AN ABSTRACT OF THE THESIS OF

Cornelia Lynde Price for the degree of Master of Science in Food Science and Technology presented on May 1, 1991.

Title: Color and Pigment Characterization of Royal Okanogan Huckleberry Juice.

Abstract Approved: _____
Ronald E. Wrolstad

Royal Okanogan Huckleberry juice was found to have a high pH (4.4), titratable acidity of 0.61% (expressed as the major non-volatile acid, citric), an average brix of 5.5°. Juice yield from the raw fruit averaged 68% v/wt. The pigment content of the juice was calculated to be over 5 g/L. Blanching of the berries before inoculation with pectinase increased the amount of polymeric color in the juice but did not affect the total monomeric anthocyanin content. CIE L*a*b* measurements of the juice color describes the intense royal purple color (L*=22.66, a*=-0.06, b*=-0.46).

The major pigment in the Royal Okanogan Huckleberry (over 70% of the total) was identified as petunidin 3-(p-coumaroyl-rutinoside)-5-glucoside. Twelve anthocyanin peaks were separated by HPLC from the pigment extract and were tentatively identified as being of the same glycosidic pattern as the major pigment. All were identified as petunidin, delphinidin, and malvidin derivatives, some of

which were acylated with p-coumaric, ferulic, and either caffeic or an acid that contains caffeic as a constituent (i.e. chlorogenic).

The Royal Okanogan Huckleberry was identified as belonging to the Solanaceae family after a chemotaxonomic survey of the pigments separated by HPLC from the berries of Solanum melanocerasum (re-named S. scabrum), S. burbankii, S. nigrum and Physalis ixocarpa. Investigation into the placentation pattern and seed shape and orientation confirmed the membership of the Royal Okanogan Huckleberry in the family Solanaceae and placed it as a variety of S. scabrum.

Color and Pigment Characterization of Royal Okanogan Huckleberry Juice

by

Cornelia Lynde Price

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed May 1, 1991

Commencement June 1991

Approved:

Professor of Food Science and Technology in charge of major

Head of the Department of Food Science and Technology

Dean of Graduate School

Date thesis is presented May 1, 1991

Words processed by Cornelia Lynde Price

ACKNOWLEDGEMENT

I would like to acknowledge and thank some of the many people and organizations that have made this research project possible. The Department of Food Science and Technology made it possible for me to continue working towards an advanced degree by granting me an assistantship. Tree Top, Inc, kindly donated the Royal Okanogan Huckleberries used in this study.

I would like to thank Dr. Ronald E. Wrolstad for his kindness, wisdom, tenacity, and encouragement during the course of this study. I would also like to thank Dr. Mina R. McDaniel for her wisdom and advice in the shaping of curricular choices and opinions regarding the importance of sensory analysis. For these two people there are not words enough to express my gratitude.

I am appreciative of the expertise freely dispensed by Bob Durst in the area of HPLC and computer analysis. I am also appreciative of the advice and and support of my friends and colleagues Matt Boyles, Vibeke Breinholt, Dr. Brett Luick, Amy Cameron Luick, Dr. Daniel P. Selivonchick, JoLynne Wightman, and many others. These people gave me moral support, good advice and goaded me on when 'the light at the end of the tunnel' appeared to wink out. I am grateful to have had them a part of my life over the past two and a half years.

Last, and certainly not least, I would like to thank my family, especially my mother and father, for their unwavering support and belief in me. I am blessed with a wonderful family and am grateful every day of my life for them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES
(continued)

LIST OF TABLES

PREFACE

The fruit of the Royal Okanogan Huckleberry was first investigated for its properties as a colorant and as an ingredient to be used in blended juices, juice drinks, and mixed berry cocktails. Therefore, the primary interest was in determining the pH, titratable acidity, amount of monomeric anthocyanin, and yield of the juice. The intense purple color of the juice was intriguing because most berry juices are actually more red than purple and lose much of their coloring properties near the pH of this juice (4.4).

The processor providing the fruit had very limited and somewhat anecdotal information as to the botanical nature of the fruit. Their understanding was that the fruit had parentage from a number of Vaccinium species, cranberries, blueberries, and wild native huckleberries. However, morphological investigation indicated that the fruit resembled the family Solanaceae (tomatoes, peppers, garden nightshade, eggplants) more than it resembled members of the family Ericaceae (blueberries, cranberries). Further, the intense purple-black color of the fruit and the juice was similar in shade (to the naked eye) to that of a dark purple eggplant, a purple tomatillo, or the wild garden nightshade (S. nigrum) found in the author's garden.

This resulted in an investigation into the pigments common to both of these families as well as to an

investigation of fruit morphology of berries thought to be closely related to the Royal Okanogan Huckleberry. The pigments of berries from the Solanum genus (grown in the author's garden) closely resembled the pigments extracted from the Royal Okanogan Huckleberry and was found to most closely resemble S. melanocerasum in pigment and morphological characteristics.

The objectives of this study were to determine the qualities of a juice made from Royal Okanogan Huckleberries in terms of pH, total monomeric anthocyanin content, and yield. Two different enzymes were used in the juice making process utilizing blanched and unblanched fruit for comparative purposes.

Characterization of the pigments in the fruit was undertaken in order to determine which anthocyanins were present. This was done with an eye towards the chemotaxonomic significance of the pigments present in order to determine to which family or genus this berry belongs. Further, the identification of the pigments was necessary in order to predict the stability of the anthocyanin pigments relative to pigments found in other highly pigmented fruits.

Finally, the color characteristics of the juice at different pH's was studied in order to be able to recommend the use of this juice as a food colorant. Since most foods have a pH of less than 8.0 (Banwart, 1981), juice samples were adjusted to reflect this range and its color recorded

using the CIE L*a*b* system. Changes in spectra due to pH were also recorded in order to determine the effect of pH on the total pigments present.

Color and Pigment Characterization of Royal Okanogan Huckleberry Juice

Literature Review

Huckleberry nomenclature

Information concerning the parentage was anecdotal and incomplete, so it became important to search the literature for an adequate definition of the term 'huckleberry'. Webster's second edition dictionary (1979) defines the term huckleberry as 'any number of related shrubs having dark-blue berries resembling blueberries' and 'loosely, a blueberry'. Medsger (1947) puts huckleberries into two different genera within Ericaceae or blueberry family. The black huckleberry, also known as Gaylussacia baccata, and the Blue huckleberry, Vaccinium vacillans, are both shrubs (i.e., non-herbaceous plants) that produce berries similar to the cultivated high and low bush varieties of blueberry. Young (1964) reports that the California Huckleberry is also known as Vaccinium ovatum. Underhill (1989) reports that all members of the Vaccinium species are known as huckleberries in the Western part of the United States including the red huckleberry which is evidently a type of wild cranberry. Underhill blames the people in the eastern United States for calling huckleberries the fruit of an

'entirely unrelated plant'.

