYD-3-RUT from CYD-3-GLURUT. This showed that depectinization or liquefaction enzymes can alter the pigment distribution in red raspberries.

MATERIALS AND METHODS

FRUIT AND JUICE SAMPLES

Sources-Red raspberry samples used in this study were supplied by commercial growers, juice producers, and agricultural research stations in the United States, Canada, and Poland. Fruit samples from Oregon were obtained through the Oregon Caneberry Commission. The fruit was grown at the North Willamette Experiment Station at Aurora, Oregon, and by commercial growers in the area. They include machine and hand harvested fruits of varying maturity levels (underripe, ripe, or overripe). One sample of extremely moldy fruit was also obtained. The samples were supplied as fresh whole fruits and were washed, block frozen at -30°C, and then stored at -23°C until made into juices.

Twelve samples of freeze dried red raspberries were obtained from Dr. Witold Plocharski of the Research Institute of Pomology and Floriculture, Skierniewice, Poland. There were three sub-samples (each representing a single picking) of four different varieties. The fruit was grown at the Polish Research Station in Prusy. They were freeze dried prior to shipment to the United States.

Four juice samples and two block frozen fruit samples were obtained through Agriculture Canada's Food Processing Laboratory in Summerland, British Columbia. Three of the juice samples were prepared by high speed centrifugation both with and without enzymic liquefaction. One juice sample was made in a pilot diffusion extractor. All the juices were prepared from the same batch of Willamette variety fruits grown in Chilliwack, B.C. Two block frozen samples of fruits (Chilcotin and Skeena varieties) were also supplied by this group.

A more detailed description of the samples appears in Table 1. Commercial frozen strawberries (variety unknown) and Concord grape juice (Welches Foods, New York) were purchased at a local supermarket. Commercial blackberry juice concentrate was available from a previous study (Hong and Wrolstad, 1990).

Cultivars- Ten different varieties of raspberries were included in this study. Willamette, Meeker, Heritage, and Golden variety fruits were obtained from Oregon and Washington, USA. Willamette, Chilcotin, and Skeena varieties were supplied by Canadian processors. The varieties Norna, Veten, Malling Promise, and Malling Seedling were provided by researchers in Poland. The samples were picked during the 1988, 1989, and 1990 growing seasons. One fall bearing variety, Heritage, was included. All others were summer bearing varieties. Fall bearing varieties produce a small crop in the summer, and a heavier crop in the Fall. One sample was of a variety that produces yellow colored fruits, named Golden.

JUICE PROCESSING METHODS

Standard- This method, representing widely used commercial processing methods, is outlined in Figure 1. It was performed in the Oregon State University Department of Food Science and Technology pilot plant. This method consists of taking thawed fruits and adding depectinizing enzymes. Rice hulls were added as a press aid, and the juice expressed using a bag press. The juice was tested for pectin, and more enzyme added and incubated until adequately depectinized. The juice was then HTST (High Temperature Short Time) pasteurized, fined with bentonite and gelatin, and

left to settle overnight. The juice was then pad filtered, and stored frozen at -20°C until analysed.

Samples of fruit too small to be processed with this equipment (all Polish samples) were made into juices using a laboratory simulation of the standard processing method. The samples were received in freeze dried form. Two kilogram lots of fruit were freeze dried in a Heraeus-Leybold laboratory freeze drier (temperature less than 30°C). The samples were stored at -23°C until processed. They were rehydrated to their original weight with distilled water one day before being made into juices. Portions of the same batch of fresh Willamette variety fruit were prepared by the standard and simulated standard methods for comparison (Samples 34A-D, Table 1).

Enzymic Liquefaction and Centrifugation- A set of Willamette variety fruits from British Columbia, Canada (Samples 35B&C) were prepared using enzymic liquefaction of the berries followed by centrifugation (Figure 2). Thawed, bulk frozen fruits were treated with one of two different commercial pectolytic enzyme preparations (Novo, Switzerland). After incubation, the juice was separated using high speed centrifugation. The juice was HTST pastuerized, and stored frozen. A control sample was similiarly prepared, but without the addition of enzymes (Sample 35A).

Diffusion Extraction- A portion of the same fruit as in the liquefaction/centrifugation samples (Sample 36) was made into a juice using a pilot plant sized diffusion extractor (Figure 3). Water at 37°C (450 ml water per 0.6 kg thawed fruit) was run countercurrent to the fruit to extract the solubles from the fruit. The extract was then depectinized, HTST pasteurized, and the resulting diluted juice stored frozen.

JUICE CONCENTRATING METHODS

Vacuum Concentration- A portion of juice prepared by the standard processing method was concentrated to 43.5°brix (Sample 37E) using a Centritherm model CT-1B centrifugal film evaporator (Alfa-Laval,). It uses heat and reduced pressure to remove water from juice spread out as a thin film within the evaporator. This is a standard commercial type of juice concentrator. A sample of unconcentrated juice was kept as a control (Sample 37D).

Osmotic Concentration- Portions of the same juice used in the vacuum concentration experiment were concentrated using a pilot direct osmosis concentrator (Osmotek, Corvallis, Oregon). It uses concentrated corn syrup as an osmotic agent to remove water from the juice. A thin semi-permeable membrane with a molecular weight cutoff of about 100 is used to keep the juice and concentrating medium separate. A more complete description of the process and apparatus has been reported by Beaudry and Lampi (1990). Two different membranes and two operating time/temperature combinations were used. Juice concentrates of approximately 45°brix were prepared, and stored frozen (Samples 37A-C).

COMMERCIAL SAMPLES

Commercial red raspberry juice concentrates were obtained from the following companies: Clermont Inc., Hillsboro, OR; Endurance Fruit Processing Inc., Wapato, WA; Kerr Concentrates Inc., Salem OR; Milne Fruit Products Inc., Prosser, WA; Rudolf Wild GmbH & Co. Kg, Eppelheim-Heidelburg, Germany; Sanofi Bio-Industries Division, Wapato, WA; and the J.M. Smucker Co., Woodburn, OR. These samples were also used in a study comparing the sensory qualities of osmotically concentrated red raspberry juice to conventionally concentrated juices (Wrolstad *et al*, 1992). They varied between 39 and 66 brix. In soliciting the samples, the companies were assured that individual sample identities would be kept confidential. These samples were coded A through I in Table 5. Samples were stored at -23°C prior to analysis.

Five samples of commercial red raspberry juice concentrates were supplied by Minot Food Packers, Inc. (Bridgeton, NJ.). These samples were coded J through N (Table 5) and their origin and processing history were unknown. We were asked by Minot to evaluate them for authenticity and quality.

EXPERIMENTAL

General Sample Preparation for Anthocyanin and Anthocyanidin Analyses- Samples supplied as juice concentrates were diluted to approximately 10° brix with distilled water prior to analysis. Samples consisting of single strength juices were thawed and used as is, or filtered through a 0.45 micron Type HA Millipore filter (Millipore Corp., Bedford, MA) if turbid. Frozen strawberries were thawed at room temperature, crushed, and extracted with

acidified methanol overnight (.01% HCl in methanol) in a refrigerator. The methanolic extract was filtered through a Millipore 0.45 micron filter, and the methanol removed in a rotary evaporator at room temperature. Concord grape juice was used as purchased.

Determination of Total Monomeric Anthocyanins and % Polymeric Color- The anthocyanin pigment content and % polymeric color were determined as described in Oregon State University Agricultural Experiment Station Bulletin #624 (Wrolstad, 1976). Pigment concentration is expressed as mg/l cyanidin-3-glucoside (E=29,600). A Beckman DB-GT grating spectrophotometer was used in these analyses.

Sample Preparation for HPLC Analysis of Anthocyanidins- The hydrolysis and concentration method described by Hong and Wrolstad (1986) was used in this study.

Sample Preparation for HPLC Analysis of Anthocyanins- The sample preparation method for quantitation of anthocyanin pigments (minimal cleanup) described by Hong and Wrolstad (1987) was used.

HPLC Separation of Anthocyanidins- Liquid Chromatograph: Varian Model 5000 liquid chromatograph fitted with a Hewlett Packard 1040A diode array detector and a Hewlett Packard 9000 computer. Column: Supelcosil LC18 (5 mm x 25 cm) 5 micron particle size with a Bio-Rad ODS-10 guard column. Solvent: A= 15% acetic acid (aq), B= 100% acetonitrile. Conditions: isocratic

elution with 85% A and 15% B at 1.5 ml/min. Injection volume 25 ul, detection at 520 nm, 20 nm bandwidth. UV-Vis spectra taken directly from chromatographic runs.

HPLC Separation of Anthocyanins- Liquid Chromatograph: The same HPLC and detector/integrator used for anthocyanidin analyses were used for anthocyanin analysis. Two chromatographic methods were evaluated for separation of anthocyanins in red raspberries. 1) Column: Supelcosil LC18 (5mm x 25 cm) 5 micron particle size with a Bio-Rad ODS-10 guard column. Solvent: A= 15% acetic acid (aq), B= 100% acetonitrile. Conditions: 0-5 minutes 100% A, 5-15 minutes 0-5% B linear gradient, followed by 5 minutes equilibration at starting conditions between injections. 2) Column: Polymer Labs PLRP-5 (5 mm x 25 cm) 5 micron particle size with a Polymer Labs PLRP guard column. Solvent: A= 4% phosphoric acid (aq), B= 100% acetonitrile. Conditions: 0-10 minutes isocratic at 6% B, 10-55 minutes 6-20% B linear gradient, 55-65 minutes isocratic at 20% B, followed by 10 minutes equilibration at starting conditions. 1.5 ml/min flow rate, 25 ul injection volume, detection at 520 nm, 20 nm bandwidth. UV-Vis spectra taken directly from chromatographic runs.