Another variety of huckleberry is one studied by Francis and Harborne (1966) that is of the family Solanaceae. This type of huckleberry is a nightshade and is more closely related to the potato, tomato, tobacco, petunia or eggplant than it is to the blueberry (Heiser, 1987; Harborne, 1986). This plant is described as a member of a group of 'annual or short lived perennial, herbaceous species of weeds' (Schilling, 1981).

Since huckleberries were to be found in two distinctly different families (Vaccinaceae and Solanaceae), it became necessary to differentiate between the two in the lab. This was done early in the investigation (see Figure 3) and it was decided that the family Solanaceae should be investigated further.

Methods of classification of the Royal Okanogan Huckleberry

Classification of the Royal Okanogan Huckleberry had to be done by comparing the fruit of the plant to other samples of fruit and/or pigments present in other fruits, since the plant itself was not available for study. This meant that the author had to rely on some basic premises involving the similarity of pigments present within the same family of plant. Much work has been done by Harborne in the study of the flavonoids of the family Solanaceae.

Harborne and Swain (1979) reported that the most prevalent pigments in this family are anthocyanins and that the most common aglycon, petunidin, is usually acylated and containing rutinose at the 3 position. Petanin, petunidin-3-(p-coumaroyl-rutinoside)-5-glucoside, is found in both the flowers and fruit of Atropa belladonna (deadly nightshade), in the stem and leaf of Lycopersicon esculentum (tomato), Petunia hybrida (petunia flowers) and in the petals, stems, fruits, and tubers of the Solanum subspecies (Harborne and Swain, 1979). Saito et al. (1965) identified petanin in S. nigrum, var. guineense. Harborne and Francis (1966) identified petanin in the garden huckleberry (S. guineese).

Pigments that resemble petanin but based on delphinidin and malvidin are also common in the Solanaceae family (Harborne and Swain, 1979) in both the eggplant and the potato. Anthocyanin pigments present in potatoes were reportedly all 3-(p-coumaroyl-rutinoside)-5-glucosides of petunidin, malvidin, and peonidin and could be used, along with other morphological features, to differentiate between cultivars (Brown and Moss, 1976). While the most common acylating acid is p-coumaric, ferulic and caffeic acids have been identified, although not bound to anthocyanins (Harborne and Swain, 1979).

Problems with classification of the Solanum genus

The genus Solanum does not seem to be laid out in a straightforward manner. Fruit color ranges from black-purple to red to green at maturity within this genus (Schilling, 1981). The pigment may depend on the ploidy level according to the findings of Rao (1978) where the hexaploids produce larger purple-black fruits, diploids produce smaller blue-black fruits (both due to anthocyanins in the fruit) and the tetraploids produce orange-red fruit (due to carotenoids).

The genus Solanum is loosely assembled but has been subject to debate for many years (D'Arcy, 1972; Bohs, 1990; Bhiravamurty and Rethy, 1984; Ganapathi and Rao, 1987). It is unclear to the author how the genus Solanum is differentiated from other genuses since the membership is so varied. S. dulcamara is a woody deciduous vine that produces orange-red, elongated and pointed berries. S. melongena (eggplant) is an herbaceous shrub which produces a large purple-black, wine-red ,or white, ovoid fruit that is pithy and light colored in the middle. S. melanocerasum (a garden huckleberry, also called S. scabrum), another herbaceous shrub, produces purple-black, umbellate, and globular fruits approximately 1 cm in diameter that is divided into two sections and carries its pigment throughout the fruit (personal observations). As Bohs states (1990), "The taxonomy of this group is likely to be in a state of flux for

many years to come" due to the lack of basic information regarding the morphology and chemistry of the species involved. Hawkes and Tucker (1968) agreed that the divisions within the family Solanaceae were in need of revision, especially in view of the economic importance of the family.

On defining a genus

The genus Solanum is comprised of 1,000 (Heiser, 1987), 1,400 (D'Arcy, 1979), 2,000 (Hawkes and Tucker, 1967) or 1,500 (Hunziker, 1979) species depending on who is proposing the grouping. This comprises roughly half of all species found within all of the genuses in the family Solanaceae (Heiser, 1987). With such a huge number of plants that are supposed to be closely related, it is clear that some confusion exists. Proposals to break the genus into smaller groups have been made from time to time but a system has not been agreed upon by all involved. D'Arcy (1979) in 'the Classification of the Solanaceae' gives an excellent discussion of the previous attempts to classify and subdivide the Solanaceae and proposes one of his own methods of classification. Since then, further work has been done to clarify the systematics used to differentiate between different members of the Solanaceae family.

Schilling (1981), undertook the project of clarifying the interrelationship of the genus Solanum section Solanum (also

known as the Solanum nigrum complex) by comparing 97 samples of this section comprised of 11 different species. Cluster analysis of 26 attributes of the different plants was performed which differentiated between the different groups. This method of division is probably the closest method to that of Linneaus in that many factors are involved in the division of this genus and the basis for naming the different plants based on factors such as fruit color, leaf shape, height of plant, anther length and color, presence of hairs on leaves, etc.. Of course, Linneaus did not employ statistical analysis for his assignment of plant species but the initial basis for taxonomic studies was the outward, observable differences between plants.

Tetenyi (1987) has proposed the use of alkaloids as markers for the different groups or species within the Solanaceae family since one of the more remarkable chemical features of this family is the presence of alkaloids and the biosynthetic routes by which these compounds are formed. Phenolic acids were used by Bhiravamurty and Rethy (1984) to differentiate between 18 members of the Solanum nigrum complex. The use of cluster analysis of 5 different phenolic acids and 10 unidentified compounds, separated by thin layer chromatography, allowed the 18 taxa to be classed into 5 different groups. Cytogenic studies carried out by Ganapathi and Rao (1987) were used to determine the relationship between members of the Solanum nigrum complex in order to

determine the evolution of the plant. Finally, Rao (1978) used pigments to demonstrate the interrelationships between certain members of the Solanum nigrum complex. He found that tetraploids (n=24) were orange and contained carotenoids. Diploid and hexaploid members were blue-black in color and were identified as containing petunidin-3-(p-coumaroyl)-rhamnosyl-glucoside as the major pigment. Comparison of the pigments indicated that tetra-, hexa-, and di-ploid members of the Solanum nigrum complex are not closely related.

The confusion surrounding the naming of a berry

Solanum guineese, as it is called in Francis and Harborne's paper (1966), has been the subject of taxonomic misinterpretation and confusion since Linneaus first named it in 1753 (Solanum guineense). A vague lab notebook identified the plant as a native of Guinea, but although cultivated there, it is not believed to have originated there. This plant has since that time been called Atropa solanaceae, Atropa guineensis, Solanum aggregatum, S. intrusum, S. melanocerasum, and S. tinctorium, S. nigrum var. guineense, (Heine, 1960) and S. scabrum (Heiser, 1987; D'Arcy, 1979; Schilling, 1981).

The names may all refer to the same plant, or they may be variants of the 30 or so known varieties of S. nigrum reported by Schilling (1981). It is unclear whether these

plants are actually all the same, but there is still some confusion that may (or may not) be cleared up by chemical investigation of the individual plants in question. Many species can no longer be located or preserved samples have been destroyed (Edmonds, 1979).

Chemotaxonomic principles

There have been numerous methods proposed for the comparison of plant constituents with the goal of being able to more accurately tell the difference (or similarities) between any two groups of plants. Recent advances in available technology have facilitated the gathering of data that will allow us to make more incisive comparisons. While the phytochemical evidence as to the makeup of the individual plant species cannot be overlooked, neither can the morphological data which has traditionally been of primary importance (Heywood, 1966). Phytochemists may classify plants by different methods than traditional taxonomists, but due to the biochemical complexity needed to determine any given morphological attribute, the two disciplines should be able to come to an agreement as to the grouping of plant species and genuses.

A beginning into the chemotaxonomic survey has been made by Francis and Harborne (1966) with a study of the pigments present in Solanum guineese. Bhiravamurty and Rethy (1984)

looked at acids present in 18 taxa of the S. nigrum complex with hopes of differentiating the taxa by phenolic acids present. (Of these taxa, S. melanocerasum and S. guineense yield different results in the analysis of phenolic acids present.) This author has attempted to use pigments present in some members of the Solanum and Physalis genuses, as well as pigments present in the Vaccinium genus, to identify the genus to which the Royal Okanogan Huckleberry belongs. Pigments from the purple fruited Solanaceae have been demonstrated to have uniquely similar HPLC profiles.

The classification of plants into groups or taxa has been done, since the beginning, through the use of chemotaxonomy, although the term is rather recent. It is the chemicals in a plant system that confer upon that plant characteristics that determine its fragrance, taste, or color that makes it different from other plants. These qualities are often the key factors in how a plant is classified (Harborne, 1967; Bate-Smith, 1967). The more closely related two plant species are, the more characteristics they will have in common (Harborne and Turner, 1974).

Secondary metabolites, such as anthocyanins or other phenolics that exists only within a small group of plants, help to further separate the plant from other like species (Harborne and Simmonds, 1964). The anthocyanins of the Solanaceae are distinctive in that they appear as acylated pigments and most frequently are 3-rutinoside-5-glucosides,

although other sugar patterns in the family exist (Harborne and Swain, 1979). Anthocyanidin 3-rutinoside-5-glucosides are found throughout the Solanaceae family, but are also found in the closely related Gesneriaceae family and sporadically in the Iridaceae and Violaceae families (Harborne, 1964). Acylated anthocyanins are found in the Solanaceae and many other families and orders among the angiosperms of the mono- and dicotyledonous plants (Harborne, 1964). The only reported instances of fruits producing 3-rutinoside-5-glucosides acylated with cinnamic acids is in the family Solanaceae.

Acylation with a hydroxycinnamic acid is considered to be an advanced character in plant evolution (Harborne, 1986), as is the appearance of delphinidin or its derivatives. Petunidin and malvidin, because of their o-methylated structure are thought to be even more highly evolved than their parent, delphinidin (Harborne, 1977). The appearance of a highly evolved anthocyanidin in conjunction with a 3-rutinoside-5-glucoside sugar pattern, acylated with cinnamic acids such as p-coumaric, ferulic, or caffeic acid should indicate membership into a unique plant taxa, the Solanaceae family.

Other pigments have characterized the Solanaceae family. Carotenoids are prevalent in this family and are well represented in the Capsicum, Atropa, Physalis, Lycopersicon, and Solanum genuses (Goodwin, 1966). Solanum dulcamara and

S. luteum, although close relatives to other solanums by nomenclature, produce carotenoids rather than anthocyanins which are evident in the black berried forms of the Solanum genus (Rao, 1978). These members of the Solanum genus produce no anthocyanins although given the correct conditions, they should be able to produce anthocyanins consistent with the rest of the Solanum genus if they are indeed closely related (Harborne, 1965). (No reports of black or purple berried varieties of these species have been found.)

Other secondary plant metabolites such as alkaloids have been used to describe the differences between plant families. The Solanaceae family is one which is known for its alkaloids and therefore much study has gone into this area of research (Adesina and Gbile, 1984; Carman et al., 1986; Kawai et al., 1987; Liener, 1969; Schreiber, 1979; Bradley et al., 1979; Roddick, 1979; and many, many more). Tetenyi (1987) looked at alkaloids and steroids as a means of subdividing the Solanaceae family into groups describing the evolution of the family. The taxonomic markers used were steroidal alkaloids and lactones that were derived from cholesterol. Alkaloids are distributed unequally throughout the angiosperms (Hegnauer, 1966) which may increase their significance as taxonomic markers. Like anthocyanins however, similar alkaloids may be produced by two otherwise unrelated species. An example of this is nicotine which is produced by both

Equisetum and Nicotiana genuses or bufotenine, produced by the bufo toad and the legume Piptadenia (Erdtman, 1967).

If the presence of a specific chemical compound exists within a plant species, it does not necessarily follow that a morphological change is to be observed, unless the compound (i.e. a pigment) itself is the cause of that observable change (Tetenyi, 1970). However, the presence or absence of one compound alone, let alone one physical trait, is not enough information by which to classify a plant. The correlation of many similarities is necessary to determine the taxonomic classification of a plant, including morphology but not excluding chemical constituents (Harborne and Turner, 1984). Parallelism, the appearance of the same trait arising from different evolutionary paths, is 'rampant' in the angiosperms and therefore the taxonomic significance of any single morphological or chemical trait alone must be viewed with suspicion (Cronquist, 1980). While it is important to be able to classify plants by their outward appearance, biochemical data can add to our knowledge of the systems involved and will certainly have an effect on the classification of plant taxa (Harborne and Turner, 1984).

Anthocyanins as chemotaxonomic markers

Cronquist (1980) recognized the problem of trying to apply one general rule as a means to classify all plants into

groups by means of chemical data. He found that in using chemistry to define taxa, that:

> "Chemical characters are like other characters: they work when they work, and they don't work when they don't work. Like all taxonomic characters, they attain their value through correlation with other characters, and perfect correlations are the exception rather than the rule.".