Statistical Analyses- Duplicate analyses of each measurement (total monomeric anthocyanins, percent polymeric color, anthocyanidins, and anthocyanins) were taken for the 46 database samples and 14 commercial juice concentrate samples. To establish the reproducibility of the analytical method, sample preparation for anthocyanin analysis was repeated five times on one of the samples (Table 7).

RESULTS AND DISCUSSION

ANTHOCYANINS

General- Six different cyanidin and pelargonidin pigments were separated and quantified in this study. They all have previously been reported by others to occur in red raspberries. Two chromatographic methods were evaluated for the separation of the anthocyanin pigments. The first method employed a polymer based reverse-phase (C18) column and a 4% phosphoric acid/ acetonitrile gradient elution program. This system had been previously used in our lab to sucessfully separate cranberry anthocyanins (Hong and Wrolstad, 1990). The second method used a silica based reverse-phase (C18) column and a 15% acetic acid/ acetonitrile solvent program (Rommel and Wrolstad, 1990). The first method was initially preferred, as the polymer column has a longer working life at the low pH necessary for analysing anthocyanins. Also, the phosphoric acid eluant is less corrosive to the HPLC system than the acetic acid. It was found, however, that the polymer column was not able to separate all of the pigments. Only four major peaks were produced with this system (see Figure 4). The pelargonidin pigments co-eluted with cyanidin pigments. The second method, using the silica based column and acetic acid mobile phase sucessfully separated all of the pigments (see Figure 5). Apparently the polymer column was not selective enough to separate the raspberry pigments, or the acetic acid acted as an organic modifier that allowed their separation. This problem was not encountered with cranberry anthocyanins using a polymer column. One must be careful when choosing a chromatographic system for separating anthocyanins in different fruits, as

there is the possibility that a system that works for one fruit may not work for another.

After it was determined that the HPLC system adequately separated the anthocyanins, repetitions of the samplepreparation and anthocyanin analysis was performed to determine the reproducibility of the method. a sample of Meeker variety juice (sample # 15) was used, as it has all six anthocyanins present. The results are summarised in Table 7. It was found that the greatest variation was in the CYD-3-SOP area, but was only about +/- 1% of the total peak area. This level of reproducibility was acceptable for use in this study.

Once an adequate HPLC analytical method was found, it was applied to all of the 46 database samples and 14 commercial samples. The results are summarized in Tables 2, 3, and 4. Peak identities in the chromatograms were made by comparison with others' results (Spanos and Wrolstad, 1987; Rommel and Wrolstad, 1990), co-elution of pigments of known composition from blackberry, and by UV-Visible spectra taken during the runs. The wavelength maximum in the visible region can be used to identify the anthocyanidin base of each peak, but not the sugars attatched (Hong and Wrolstad, 1990). These were identified by co-elution with blackberry anthocyanins.

Experimental Samples- The anthocyanin pigment profiles of the database samples fell into three general patterns. The first group is exemplified by Willamette variety raspberries (figure 6). They avaraged 81% cyanidin-3-sophoroside (CYD-3-SOP), 14% cyanidin-3-glucoside (CYD-3-GLU), 4% pelargonidin-3-sophoroside (PGD-3-SOP), and less than 1% each of cyanidin-3-glucosylrutinoside (CYD-3-GLURUT), cyanidin-3-rutinoside

(CYD-3-RUT), and pelargonidin-3-glucosylrutinoside (PGD-3-GLURUT). Other varieties in this general category were Malling Seedling, Malling Promise, Heritage, and Skeena. They all have CYD-3-SOP as the major pigment, and CYD-3-GLU as the next major pigment. The second group follow a pattern similiar to Meeker variety (Figure 5). The Meeker variety samples averaged 64% CYD-3-SOP, 13% CYD-3-GLURUT, 13% CYD-3-GLU, 4% CYD-3-RUT, 5% PGD-3-SOP, and 1% PGD-3-GLURUT. All varieties in this category have CYD-3-SOP as the major pigment, and lesser amounts of all of the other five pigments detected. Other varieties falling into this category are Chilcotin and Golden. The third group consists of varieties whose anthocyanin patterns do not fit into either of the other two groups. While in the first two groups CYD-3-SOP was the major pigment (over 50% of the total), the varieties in the third group did not show this characteristic. The samples falling into this group were the Polish varieties Veten and Norna. A chromatogram of Veten variety anthocyanins appears in Figure 7.

Two samples supplied by commercial packers in Oregon appeared to have been mislabeled as to the variety of fruit. One sample (#3) was labeled as Willamette variety, but had an anthocyanin distribution like Meeker variety fruits. Sample number 7 was labeled as a Meeker sample, but had a pigment distribution typical for a Willamette. They were obtained from different sources. These varieties are readily distinguished from each other by their pigment profiles. It was assumed that they were inadvertantly misidentified, and are listed in Tables 1, 2, and 3 by their apparent true cultivar names.

Commercial Samples- The analytical results of the commercial samples appear in Table 5. Two of the samples (Samples G and L, Table 5) were

found to contain delphinidin pigments. They also had small amounts of CYD-3-SOP and CYD-3-GLU. This suggests that they do have some red raspberry juice in them, but in low amounts. They are high in polymeric color and low in anthocyanin content, indicating degradation of pigments, possibly caused by poor processing or storage abuse. The presence of foreign anthocyanins indicates adulteration with other anthocyanin containing juices or colorants.

Samples H, K, and M (Table 5) had unusually high percentages of CYD-3-GLU, low anthocyanin content, and high polymeric color. The anthocyanidin profiles for these samples were within the range found in the experimental samples. Based on the pigment results they appear to be authentic, but of poor quality. A study done on the pigment changes on storage of red raspberry juice concentrates (Withy, 1991) showed increases in CYD-3-GLU percentage and polymeric color, and decreases in anthocyanin content when juice concentrates were stored for three months at 20°C. The pigment analysis results for samples H, K, and M could be explained as storage abused samples.

The remaining nine commercial samples were found to contain only pigments known to occur in red raspberries. They all had anthocyanin profiles similiar to Meeker variety raspberries, having CYD-3-SOP as the major pigment, and greated than 3% CYD-3-GLURUT. These samples all appear to be authentic red raspberry juice concentrates based on these results.

ANTHOCYANIDINS

General- Anthocyanin extracts from each of the samples were hydrolysed to determine the anthocyanidins present. The individual results are shown in

Tables 2-4. All previous studies on red raspberries detected only cyanidin, and small amounts of pelargonidin (Spanos and Wrolstad, 1987; Barrit and Torre, 1975; Misic, 1972; Francis, 1972; Barritt and Torre, 1973). Only six anthocyanidins are known to occur in most fruits (Figure 4). Peak assignments in the anthocyanidin analyses were made by comparison with Concord grape and strawberry anthocyanidins. Concord grape contains five of the six anthocyanidins (no pelargonidin), and strawberry has only pelargonidin and cyanidin. The individual anthocyanidins were identified by their retention times and their visible wavelength absorbance maxima (Hong and Wrolstad, 1990).

All of the samples were found to contain both cyanidin and pelargonidin. When the same samples were analysed for anthocyanins using the polymer based HPLC column and phosphoric acid eluant, no pelargonidin pigments were isolated. This led to the investigation of other HPLC methods. It was determined that the pelargonidin pigments were co-eluting with cyanidin compounds with close retention times. The silica column and acetic acid eluant system was found to resolve all of the pigments.

Experimental Samples- The results of the anthocyanidin analyses showed a range of 90-97% cyanidin, and 3-10% pelargonidin. No other anthocyanidins were found in any of the samples. The different cultivars had varying amounts of pelargonidin. A summary of the ranges of pelargonidin content by cultivar appears in Table 6. The cultivars with the highest pelargonidin content were Meeker and Malling Seedling, with 7.1% pelargonidin (excluding the moldy sample). The variety with the lowest pelargonidin content was Skeena, which had 2.4% . A study by Spanos and

Wrolstad (1987) found higher pelargonidin content in Meeker than in Willamette, and found low pelargonidin levels in Skeena variety.

The sample of juice made from moldy Meeker variety berries had the highest percentage of pelargonidin, 9.5%. It had a low monomeric pigment content (56 mg/l) and was low in soluble solids content (2.5°brix) indicating that considerable fermentation had taken place. Molds are known to contain enzymes capable of degrading anthocyanins through hydrolysis of the sugar groups (Blom, 1983; Jiang *et al*, 1990). This sample also had the highest percentage of pelargonidin-3-sophoroside of all the samples. It may be due to a higher resistance to degradation for pelargonidin pigments than for corresponding cyanidin pigments. It would be worth investigating whether the high pelargonidin content is caused by mold contamination and could be used as a quality indicator in commercial raspberry products.