This comment has proved to be quite true with the use of anthocyanins as a means of classifying angiosperms and their fruit.

The anthocyanins found in blueberries fall into separate classes (Sapers et al., 1984; Ballinger et al., 1979) and may indicate that the species designation Vaccinium should be further subdivided to reflect those differences. Analysis of a pink fruited blueberry (Ballinger et al., 1972) indicated that the blueberry produced fewer, rather than different, anthocyanins than would be found in another variety.

Once the anthocyanic patterns are set it appears that even ecological abuse is ineffective in changing the types of anthocyanins that are produced by a plant. Chandel and Singh (1985) found that exposing members of the Solanum nigrum complex to gamma radiation, at levels of 10 to 50 krad, caused no change in color of the berries. Lees and Francis (1972) took this farther and found that by exposing cranberries (Vaccinium macrocarpon) to doses of 150 to 300 krad gamma radiation, anthocyanin levels were enhanced, but the relative amount of each kind of anthocyanin was not

altered. Asen (1982) found that changes in rate of fertilizer application and daylength affected the amount of flavonoids found in roses but did not affect the qualitative distribution of those flavonoids. It has been shown that amounts of anthocyanin produced in Brassica oleracea are affected by the amount of light and temperature that the plant receives (Rabino and Mancinelli, 1986). However, there is no evidence to suggest that the plant produces a different type of anthocyanin when exposed to different levels of light and temperature.

It is an important point that the qualitative anthocyanin character does not appear to be altered by ecological forces. This allows for the use of pigments present as taxonomic markers or 'fingerprints' at the species level. In a broader sense, these fingerprints can often be used at the genus or family level although variations are to be expected. The amount of variation should depend on the taxa of interest and the amount of cross breeding that has taken place. Asen's work on roses (1982) supports this in that the use of HPLC techniques has for the first time allowed for rapid separation and quantitation of compounds present. This makes it possible to identify new cultivars by their 'flavonoid fingerprint' in order to protect plant patents. This is the same basis for the detection of adulterants in cranberry juice by Hale et al. (1986) where deviations from the expected flavonoid HPLC profile for cranberry juice indicated

adulteration with other compounds.

Anthocyanin pigments present in the Solanaceae family are derivatives of the six major anthocyanidins: delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin (Harborne, 1979). Within the Solanum genus, (the genus to which garden huckleberries belong) the predominant glycosylation pattern is reported to be 3-rutinoside-5-glucoside and 3-rutinosides. Acylation with one mole p-coumaric acid to the rutinoside at the 3 position is also common (Harborne, 1979). Francis and Harborne (1966) identified petunidin-3-(p-coumaroyl rutinoside)-5-glucoside (petanin) as the major pigment in Solanum guineese. Of the 4 anthocyanins detected in S. guineese, all were identified as 3-rutinoside-5-glucosides of petunidin or malvidin, 3 of which were acylated pigments.

The anthocyanins present in the blueberry (Vaccinium spp.) are also derivatives of the six major anthocyanins listed above but exist as 3-glucosides, 3-arabinosides, or 3-galactosides only (Francis et al., 1966). Ballinger et al. (1979) found that of 11 cultivars of blueberries studied, the anthocyanin content of the berries differed both quantitatively and qualitatively. Sapers et al. (1984) used HPLC to characterize the pigments present in 11 cultivars of the highbush blueberry (Vaccinium corymbosum) and found that the profiles fell into three distinctly different categories. Data presented in the paper indicates that some of the peaks

either disappeared or were evident in amounts below the detectable threshold for different cultivars. In other cases the proportions of the pigments changed in relation to each other. The data does not indicate that the pigment profiles could in any way mimic the profile of the pigments from the Royal Okanogan Huckleberry.

The importance of color

There are over 300 known structures of anthocyanins (Harborne and Turner, 1984) found in leaves, tubers, flowers, fruits and other parts of various plants. Flower pigments are generally thought to have evolved as a means of attracting pollinators. Bees appear to prefer the blue colored flowers while birds seem to prefer red (Harborne, 1975). Neither of these arguments explains why fruits are colored except as a means of seed dispersal when preferred by one type of animal over another (Harborne, 1975).

Anthocyanins are concentrated in the skin of most fruits where the primary reason for them to be there is, theoretically, to attract animals. It is unclear why a berry would contain pigment throughout its flesh (Gross, 1987). It may be that secondary metabolites accumulate in the fruit to protect the plant from predators (Mabry, 1972), rather than for the purposes of seed dispersal. The evolution of the many alkaloidal components reported for this family (Tetenyi,

1987) could be explained in this way. The flavor of the anthocyanins themselves could be the slightly bitter component present in the berry due to their slightly hydrophobic character (Daniel, 1991). (Although it is unproven that the taste of the compounds present in the Royal Okanogan Huckleberry or other members of the Solanum nigrum complex are repugnant to some animals, the berries grown in the author's garden remained untouched in spite of numerous birds in the area.)

If the plant evolved with a defense mechanism that made it distasteful to predators, the plant would have to reproduce by spreading its seeds over the immediate area. S. nigrum produces around 40 seeds per berry, approximately 200 berries per plant (Weller and Phipps, 1978/79), and the seeds can remain viable in the soil for up to 39 years (Toole and Brown, 1946). The longevity of the seed material and the number of seeds produced could have evolved as a means of ensuring survival of the plant species.

While the reason for intense pigmentation of the berry is unclear, the nearly black color of the fruit may serve to absorb large amounts of solar energy which could then be used to catalyze the synthesis of other compounds. Since the pigments found in the plant are of a highly evolved nature (Harborne, 1977), they require considerable biochemical modification that sets these pigments apart from most others. Further, secondary metabolites found in this genus, such as

alkaloids (Tetenyi, 1987; Adesina and Gbile, 1984), phenolic acids (Bhiravamurty and Rethy, 1984) and of course pigments, are synthesized at the expense of energy.

Heat energy, such as would be absorbed by a dark berry on a sunny day, would speed up the action of the enzymes present in the plant. Plants grown at low temperatures produce a smaller amount of anthocyanin pigments (Rabino and Mancinelli, 1986). While this may be linked to the reduced growth and development of the plant tissues at lower temperatures, it may also be tied to the speed at which the enzymes that produce the pigments work. More sunlight means more energy supplied to the plant, which in turn means that more energy is absorbed as heat. Harborne (1977) reported that angiosperms in temperate (e.g.cooler) climates produce more delphinidin derivatives, with a trend towards the o-methylated and/or complex glycosidic patterns. This could be the result of those races surviving due to pollinators that prefer the color of the flower, as Harborne contends, but it could also be due to the ability of the plant to absorb enough heat energy into the dark berry to catalyze the reactions necessary to make a very complex anthocyanin. While it would be necessary for the plant to have evolved the right enzymes to make this reaction proceed, the absorption of energy through the skin would greatly facilitate the reactions necessary to make the secondary metabolites that set one plant apart from another.