The sample showing the lowest concentration of pelargonidin was the juice made from Skeena variety fruits grown in Canada, which had 2.4% pelargonidin. A study done in our lab by Spanos and Wrolstad (1987) found only trace amounts of pelargonidin (less than 2%) in a sample of juice made from Skeena variety fruit. In the same study they found between 96 and 98% cyanidin in samples of Meeker (n=3) and Willamette (n=2) juices. These values are somewhat higher than the levels found for these varieties in this project (91-93% for Meeker, n=7, and 93-96% for Willamette, n=21). The same hydrolysis and extraction procedure was used in both studies, but different HPLC methods were used. This could account for some of the differences found. Anthocyanidin extracts are also very unstable to light and heat, and differences in sample handling could affect the results.

The percentages of cyanidin and pelargonidin may not in themselves prove the authenticity of a raspberry juice sample, but samples containing

large amounts of pelargonidin may indicate mold degradation or storage abuse. None of the database samples were without pelargonidin, so very low or no pelargonidin could indicate the addition of other colorants. Anthocyanin colorants containing cyanidin pigments are commercially available, but they generally lack pelargonidin (Hong and Wrolstad, 1990).

Commercial Samples- Two of the commercial samples analysed were found to contain large amounts of delphinidin (see Figure 8). Delphinidin has not been reported to occur in red raspberries of any variety. Both samples appear to have some raspberry pigments in them, but in low amounts. The presence of anthocyanidins other than cyanidin and pelargonidin are strong evidence of adulteration with delphinidin containing fruits or colorants, and have been labeled as adulterated in Table 4. The other commercial samples had only cyanidin (93-97%) and pelargonidin (3-7%). Based on these results, they do not appear to have other fruits or colorants added.

TOTAL ANTHOCYANIN CONTENT

The total anthocyanin content found in these samples varied from 1101 mg/l for one Willamette sample, to 4 mg/l for a sample of Golden variety (calculated as cyanidin-3-glucoside). The pigment concentrations were calculated as cyanidin-3-glucoside because of the lack of a molar extinction coefficient for cyanidin-3-sophoroside, the major pigment in most red raspberry varieties. Total anthocyanin content values were normalized to 10° brix in all samples. The pigment concentrations were found to be, on average, highest in Willamette variety. The average for the 21 Willamette samples was 624 mg/l. This level is about 2-5 times higher than the levels found in the other red raspberry varieties studied (excluding Golden variety).

This agrees with results reported by other researchers (Barritt and Torre, 1975; Torre and Barritt, 1977; Spanos and Wrolstad, 1987). Fruits with a high pigment content are very desirable for making juices, as color level is probably the single most important quality factor in a red raspberry juice.

Samples of juices from ripe Willamette variety fruits grown in British Columbia, Canada and in Oregon, USA showed essentially the same total pigment concentration . The total amount of pigments were found to increase with degree of maturity, as seen in the 21 Willamette samples (Table 2). This effect of ripening has been shown by others in red raspberries and blackberries (Sapers, *et al* 1986; Sagi, 1974). This was not as evident in the eight samples of Meeker variety. This is probably due to the smaller number of samples of different maturity levels in this subset, and the subjectivity in grading the maturity level by different people, as these samples came from different sources.

PROCESSING METHODS

Standard vs. Laboratory Simulated Standard Processing- Samples 34A and 34B were prepared using the "standard" processing method outlined in Figure 1 in our pilot plant. Samples 34C and 34D were prepared using a simulated standard method in the laboratory. All four were made from the same batch of ripe Willamette fruits. The simulated standard method was used to prepare juices from batches of fruits too small to be processed using the equipment in our pilot plant. Both methods produced juices with typical anthocyanin concentrations (for Willamette variety) and low percentages of polymeric color. The juices prepared using the laboratory method had lower percentages of CYD-3-GLU than those prepared using the standard method. The juices prepared using the standard method had about 15% CYD-3-

GLU, and the simulated standard processed juices had about 10% CYD-3-GLU.

Enzymic Liquefaction and Centrifugation- One batch of Willamette variety fruits was used to observe the effects of enzymes on the content and distribution of anthocyanins in juices. The samples were prepared by enzymic liquefaction of the fruits followed by centrifugation of the resulting mash (see Figure 2). Two commercial enzyme preparations were used, Pectinex Ultra SP, and Pectinex BE (Novo, Switzerland). A control was also prepared using the same processing method, but without the addition of enzymes. The two juices prepared using enzymes showed 10 to 20% higher total anthocyanin content than in the control. Prepress enzymes are used to increase the yield of both free run juice and pigment content.

The sample prepared using Pectinex BE did not differ in pigment distribution, as compared to the control sample. However, this sample showed a lower percentage of polymerized pigments. The control and Pectinex Ultra SP treated samples both showed about 25% polymeric color. This is a relatively high level for a freshly prepared juice. The polymeric color level of a freshly prepared Willamette variety juice should be around 10% or less, as seen in Table 2. Since the control and one of the enzyme treated samples had high polymeric color, it seems that the juice making process itself produced the polymeric color, and that the Pectinex BE enzyme preparation had some kind of protective effect on the anthocyanins.

The sample prepared using Pectinex Ultra SP showed a 3% decrease in CYD-3-SOP, and a 3% increase in CYD-3-GLU. In a study by Jiang *et al* (1990), red raspberries were treated with pectolytic enzymes and the changes in anthocyanin concentrations and distributions were followed. One

enzyme preparation (Pectinex Ultra SP-L) was found to cause decreases in CYD-3-SOP and CYD-3-GLURUT, and corresponding increases in CYD-3-GLU and CYD-3-RUT. This was attributed to beta-glycosidase activity that converted sophorosides to glucosides and glucosylrutinosides to rutinosides by cleavage of a beta-1-2-glucosidic bond. This effect could account for the changes in the sample treated with Pectinex Ultra SP. Under the conditions used to prepare our juice samples (0.02% enzyme for 3 hours at 40°C) the changes observed would not affect the overall juice quality. In some previous studies (Jiang *et al*, 1990, Tranchev *et al*, 1969), higher enzyme concentrations, higher temperatures, and longer incubation times were used, and greater levels of pigment destruction were observed. The increases in juice and pigment yield obtained by enzymic liquefaction probably outweigh the slight pigment changes observed.

Diffusion Extraction- One sample was prepared using a commercial diffusion extraction system (Figure 3). The resulting juice had a low CYD-3-GLU content compared to the three other samples prepared from the same batch of fruit (samples 35A, 35B, and 35C). It also had a fairly high percentage of polymeric color (30%) and a high monomeric anthocyanin concentration. The high polymeric color and low concentration of CYD-3-GLU can be attributed to the use of hot water to extract the juice. Cyd-3-GLU has been shown to be the most unstable anthocyanin in red raspberries (Withy et al, 1991; Rommel et al, 1990). Also, this juice making method extracts more phenolic compounds (Rommel and Wrolstad, 1991), which may contribute to polymerization of the anthocyanin pigments. Juices produced commercially using this method (apple, pear) require concentration, as they are diluted by the water used to extract the juice. This

was not done so as to determine the effect of this type of processing on the pigment composition of the juice, separate from the effects of juice concentrating.

Effect of Osmotic vs. Vacuum Concentration- A batch of juice prepared using the standard processing method with ripe Willamette berries was divided into five portions. Three were concentrated using a direct osmosis concentrating unit, and one was concentrated using a commercial vacuum concentrator. The last portion was left unconcentrated to serve as a control to monitor the effects of the concentrating methods. Samples 37A and 37B were concentrated using the same osmotic membrane, but at two different temperatures and times. Sample 37A was concentrated at 8°C for 10.3 hours to 44.8°brix. Sample 37B was run for 5.8 hours at 26°C to a final concentration of 43.5°brix. The sample run at 26°C produced a juice with a higher anthocyanin content, but the same pigment distribution and percent of polymeric color as the one run at the lower temperature. Both samples were found to have lower percentages of CYD-3-GLU and higher percentages of CYD-3-SOP than the control sample. CYD-3-GLU has been shown to be the least stable of the red raspberry anthocyanins (Rommel, 1990). All of the samples that were concentrated had less CYD-3-GLU than the control, indicating that some pigment degradation had occurred. Sample 37C was osmotically concentrated on a different membrane for 5.8 hours at 26°C to a concentration of 45.5°brix. It had a CYD-3-GLU content close to that of the control sample, indicating the lowest level of pigment degradation of any of the concentration methods studied. It had a pigment distribution and percent polymeric color that matched the control sample, but with a lower pigment concentration. The sample that was vacuum concentrated

produced a juice that was identical to sample 37A (osmotically concentrated at 8°C) in all the parameters measured.

The results of this portion of this study show that osmotic concentration of red raspberry juice produces a concentrate similiar in all the measured characteristics to conventionally produced juice concentrates. A study on the sensory characteristics of these samples showed them to be essentially of the same flavor quality as juices prepared by standard methods (Wrolstad et al, 1991). Other factors such stability or processing costs may determine if there is any advantage to using osmotic concentration methods for red raspberry juice.

Effect of Mold on Pigments- A juice sample was made from a batch of ripe, moldy Meeker variety fruits. It was found to contain a very low pigment concentration (64 mg/l) and a low sugar content (2.5° brix). It also had a fairly high percentage of polymerized pigments (26%). It had higher concentrations of CYD-3-GLURUT and PGD-3-SOP than other Meeker juices in this study. It was also found to have the highest pelargonidin content of all the samples. Enzymes capable of degrading anthocyanins are known to occur in molds and in commercial enzyme preparations made from molds (Jiang et al 1990; Blom, 1983).

CONCLUSIONS

The results of the pigment analyses of the experimental samples show that there is conciderable variation in both the total anthocyanin content and in the anthocyanin distributions among the different cultivars. Only six anthocyanins and two anthocyanidins were found in any one of the samples, which makes them useful for determining the authenticity of red raspberry

products. The presence of other anthocyanins or anthocyanidins are strong evidence of adulteration with other fruits. While processing methods can alter the distribution of pigments in red raspberry juice, none of the methods studied here affected the pigments enough to mask their identity as authentic red raspberry juices.

Low total anthocyanin content, high percentages of polymeric color, and elevated levels of CYD-3-GLU are all indicators of degradation due to processing or storage. Mold acting on fruits destroyed most of the pigments, and appeared to degrade the cyanidin pigments more than the pelargonidin pigments. It would be of value to further investigate the effects of mold on red raspberry juice for use in the evaluation of commercial red raspberry products for quality.

STANDARD JUICE PROCESSING

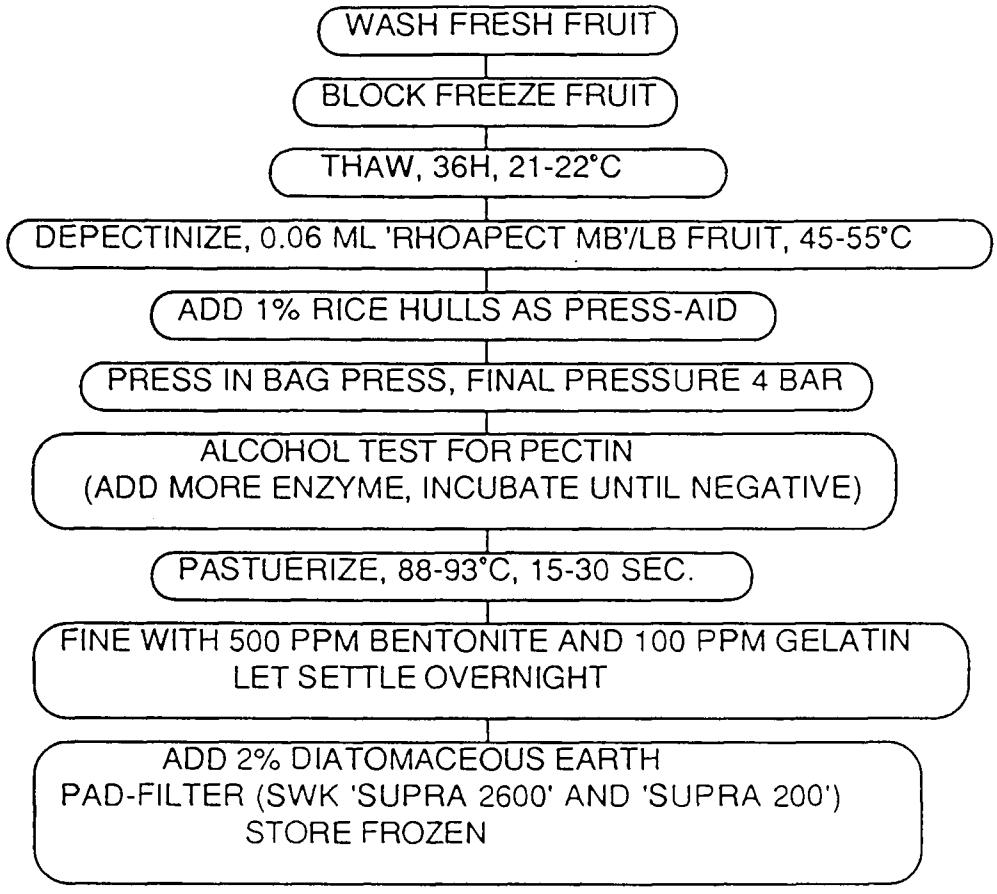

Figure 1. Flowchart of standard juice making process.

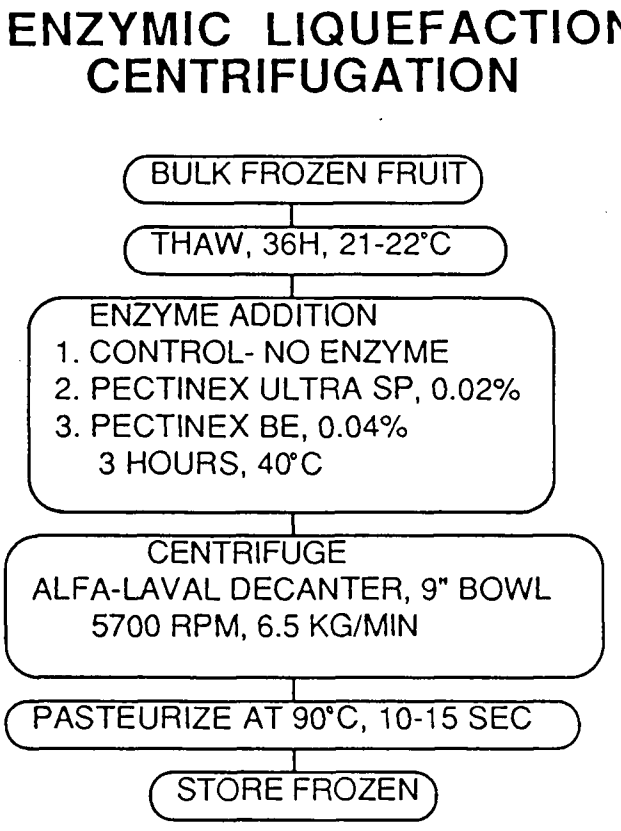

Figure 2. Flowchart of enzymic liquefaction/centrifugation process.

DIFFUSION EXTRACTION

Figure 3. Flowchart of diffusion extraction process.

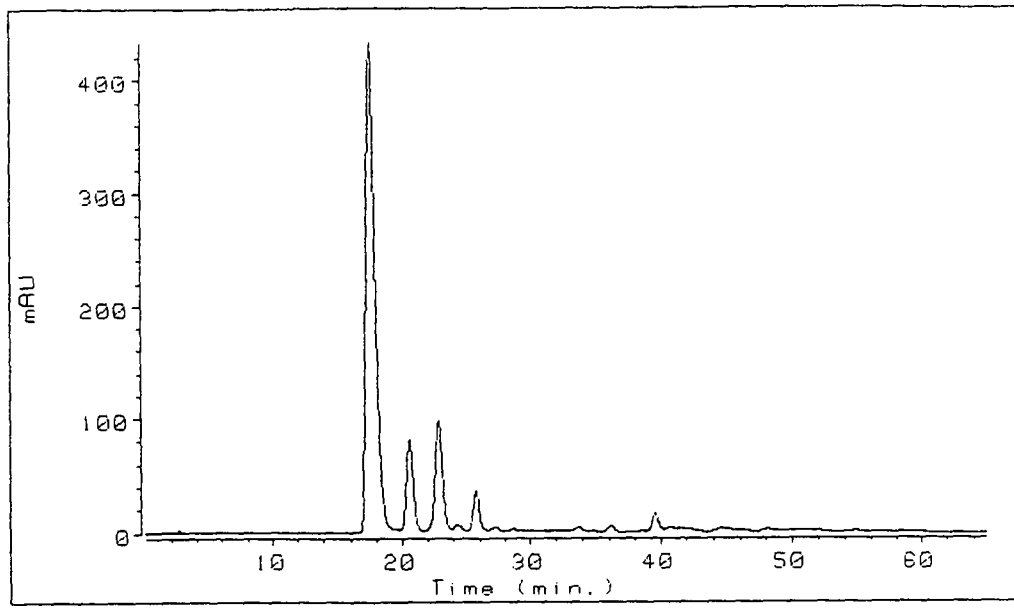

Figure 4. HPLC chromatogram of Meeker anthocyanins using polymer column/ phosphoric acid mobile phase

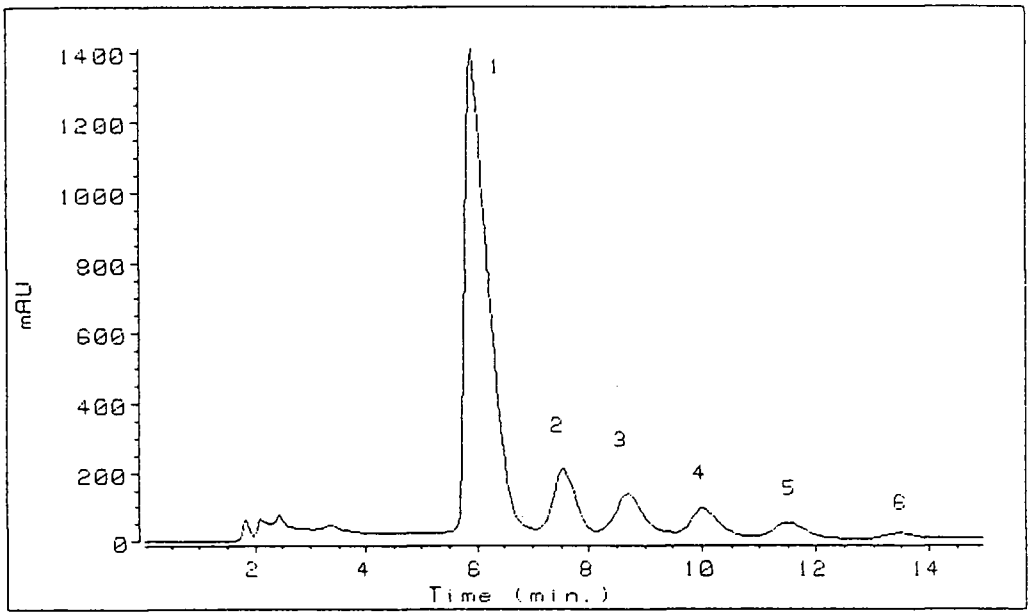

Figure 5. HPLC chromatogram of Meeker anthocyanins using silica column/acetic acid mobile phase