Acylation of anthocyanins as an indication of stability

Anthocyanins have traditionally not been used as food colorants, except where they occur naturally, because they are chemically unstable, difficult to purify, and not available commercially in large quantities (Mazza and Brouillard, 1987). The latter two objections are valid but the juice of the Royal Okanogan Huckleberry contains so much pigment that little purification would be needed in order to procure a usable quantity of the pigment. Further, the berries are grown so easily that juice could be easily obtained. The stability of the chemical species has partially been overcome by the fact that the majority of the pigments present are acylated. They therefore remain colored in neutral aqueous solutions and should demonstrate increased stability upon long term storage.

Teh and Francis (1988) reported on the exceptional stability of dicaffoylpeonidin-3-sophorside-5-glucoside (DCPSG) over its deacylated form upon storage at pH 2.8 in citric acid buffer. It was found that the peonidin derivative was uniquely stable at this pH. Asen et al. (1977) reported that DCPSG was stable in neutral aqueous solutions and speculated that the stability might be due to the grouping of the hydroxyls on the acylating caffeic acids.

Brouillard (1981) reported that anthocyanins that contained two acylating cinnamic acids resisted hydration of

the pyryllium ring, in effect not allowing the formation of the colorless pseudobase. Further, it was reported that the higher the pKa of the anthocyanin molecule, the more stable it was. (A monoglucoside has a lower pKa than a 3,5-diglucoside which in turn has a lower pKa than a 3,7,3'-triglucoside.) Brouillard and Delaport (1977) reported that malvidin-3-glucoside formed the chalcone form of the molecule at a rate twice that of malvidin-3,5-diglucoside at room temperature between pH 4 and 6. This indicates, in two different ways, that the more stable of the two species is the diglucoside. The stability of a di-glycoside is further substantiated by work done by Rommel et al. (1990) and Bronnum-Hansen and Flink (1985).

Using the same logic as was applied by Brouillard (1981) and Brouillard and Delaport (1977), the anthocyanins found in the Royal Okanogan Huckleberry should have a relatively high pKa because of the sugars attached at the 3 and 5 positions and therefore be more stable. The stability would also be enhanced by the presence of the acylating acid groups that appear in over 97% of the anthocyanins present. Chen and Hradzina (1981) reported that other flavonoids that might exist in solution can act as copigments which effectively increase the absorbance of the pigments in solution. Mazza and Brouillard (1990) reported that anthocyanins could interact with chlorogenic acid and become bound together by hydrophobic interactions. This had the greatest effect on

absorption near pH 3.6, or around the pH where the colorless anhydrobase forms. The interaction with the chlorgenic acid was presumably due to the superimposition of the aromatic ring of the chlorogenic acid (i.e. the phenyl ring of the caffeic acid portion of the molecule) over the pyrillium ring of the anthocyanin, thereby stabilizing the molecule and preventing hydration of the molecule. Mazza and Brouillard (1990) found that a 1:1 ratio of pigment to copigment formed under these conditions.

A molar ratio of 1:1 formed in the Mazza and Brouillard study (1990) between anthocyanins and the copigment indicates that only one aromatic ring is necessary to stabilize the pyryllium ring of the anthocyanin. Hoshino et al. (1980) reported that anthocyanins that were acylated participated in pigment/copigment interactions at a much higher rate than non-acylated pigments presumably due to the ability of the aromatic molecules to stack and to form a stronger hydrophobic complex. Brouillard (1981) reported on the stability of cyanidin-3,7,3'-triglucoside acylated with caffeic and ferulic acids. The explanation of its stability in aqueous media near neutral pH is that the acids stack over and under the pyryllium ring and therefor protect the ring from hydration. This same pigment did not exhibit long term color stability at pH 2.8 in a study done by Teh and Francis (1988) and was found to be less stable than DCPSG.

Sapers et al. (1981) looked at the pigments in red

cabbage (Brassica oleracea) and compared the stability of the pigments to cranberry and red beet pigments. Red cabbage contains acylated pigments and was found to be much more stable than the other pigments tested over long term storage, in light or dark, in the pH range of 3 to 4. The pigments of red cabbage contain acylated and free cyanidin-3,5,diglcosides (Harborne, 1964).

The extraction of red cabbage pigments is a lengthy process that involves purification and concentration, since the presence of cabbage in products such as yogurt is not generally accepted. The relative stability of the acylated pigments extracted makes the process feasible especially in light of the demand for natural colorants. The juice of the Royal Okanogan Huckleberry however is so rich in pigment, that further purification may not be necessary. The pigments should still exibit the same long term stability to degradation that is shown by the pigments from the red cabbage but at a lower cost. This pigment extract should still satisfy the demand for a natural colorant.

MATERIALS AND METHODS

Samples

Frozen Royal Okanogan Huckleberries were obtained from Tree Top, Inc., Selah, WA. Purple tomatillos (Physalis ixocarpa) and eggplants (Solanum melongena, var Dusky) were obtained from Dennison Farms, Corvallis, OR. Highbush blueberries (1989 growing season) were purchased from Twedt's Berry Farm, Corvallis, OR. Purple peppers (Capsicums, believed to be 'Chinese Multicolor' variety) and purple tomatillos were purchased from Cub Foods, Corvallis OR. Garden Huckleberries (Solanum melanocerasum) and Mrs. B's non-bitter Garden Huckleberries, also known as Wonderberries (Solanum burbankii) were grown in the author's home garden from seeds obtained from Peace Seeds, Corvallis, OR. Berries from wild Solanum nigrum were collected in the Corvallis, OR area and were identified by pictorial comparison of authentic samples (Underhill, 1989).

Welch's Cranberry juice was purchased at a local supermarket. Blackberry juice was available from an earlier departmental research project (Rommel, 1988).

Reagents, solvents and standards

HPLC grade methanol, HPLC grade acetonitrile, HPLC grade acetone, reagent grade dichloromethane, HPLC grade glacial acetic acid, potassium chloride, citric acid monohydrate, 'Dilut-it' sodium hydroxide standard, reagent grade concentrated hydrochloric acid (37%) and HPLC grade chloroform were purchased from J.T.Baker, Phillipsburg, PA.

Concentrated phosphoric acid was purchased from EM Science, Gibbstown, NJ.

Tris-hydrochloride, caffeic acid, p-coumaric acid, and ferulic acid were ordered from Sigma Chemical Corp., St. Louis, MO.

Potassium hydroxide, sodium hydroxide, and monobasic potassium phosphate were ordered from Mallinckrodt Chemical Works, St. Louis, MO.

Samples injected onto HPLC and non-HPLC grade solvents used were filtered through 0.45 um, type HA filters (Millipore Corp., Bedford, MA) or Biorad 0.45 um Prep-Disk membrane filters (Bio-rad Laboratories, Richmond, CA).

Pectinase enzymes used to process the juice were Novo Ultra SP (Novo Labs, Danbury, CT) and Rohapect B1-L (Rohm Tech., Malden, MA).

Apparatus

HPLC system - Varian 5000LC (Varian Instrument Group, Walnut Creek, CA) attached to an HP 9000/9153 computer system (Hewlett Packard, North Hollywood, CA).

Detectors:

(a) Hewlett Packard HP 1040 diode array detector (Hewlett Packard, North Hollywood, CA).

(b) Varian refractive index detector (Varian Instrument Group, Walnut Creek, CA).

Columns and gradients:

(a) Supelcosil C18 column (Supelco Inc., Bellefonte, PA) 250 mm X 4.6 mm ID fitted with a Biorad ODS-10 guard column (Biorad, Richmond, CA). Solvents used were 15% acetic acid (150 mL glacial acetic acid made up to 1.0 L with deionized water and filtered through 0.45 um filter) and HPLC grade acetonitrile. Gradient was isocratic at 15% acetonitrile and 85% acetic acid solution using a flow rate of 1.5 mL per min. Peaks were monitored at 520 nm, spectra was recorded from 250 to 610 nm.

(b) Spherisorb ODS-2 column (Alltech Associates, Inc., Deerfield, IL) 250 X 4.6 mm ID fitted with a Biorad ODS-10 guard column (Biorad Laboratories, Richmond, CA). Solvents used were 4% phosphoric acid (40 mL concentrated phosphoric

acid made up to 1.0 L with deionized water and filtered) and HPLC grade acetonitrile. Gradient used began at 12% acetonitrile and increased linearly to 16% over 40 min. then to 25% at 45 min. using a flow rate of 1.3 mL per min. Peaks were monitored at 280 and 520 nm.

(c) PLRP-S polymer column (Polymer Labs, Amherst, MA), 5 u particle size, 250 X 4.6 mm ID fitted with a Polymer Labs guard column. Solvents used were the same as used with the ODS-2 system. Gradient used began at 6% acetonitrile and remained there for 10 min. then increased linearly to 20% acetonitrile at 55 min. using a flow rate of 1.5 mL per min. Peaks were monitored at 520 nm.

(d) Spherisorb ODS-2 connected to a Spherisorb ODS-1 column (both from Alltech Associates Inc., Deerfield, IL) 250 mm X 4.6 mm ID. Solvents and gradient are as described for non-volatile acid determination by Hong and Wrolstad, 1986.

(e) Biorad aminex HPX-87C (Biorad Labs, Richmond, CA) 300 X 7.8 mm ID, fitted with Biorad Carbo C guard column. Solvent and conditions are as described for LC determination of sugars by Spanos and Wrolstad (1987).

GC system

Varian Aerograph series 1400 (Varian Instrument Group, Walnut Creek, CA) attached to an HP3396 A integrator (Hewlett Packard, North Hollywood, CA). A Supelcoport SP-2340 packed glass column (Supelco, Inc., Bellefonte, PA) 6 ft. X 2 mm ID

was used. Nitrogen was used as the carrier gas at a rate of 17 mL per min. The column temperature was held constant at 225° C, the detector at 262° C, and the injector at 240° C.

Juice processing

The unit operations for processing juice from frozen Royal Okanogan Huckleberries is shown in Figure 1. Half of the samples were given an initial heat treatment by heating the berries in a microwave oven to 90° C and holding for 5 min before cooling to 50° C in a water bath. Depectinization with Novo Ultra SP pectinase (0.05% v/w) was carried out at 50° C over three hours. Depectinization was monitored using the alcohol precipitation test (Rommel et al., 1990). These trials were done in triplicate.

Juices were also processed with the following modifications. Frozen berry samples (275 g) were broken in a Waring blender. Half of the samples were heated to 80° C in a microwave oven and held at that temperature for 5 min. Berry samples were brought to 50° C in a water bath before depectinizing with Rohapect B1L (0.1% v/w). Samples were held at 50° C for 8 hours then at room temperature for 14 hours before screening. Samples were allowed to incubate at 50° C for 8 hours then at 25° C for an additional 14 hours until testing negative for pectin. Gelatine and bentonite (500 ppm) were used as fining agents following the procedure

of Rommel et al. (1990). Samples were stored at -15° C before final filtering. Juice samples were processed in duplicate.

Both juice trials received a final filtering through Whatman #4, 'Polarlite' polyester fleece material (Fabricland, Corvallis, OR), and a 1 cm layer of hy-flo super cell (filter aid) under vacuum.

Analytical determinations

Titratable acidity was measured as citric acid, according to AOAC 22.059, glass electrode method using 0.01 normal NaOH (1/100 dilution of 1.0 normal 'Dilut-it' standard solution with deionized water).

Juice pH was measured directly using a Corning 125 pH meter (Corning Glass Works, Medfield, MA) with a Sensorex combination electrode (Stanton, CA.).

Brix of the juice samples was determined using a hand held, 0°-90° Brix, refractometer (Atago, Japan).

Sample preparation and HPLC separation of Anthocyanins

Following the sample preparation procedure described by Hong and Wrolstad (1990), a Water Associates (Milford, MA.) C-18 Sep-pak cartridge was employed. Pigments retained on the mini-column were washed with deionized water and eluted

with HPLC grade methanol. Pigments were again dissolved in 4% phosphoric acid and passed through 0.45 um filters. Anthocyanins were separated by either the Polymer Labs PLRP-S column or the Spherisorb ODS-2 column system. Note: Pigments analyzed on the polymer column were eluted from the Sep-pak system with 0.01% hydrochloric acid in methanol (0.1 ml concentrated hydrochloric acid added to 999.9 mL HPLC grade methanol.).

Pigments were also isolated from fruit via acetone using the method of Wrolstad et al. (1990). Repeated pigment extractions of the same plant material were not carried out. Eggplant skins used for analysis were frozen in liquid nitrogen and pulverized in a Waring blender using the methods of Wrolstad et al. (1990). Portions of the powder were extracted with acetone for 24 hours. Thawed berries used for analysis were crushed in a beaker with a glass stirring rod and were also allowed to macerate in acetone 24 hours. The acetone extract was vacuum filtered through a Whatman #1 filter and subsequently partitioned with 2.4 volumes chloroform. Phase separation took place in a separatory funnel over a 5 hour period after which the lower phase was discarded. Pigments were isolated from the upper, aqueous phase using the Sep-pak method described earlier and separated on the ODS-2 column system.