1. Cyanidin-3-sophoroside
2. Cyanidin-3-glucosylrutinoside
3. Cyanidin-3-glucoside
4. Pelargonidin-3-sophoroside
5. Cyanidin-3-rutinoside
6. Pelargonidin-3-glucosylrutinoside

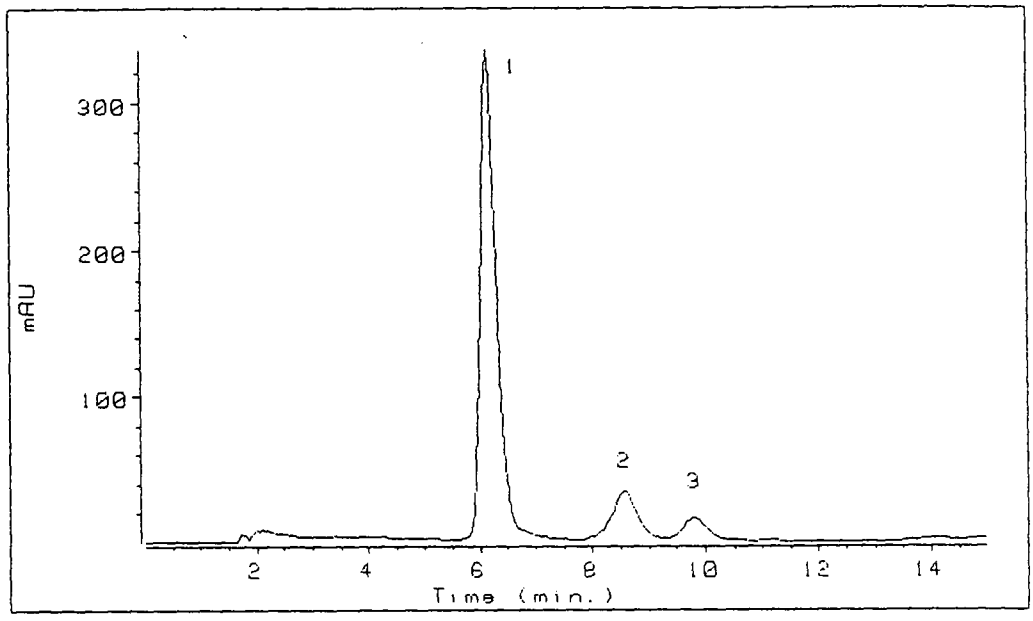

Figure 6. HPLC chromatogram of Willamette anthocyanins

1. Cyanidin-3-sophoroside
2. Cyanidin-3-glucoside
3. Pelargonidin-3-sophoroside

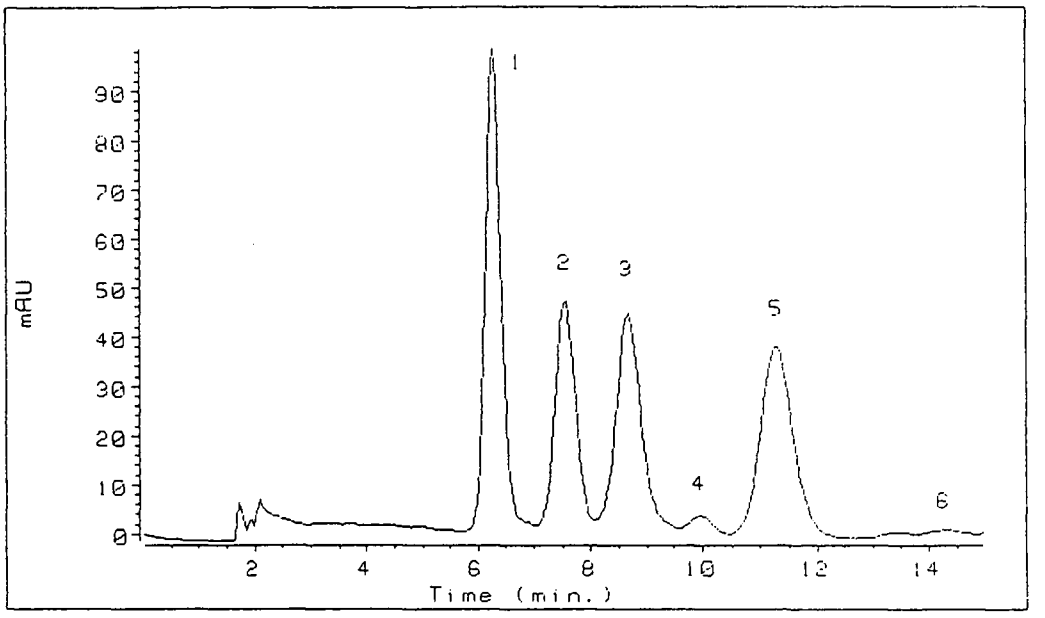

Figure 7. HPLC chromatogram of Veten anthocyanins

1. Cyanidin-3-sophoroside
2. Cyanidin-3-glucosylrutinoside
3. Cyanidin-3-glucoside
4. Pelargonidin-3-sophoroside
5. Cyanidin-3-rutinoside
6. Pelargonidin-3-glucosylrutinoside

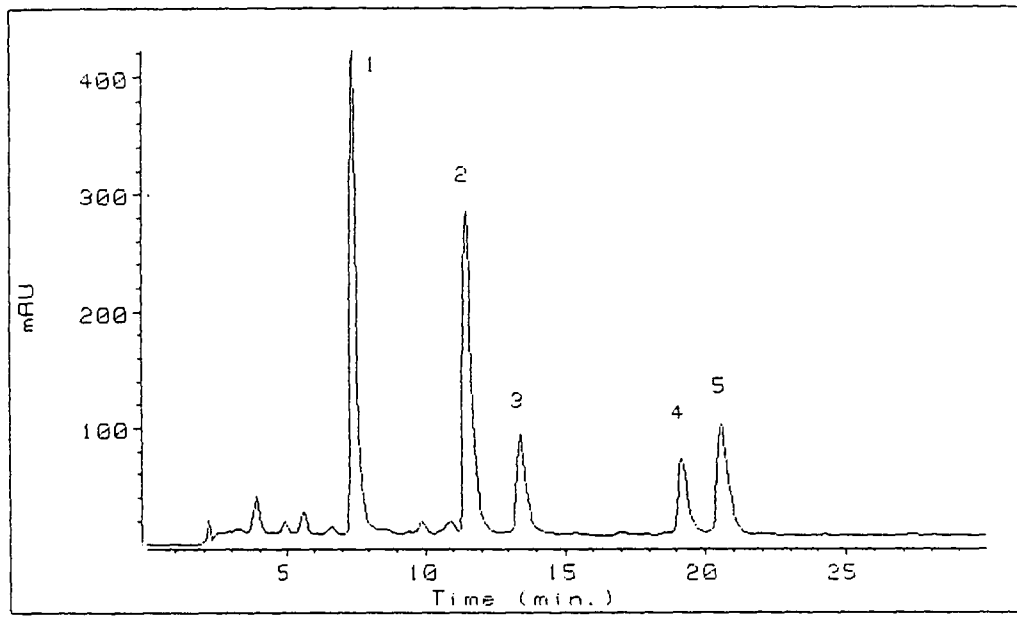

Figure 8. HPLC cromatogram of grape anthocyanidins

1. Delphinidin
2. Cyanidin
3. Petunidin
4. Peonidin
5. Malvidin

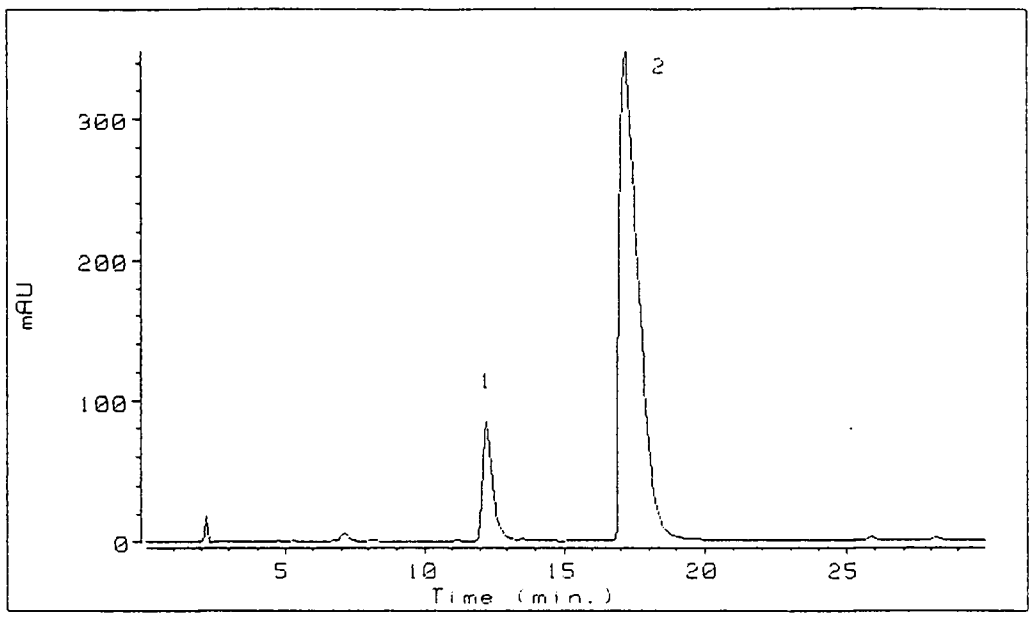

Figure 9. HPLC chromatogram of strawberry anthocyanidins

1. Cyanidin
2. Pelargonidin

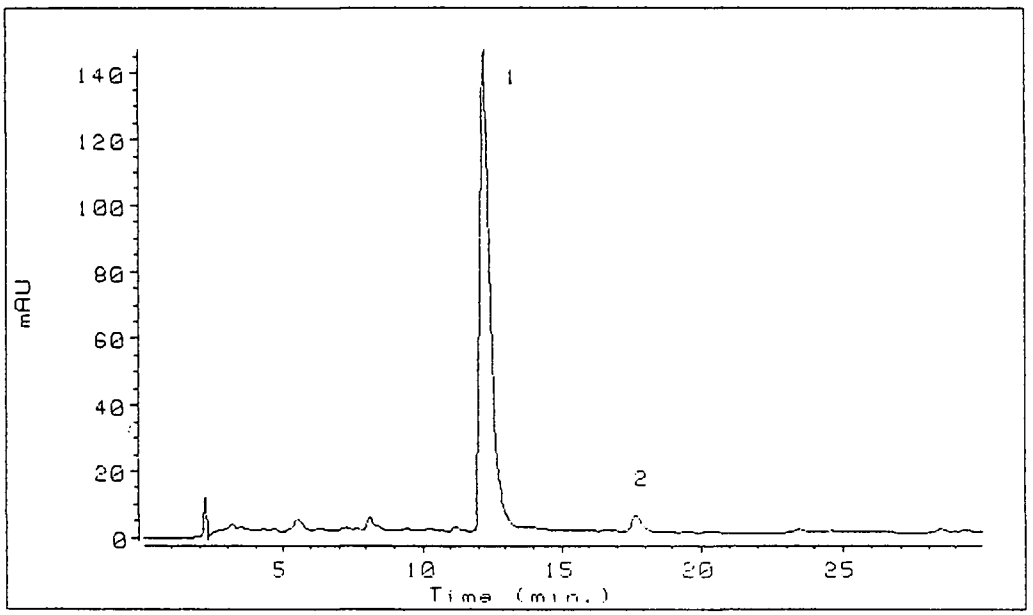

Figure 10. HPLC chromatogram of Meeker red raspberry anthocyanidins

1. Cyanidin
2. Pelargonidin

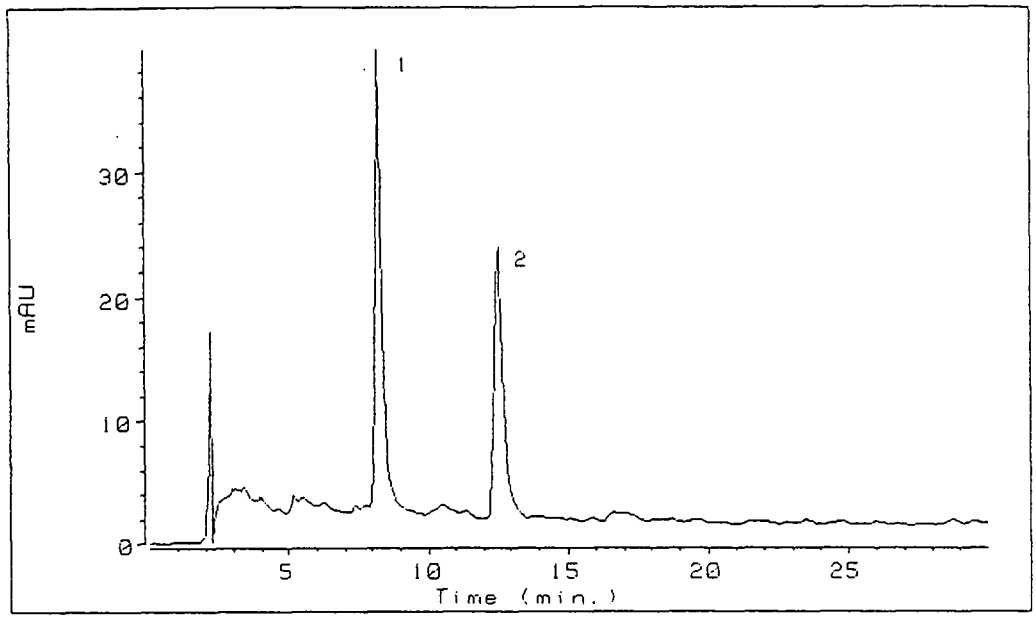

Figure 11. HPLC chromatogram of commercial sample "L" anthocyanidins

1. Delphinidin
2. Cyanidin

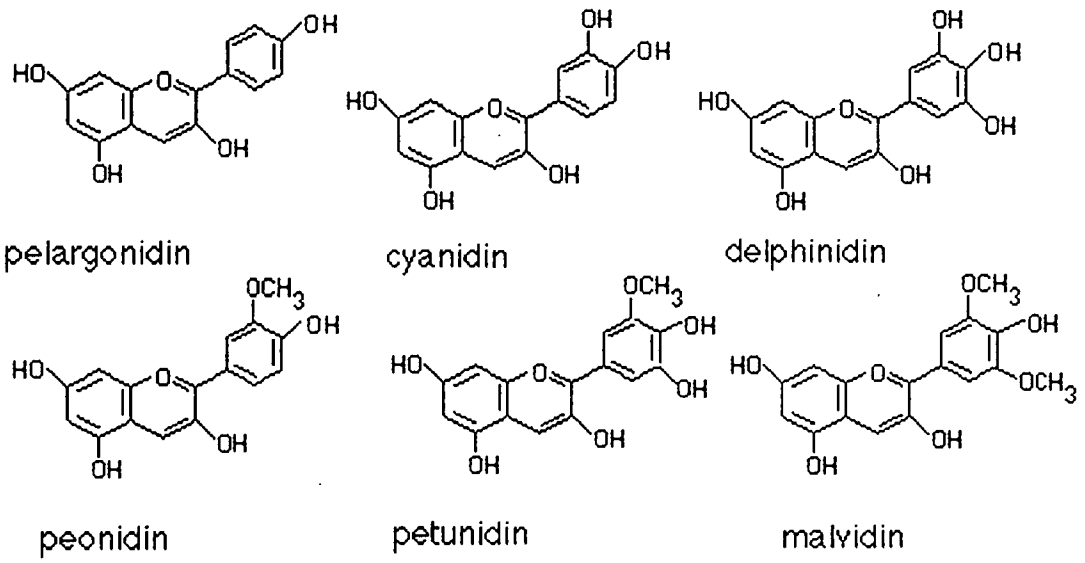

Figure 12. The six common anthocyanidins

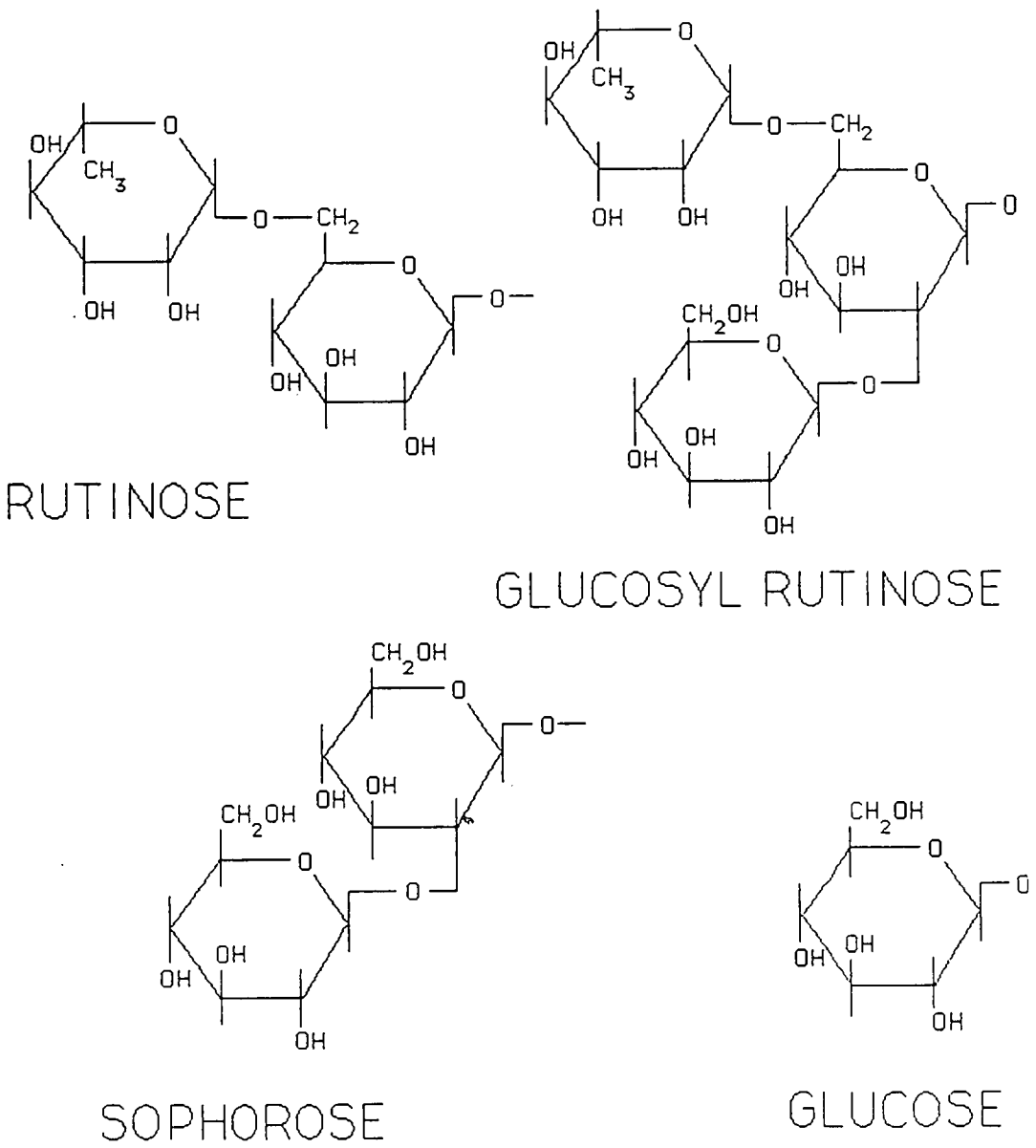

Figure 13. Sugars occuring in red raspberry anthocyanins

Table 1. Description of samples

SAM-PLE#	VARIETY	ORIGIN	PICKING DATE	HARVESTING METHOD	MATURITY	PROCESSING METHOD (1)	DEGREE BRIX
18	WILLAMETTE	OREGON	22-Jul-88	HAND	UNDERRIPE	A	8.4
20	WILLAMETTE	OREGON	1-Jul-88	HAND	UNDERRIPE	A	9.0
34A	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	A	9.1
34B	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	A	8.0
34C	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	B	9.2
34D	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	B	10.0
35A	WILLAMETTE	B.C. CANADA	20-Jul-88	MACHINE	RIPE	C	11.9
35B	WILLAMETTE	B.C. CANADA	20-Jul-88	MACHINE	RIPE	D	12.4
35C	WILLAMETTE	B.C. CANADA	20-Jul-88	MACHINE	RIPE	D	12.2
36	WILLAMETTE	B.C. CANADA	20-Jul-88	MACHINE	RIPE	E	7.6
37A	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	F	44.8
37B	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	F	43.5
37C	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	F	45.5
37D	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	A	12.2
37E	WILLAMETTE	OREGON	19-Jun-90	HAND	RIPE	A	43.5
19	WILLAMETTE	OREGON	1-Jul-88	HAND	OVERRIPE	A	8.0
14	WILLAMETTE	OREGON	22-Jul-88	HAND	OVERRIPE	A	9.2
4	WILLAMETTE	OREGON	8-Jul-88	MACHINE	OVERRIPE	A	9.8
6	WILLAMETTE	OREGON	11-Jul-88	HAND	OVERRIPE	A	10.5
9	WILLAMETTE	OREGON	21-Jul-88	MACHINE	OVERRIPE	A	13.0
7	WILLAMETTE	OREGON	21-Jul-88	MACHINE	OVERRIPE	A	13.4
16	MEEKER	OREGON	21-Jul-88	MACHINE	UNDERRIPE	A	9.8
8	MEEKER	OREGON	11-Jul-88	HAND	UNDERRIPE	A	8.5
11	MEEKER	OREGON	12-Jul-88	MACHINE	RIPE	A	7.5
10	MEEKER	OREGON	23-Jul-88	HAND	OVERRIPE	A	13.0
15	MEEKER	OREGON	11-Jul-88	HAND	OVERRIPE	A	10.0
3	MEEKER	OREGON	23-Jul-88	MACHINE	OVERRIPE	A	13.0
5	MEEKER	OREGON	8-Jul-88	MACHINE	OVERRIPE	A	10.2
17	MEEKER	OREGON	12-Jul-88	HAND	RIPE, MOLDY	A	2.5
12	VETEN	POLAND	6-Jul-88	HAND	RIPE	B	8.5
23	VETEN	POLAND	8-Jul-88	HAND	RIPE	B	8.8
25	VETEN	POLAND	11-Jul-88	HAND	RIPE	B	9.8
21	NORNA	POLAND	11-Jul-88	HAND	RIPE	B	7.9
22	NORNA	POLAND	8-Jul-88	HAND	RIPE	B	9.0