Anthocyanidins

Anthocyanidins from juices and from purified fractions were analyzed following the methods of Hong and Wrolstad (1986). Pigments were isolated on a Sep-pak mini-column, rinsed with water, eluted with methanol and dried under vacuum. Isolate was hydrolyzed in 2 N HCl at 100° C for 30 min. Modifications of the procedure include the use of pure methanol to elute pigments from the Sep-pak and the omission of NaCl. Sugars obtained from the hydrolysis procedure were reserved for further analysis after being separated from pigments retained on the Sep-pak. Anthocyanidins obtained by this method were diluted in 4% phosphoric acid (40 mLs concentrated phosphoric acid diluted to 1.0 liter with deionized water) and filtered. Pigments obtained were stored in a lab freezer at -15° C until analyzed and were injected onto the HPLC within 12 hours. Anthocyanidins were separated on the Supelcosil C-18 column system.

Alkaline hydrolysis of anthocyanins

The procedure described by Hong and Wrolstad (1990) was used to saponify the acylating acids and to separate them from the glycosylated anthocyanins. The pigments and acids were washed with deionized water and eluted with HPLC grade methanol before redissolving in 4% phosphoric acid and

filtering. Pigments and acids were separated on the Spherisorb ODS-2 column.

Nonvolatile and phenolic acid analysis

Organic acid analysis was carried out using the methods and equipment of Hong and Wrolstad (1986) using ODS - 1 & 2 columns. Standards were used to identify retention times of acids present. Samples and standards were passed through Sep-pak cartridges and filtered through 0.45 um filters before injecting into the HPLC system.

Acylating phenolic acids were separated and identified during the alkaline hydrolysis procedure. Separated peaks were monitored at 280 nm and authentic samples of caffeic, p-coumaric, and ferulic acids were used as standards.

Sugars

Using the method of Blakeney et al. (1983), sugars isolated during the acid hydrolysis procedure were transformed into their corresponding alditol acetates for analysis by gas chromatography. Modifications of the procedure include the use of HCl for sugar hydrolysis as well as lyophilization to concentrate the sugars after removing them from the pigments.

Major Sugars of Royal Okanogan Huckleberry Juice were

separated by HPLC and identified by retention time according to the methods of Spanos and Wrolstad (1987) using the Varian 5000LC to deliver the mobile phase. Sugars were monitored with a Varian refractive index detector.

Peak purification

Pigment peaks (repeated HPLC separations made on the ODS-2 system) were collected manually. The individual peaks were pooled and a portion re-injected in order to confirm purity. Samples were stored at -15° C until used. Purified pigments were separated from the HPLC mobile phase by using a C-18 Sep-pak mini-column. Each purified peak was analyzed according to a procedure described earlier in order to determine the identity of its chemical constituents. Anthocyanidins were determined through removal of the sugars and acylating acids by acid hydrolysis and identified by comparison of HPLC peaks to known standards by retention time and spectral comparison. Sugars recovered from acid hydrolysis procedure were identified by GC according to retention time. Acylating acids were separated from the pigments by alkaline hydrolysis before separation by HPLC. Acylating acids were identified by comparison of retention time and spectra to known standards.

Anthocyanin content of the juice

Total monomeric anthocyanin content was determined according to the method described by Fuleki and Francis (1968). Tannin contribution (%) to the color of the juice was determined using the method described by Somers (1971). Absorptions were measured on a Varian DMS-80 double beam spectrophotometer using quartz cuvettes and appropriate buffer systems as blanks. A 1/100 dilution of single strength juice was necessary to accurately measure absorbance at pH 1 in the measurement of monomeric anthocyanin and a 1/75 dilution for the measurement of polymeric color.

Molar absorption (E) of the major pigment was determined from Beer-Lambert's law after isolation and identification of the major pigment. The purified pigment was rinsed onto a sep-pak mini column, rinsed three times with deionized water, and eluted with methanol. The solution was evaporated to dryness in a rotary evaporator at 50° C. Once dry, the pigment was allowed to dry further in a desiccator for 2 hours.

The pigment was weighed, and removed from the evaporating flask by dissolution in successive rinsings of 5.0 mL 1% HCl in deionized water, 0.5 mL 1% HCl in methanol, 1.0 mL 1% HCl in deionized water. The pigment solution was brought up to 10.0 mL in a volumetric flask with 1% HCl (aqueous). A further dilution of 1.0 mL pigment solution in

10.0 mL 1% HCl (aqueous) was made in a separate volumetric flask. This procedure was done twice to determine the molar absorptivity constant ( ) at 520 nm and 510 nm.

Absorbance (A) of the solutions were measured on the Varian DMS80 using 1% HCl (aqueous) as a blank in 1.0 cm quartz cuvettes. The molecular weight of the major pigment, (identified as Petunidin-3-(p-coumaroyl-rutinoside)-5-glucoside) is 934 g/mol. The weight of the pigment used to determine at 520 nm was 2.80 mg, at 510 nm, 2.20 mg. The derived values for were 1.65 X 10^4 and 1.74 X 10^4 respectively. at 510 was used to determine the amount of monomeric anthocyanin contained in the juice of the Royal Okanogan Huckleberry.

Color and spectral analysis of juice pigments

Juice was diluted 1/50 with buffers in order to determine color and spectral properties at different pH's. Buffers were made by diluting the following compounds in 75 mLs water, adjusting the pH with either 1 N HCL or 10% NaOH, and bringing the volume to 100 mLs with deionized water. PH 1.0 buffer, 1.49 grams potassium chloride; pH 2 buffer, 1.28 grams monobasic potassium phosphate; pH 3 buffer, 2.38 grams citric acid monohydrate; pH 4 buffer, 1.49 grams succinic acid; pH 4.5 buffer, 13.6 grams sodium acetate; pH 5.0 buffer, 1.45 grams succinic acid; pH 6 buffer, 1.27 grams

succinic acid; pH 7 buffer, 1.33 grams monobasic potassium phosphate; pH 8 buffer, 1.64 grams tris-hydrochloride. Final pH's of the diluted juice solutions were 1.17, 2.00, 3.03, 4.05, 4.58, 5.02, 6.03, 7.13, and 8.01.

CIE L*a*b* values were measured using a Hunter ColorQUEST (Hunter Instruments, Reston, VA) attached to an IBM pc using Hunter Instruments software version 2.5. Transmittance values using standard illuminant C in a 5 mm acrylic cuvette were recorded.

UV-visible spectra of the solutions were recorded using an HP 8452 Diode Array Detector Spectrophotometer attached to an HP Vectra scanning computer system (Hewlett Packard, North Hollywood, CA.). Absorbance spectra of the solutions in 1.0 cm pathlength quartz cuvettes were recorded from 400 to 700 nm.