26	NORNA	POLAND	6-Jul-88	HAND	RIPE	B	9.0
13	MALLING SEEDLING	POLAND	6-Jul-88	HAND	RIPE	B	8.5

Table 1 Continued

SAM-PLE#	VARIETY	ORIGIN	PICKING DATE	HARVESTING METHOD	MATURITY	PROCESSING METHOD (1)	DEGREE BRIX
27	MALLING SEEDLING	POLAND	11-Jul-88	HAND	RIPE	B	8.5
28	MALLING SEEDLING	POLAND	8-Jul-88	HAND	RIPE	B	7.0
24	MALLING PROMISE	POLAND	11-Jul-88	HAND	RIPE	B	11.0
29	MALLING PROMISE	POLAND	8-Jul-88	HAND	RIPE	B	8.0
30	MALLING PROMISE	POLAND	6-Jul-88	HAND	RIPE	B	9.4
31	HERITAGE	OREGON	23-Sep-89	HAND	RIPE	B	15.0
32	HERITAGE	OREGON	23-Sep-89	HAND	RIPE	B	13.2
33	GOLDEN	OREGON	1-Jul-89	HAND	RIPE	B	11.3
1	CHILCOTIN	B.C. CANADA	20-Jul-88	MACHINE	RIPE	A	9.4
2	SKEENA	B.C. CANADA	20-Jul-88	MACHINE	RIPE	A	9.0

(1) A= Standard processing
B= Simulated standard processing
C= Pressing + centrifugation
D= Enzymic liquifaction + centrifugation
E= Diffusion extraction
F= Osmotic concentration

Table 2. Willamette sample results

		ANTHOCYANINS (% by peak area)						ANTHOCYANIDINS (% by peak area)					
SAM-PLE#	MATURITY LEVEL	CYD-3-SOPH	CYD-3-GLURUT	CYD-3-GLU	PGD-3-SOPH	CYD-3-RUT	PGD-3-GLURUT	CYANIDIN	PELAR-GONIDIN	CYD/PGD	BRIX	ANTHOCYANIN CONTENT*(1)	% POLYMERIC COLOR
18	UNDERRIPE	84.9	2.9	7.5	3.7	1.0	0.0	95.7	4.3	22.3	8.4	116	7
20	UNDERRIPE	86.8	1.0	7.8	3.8	0.4	0.2	95.7	4.3	22.3	9.0	513	6
34A	RIPE	82.3	1.0	14.1	2.8	0.5	0.1	95.4	4.6	20.6	9.1	461	7
34B	RIPE	79.1	1.0	16.0	2.7	0.8	0.4	95.4	4.6	20.6	8.0	682	0
34C	RIPE	83.8	1.5	10.7	3.0	0.6	0.5	95.3	4.7	20.3	9.2	533	6
34D	RIPE	85.3	0.9	10.1	3.1	0.3	0.3	94.9	5.1	18.5	10.0	573	6
35A	RIPE	77.4	0.0	17.6	4.2	0.3	0.0	93.8	6.2	15.1	11.9	564	24
35B	RIPE	73.7	0.0	20.7	3.9	0.4	1.3	94.0	6.0	15.6	12.4	621	25
35C	RIPE	76.9	0.0	17.4	4.6	0.4	0.8	94.2	5.8	16.3	12.2	676	8
36	RIPE	86.4	1.6	7.2	4.2	0.4	0.3	93.7	6.3	14.8	7.6	700	30
37A	RIPE	78.3	1.6	16.1	3.1	0.5	0.4	94.8	5.2	18.1	44.8	720	4
37B	RIPE	77.6	1.5	14.4	2.5	0.5	0.5	94.2	5.8	16.2	43.5	816	0
37C	RIPE	75.4	1.5	19.4	2.6	0.7	0.4	94.9	5.1	18.5	45.5	636	4
37D	RIPE	74.2	1.5	20.3	2.6	0.7	0.7	94.6	5.4	17.5	10.8	726	3
37E	RIPE	78.8	1.4	15.7	3.1	0.5	0.5	94.6	5.4	17.5	43.5	716	3
19	OVERRIPE	85.7	0.0	10.9	3.2	0.0	0.2	94.4	5.6	16.8	8.0	1101	7
14	OVERRIPE	84.0	0.0	10.0	5.7	0.3	0.0	92.9	7.1	13.1	9.2	646	6
4	OVERRIPE	84.0	0.0	9.5	5.5	0.5	0.2	94.0	6.0	15.7	9.8	613	4
6	OVERRIPE	83.1	0.6	10.9	5.1	0.4	0.0	93.5	6.5	14.3	10.5	640	6
9	OVERRIPE	78.2	0.0	14.3	6.7	0.6	0.1	93.5	6.5	14.3	13.0	421	10
7	OVERRIPE	78.5	0.7	12.3	5.7	1.1	1.7	93.6	6.4	14.6	13.4	553	4
	Minimum	73.7	0.0	7.2	2.5	0.0	0.0	92.9	4.3	13.1	8.0	116	
	Maximum	86.8	2.9	20.7	6.7	1.1	1.7	95.7	7.1	22.3	13.4	1101	
	Mean	80.7	0.9	13.5	3.9	0.5	0.4	94.4	5.6	17.3	10.3	620	
	Std. Dev.	4.2	0.8	4.2	1.2	0.2	0.4	0.8	0.8	2.7	1.8	182	

Table 3. Meeker sample results

		ANTHOCYANINS (% by peak area)						ANTHOCYANIDINS (% by peak area)					
SAM-PLE#	MATURITY LEVEL	CYD-3-SOPH	CYD-3-GLURUT	CYD-3-GLU	PGD-3-SOPH	CYD-3-RUT	PGD-3-GLURUT	CYANIDIN	PELAR-GONIDIN	CYD/PGD	BRIX	ANTHOCYANIN CONTENT*(1)	% POLYMERIC COLOR
16	UNDERRIPE	67.7	12.8	9.0	5.9	3.9	0.7	92.5	7.5	12.3	9.8	281	12
8	UNDERRIPE	68.1	11.7	9.9	5.0	3.7	1.6	93.0	7.0	13.4	8.5	313	15
11	RIPE	60.9	11.3	16.2	3.7	6.2	0.5	93.0	7.0	13.4	7.5	511	10
10	OVERRIPE	50.1	13.2	23.9	3.8	7.3	1.7	93.4	6.6	14.1	13.0	241	16
15	OVERRIPE	70.9	10.5	8.9	5.6	3.3	0.8	92.2	7.8	11.8	10.0	364	19
3	OVERRIPE	65.9	10.2	15.0	3.9	4.3	0.7	93.8	6.2	15.1	13.0	246	18
5	OVERRIPE	65.1	12.9	11.3	4.9	4.4	1.4	93.0	7.0	13.3	10.2	284	15
17	RIPE,MOLDY	62.4	18.7	11.6	7.4	0.0	0.0	90.5	9.5	9.5	2.5	56	26
	Minimum*(2)	50.1	10.2	8.9	3.7	3.3	0.5	92.2	6.2	11.8	7.5	241	
	Maximum	70.9	13.2	23.9	5.9	7.3	1.7	93.8	7.8	15.1	13.0	511	
	Mean	64.1	11.8	13.5	4.7	4.7	1.1	93.0	7.0	13.3	10.3	320	
	Std. Dev.	6.9	1.2	5.4	0.9	1.5	0.5	0.5	0.5	1.1	2.1	94	

*(1) mg/L CYD-3-GLU, normalized to 10 brix

*(2) statistical summary excludes moldy sample (#17)

Table 4. Other variety sample results

		ANTHOCYANINS (% by peak area)						ANTHOCYANIDINS (% by peak area)					
SAM-PLE #	VARIETY	CYD-3-SOPH	CYD-3-GLURUT	CYD-3-GLU	PGD-3-SOPH	CYD-3-RUT	PGD-3-GLURUT	CYANIDIN	PELAR-GONIDIN	CYD/PGD	BRIX	ANTHOCYANIN CONTENT*(1)	% POLYMERIC COLOR
12	VETEN	24.0	17.5	34.5	2.1	21.9	0.0	97.2	2.9	34.0	8.5	219	36
23	VETEN	32.7	18.9	26.5	2.5	19.0	0.6	97.1	2.9	33.8	8.8	202	36
25	VETEN	39.4	21.1	21.1	2.1	15.4	0.5	96.8	3.2	30.7	9.8	228	21
21	NORNA	31.0	19.1	22.9	2.3	23.7	0.4	96.8	3.2	30.2	7.9	177	53
22	NORNA	23.5	21.7	20.2	2.2	32.1	0.4	96.5	3.6	27.1	9.0	135	45
26	NORNA	24.5	23.6	17.6	2.3	31.5	0.5	97.2	2.9	34.1	9.0	217	19
13	MALLING	63.3	4.0	29.4	3.2	0.0	0.0	93.7	6.5	14.4	8.5	110	67
27	SEEDLING	81.6	0.0	11.1	5.6	0.0	1.7	92.3	7.7	11.9	8.5	148	17
28	"	81.0	0.0	13.2	5.5	0.0	0.3	92.8	7.2	12.9	7.0	166	18
24	MALLING	80.4	0.0	17.0	2.6	0.0	0.0	96.5	3.5	27.4	11.0	72	57
29	PROMISE	78.6	0.0	18.6	2.4	0.0	0.3	96.5	3.5	27.6	8.0	135	32
30	"	75.4	3.0	17.5	3.3	0.8	0.0	96.6	3.4	28.2	9.4	154	25
31	HERITAGE	69.8	0.0	24.0	4.7	0.3	1.1	94.1	5.9	16.0	15.0	308	19
32	HERITAGE	71.4	0.0	22.9	4.8	0.3	0.7	94.0	6.0	15.7	13.2	296	18
33	GOLDEN	62.6	9.1	17.5	0.0	10.8	0.0	93.6	6.5	14.5	11.3	4	43
1	CHILCOTIN	58.8	21.4	11.6	2.4	4.7	1.1	96.3	3.7	25.9	9.4	208	23
2	SKEENA	77.6	0.0	18.0	2.8	1.4	0.3	97.6	2.4	40.8	9.0	244	13

*(1) mg/L CYD-3-GLU, normalized to 10 brix

Table 5. Commercial sample results

	ANTHOCYANINS (% by peak area)						ANTHOCYANIDINS (% by peak area)					
SAMPLE CODE	CYD-3-SOPH	CYD-3-GLURUT	CYD-3-GLU	PGD-3-SOPH	CYD-3-RUT	PGD-3-GLURUT	CYANIDIN	PELAR-GONIDIN	CYD/PGD	BRIX	% POLYMERIC COLOR	ANTHOCYANIN CONTENT*(1)
A	72.5	8.0	13.0	4.6	1.4	0.5	94.5	5.5	17.1	45	43	125
B	62.3	16.5	13.1	3.0	4.0	1.0	95.3	4.7	20.4	65	21	249
C	76.7	4.6	12.1	5.2	1.0	0.4	93.3	6.7	13.9	45	21	501
D	77.1	3.6	13.0	5.4	0.9	0.2	93.6	6.5	14.5	45	21	510
E	64.4	12.0	14.1	4.8	3.8	0.9	93.2	6.8	13.6	45	6	284
F	73.4	6.1	14.6	4.3	1.5	0.1	94.1	5.9	16.1	45	9	412
G	10.0	0.5	25.5	0.0	0.0	0.0	46.6	0.0	*(2)	65	47	35
H	43.5	3.3	49.8	3.5	0.0	0.0	94.7	5.3	17.9	66	50	42
I	66.6	4.4	19.9	5.0	3.2	0.9	93.4	6.6	14.1	65	32	100
J	75.1	5.3	13.4	4.3	1.7	0.2	94.5	5.6	17.0	39	29	383
K	45.7	8.6	32.9	4.8	6.5	1.6	97.1	2.9	33.2	65	63	33
L	18.2	0.0	28.0	0.0	0.0	0.0	42.2	0.0	*(2)	66	70	4
M	34.6	5.8	50.4	4.1	4.5	0.6	96.3	3.7	25.8	60	53	62
N	73.2	5.9	13.8	4.9	1.8	0.4	93.4	6.6	14.1	63	18	243

*(1) mg/L CYD-3-GLU, normalized to 10 brix

*(2) contains other anthocyanidins

Table 6. Anthocyanidin and anthocyanin results summary by cultivar

CULTIVAR	# SAMPLES	% PELARGONIDIN		ANTHOCYANIN CONTENT	
		MEAN	RANGE	MEAN	RANGE
MEEKER	7*	7.1	6.6-7.8	320	241-511
MALLING SEEDLING	3	7.1	6.5-7.7	141	110-166
GOLDEN	1	6.5	-	4	-
HERITAGE	2	6.0	5.9-6.0	302	296-308
WILLAMETTE	21	5.6	4.3-7.0	620	116-1101
CHILCOTIN	1	3.7	-	208	-
MALLING PROMISE	3	3.5	3.4-3.5	120	72-154
NORNA	3	3.2	2.9-3.6	176	135-217
VETEN	3	3.0	2.9-3.2	216	202-228
SKEENA	1	2.4	-	244	-

*EXCLUDES MOLDY SAMPLE

Table 7. Repetition of sample preparation and anthocyanin analysis

REPETITION NUMBER	CYD-3-SOP	CYD-3-GLURUT	CYD-3-GLU	PGD-3-SOP	CYD-3-RUT	PGD-3-GLURUT
1	70.93	9.74	8.33	5.55	3.15	1.57
2	71.45	10.10	7.56	5.63	2.96	1.36
3	71.60	9.71	7.99	5.41	2.97	1.41
4	72.87	9.68	7.53	5.32	2.60	1.15
5	70.91	10.47	8.87	5.56	3.26	0.79
MEAN	71.55	9.94	8.06	5.49	2.99	1.26
STD DEV	0.80	0.34	0.56	0.13	0.25	0.30
95 % C I*	0.99	0.43	0.70	0.16	0.31	0.37

*95% Confidence interval estimate of the mean

is mean =/- this value

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