RESULTS AND DISCUSSION

Juice processing and characteristics

Table 1 gives the yield and some of the characteristics for various processing trials of juice made from Royal Okanogan Huckleberries. Results from a similar trial involving Garden Huckleberries (Solanum melanocerasum) are also listed.

The pH of the juice (4.4) is relatively high in comparison to many fruit juices and would be classified as an acid food rather than a low acid food because it has a pH between 3.7 and 4.6 (Banwart, 1981). Highbush blueberries were shown to have an average pH of 3.3 (Sapers et al., 1984) and cranberry juice has a pH of less than 2.5 (Coppola, 1982) or less than 2.6 (Hong and Wrolstad, 1986). Since the pH of the Royal Okanogan Huckleberry juice is so high, it would indicate that it is related to neither of these berries. In fact, most fruits have an average pH less than 4.0 with the exception of tomato juice which has an average pH of 4.3 (Banwart, 1981; Belitz and Grosch, 1986; Gould, 1983).

The average value for the titratable acidity of the juices produced, shown in Table 1, is 0.61% expressed as citric acid, the major non-volatile acid identified in the juice. This is comparable to the lower range of that given for orange or grapefruit juice and certainly within the range

of apple or grape juice (Belitz and Grosch, 1987). Gould (1974) lists the titratable acidity for tomato juice as 0.3 to 0.5%, expressed as citric acid, which is slightly less acidic than Royal Okanogan Huckleberry juice. There is also a significant difference in the titratable acidity between the two enzymes used. Using a standard t test, there is a difference at alpha = 0.10, but not at the alpha = 0.05 level. There is a yield difference between the two pectinase treatments used and statistical analysis (standard t-test) indicates that the Rohapect enzyme method produces a higher juice yield at alpha = 0.05. Unheated berry samples also produce higher juice yields at alpha = 0.05 level but both of these differences disappear at the alpha = 0.02 level of statistical comparison. The lower juice yield of the heat treated samples may be due to heating the small sample in the microwave and occasionally stirring the samples to allow for even heat distribution. Even though the samples were covered while heating, evaporation during stirring could account for some of the loss of volume. This difference may disappear when larger batches of juice are processed.

Filtration of the juice was achieved with difficulty. Results from the alcohol precipitation test indicated that the juices were free of pectin although attempts at filtration were hampered by the build up of a 'slime' coating at the juice filter-aid interface. This condition was not improved by disturbing the top layer of the filter-aid (stirring) while under vacuum. Use of the 'polarlite' fleece

material improved the process perhaps because of its flexible, porous nature. Cavitation occurring in the pores of the material during the vacuum filtration may have enhanced the efficiency of the filter aid by expanding and contracting under pressure. This would allow for the exposure of greater surface area of the filter aid and acted to counter act the build up of particles at the juice / filter-aid interface. Fining methods utilizing bentonite clay and gelatine did not improve filtration.

Freezing the samples, after initial screening, seemed to facilitate the filtration process. Samples that were not frozen were nearly impossible to filter whereas the frozen and thawed samples could be filtered with difficulty. Perhaps the freezing and thawing of the juice acted to physically break apart large molecules that interfered with the filtration process.

Juice foaming occurred with all of the techniques attempted and made vacuum filtration a difficult process. This could be due to the high molecular weight molecules, such as polysaccharides, present in the juice. Although juice filtration presented problems, perhaps these difficulties could be overcome by the use of ultrafiltration techniques, centrifugation, or treatment with enzymes designed to completely breakdown the polysaccharides present. Another alternative is the use of filters with larger pores to allow the passage of larger molecular structures, such as

those that readily clog the filters used. This could create a juice that is more viscous and lacking the clarity of a finely filtered juice or may be so thick as to resemble tomato juice.

Brix levels given for the juices average 5.5° and are partially accounted for by the high pigment content of the juice as well as free sugars, acids, and other soluble compounds found in the juice. This is similar to tomato juice which has a brix of 4.0 to 6.0° (Gould, 1974).

HPLC analysis of the free sugars indicates that there are 2.35 g glucose, 2.88 g fructose and an undetermined amount of sucrose per L juice. In any case, this is not a sweet juice and has a comparable sugar content to lemon or raspberry juice (Belitz and Grosch, 1987).

The blanching procedure did not appear to affect the amount of monomeric anthocyanin pigment present in the juice but it did affect the percentage of the tannins present in the final juice (see Table 1). Color contributed by the tannins present did not appear to affect the CIE L*a*b* readings (please see Table 2) for the full strength juices.

Berries that were not heat treated (blanched) and then subjected to incubation at 50° C to depectinize the juice, developed a higher percentage of tannins in the final juice. Berries that were blanched before innoculation with the pectinase enzyme had a lower polymeric color content. This is especially evident in the use of the Rohapect B1-L enzyme

where a 24 hour incubation period was used to achieve total depectinization and the percentage of tannins present in the juice soared to 50%. The unblanched berries treated with the Novo Ultra SP pectinase show a slightly higher incidence of tannins present in the juice, consistent with the short period of time it was allowed to incubate at 50° C. (Using a standard t test, there is a significant difference at the alpha = 0.10 level.)

The effect of blanching on total monomeric anthocyanin content is not evident in Table 1. Heating of the fruit (blanching) is used to inactivate enzymes present in the fruit that may have adverse effects on the final juice product (Wrolstad et al., 1980; Siegel et al., 1971; Sapers et al., 1984). It is used in the making of tomato juice to inactivate pectinases that would otherwise cause the juice to separate into serum and pulp (a procedure known as hot break). It is also used in the making of fruit juices where naturally occurring phenolases, which would destroy the anthocyanins, need to be inactivated before inoculation and incubation with a pectinase. The making of most juices involves the appropriate selection of the enzyme(s) present during the juice making process so that color and integrity of the juice product can be maintained, while breakdown of pectins is achieved. With Royal Okanogan Huckleberries the initial blanching did not appear to affect the monomeric pigment content, brix, or pH but did have adverse affects on

the juice yield and the polymeric color compounds formed.

Total monomeric anthocyanin content listed in Table 1 shows that the anthocyanin content of the berries is independent of either pectinase treatment or blanching procedure but the berries are extremely high in pigment. The average anthocyanin content (not including the anthocyanins present in the juice presscake) from Table 1 is 350.2 mg/100 grams fruit.

Ballinger et al. (1972 and 1979) reported that ripe blueberries (Vaccinium) of different genuses and varieties produced from 2.5 to 764 mg anthocyanin/100 g fruit. Pigment content of the cranberry (Vaccinium macrocarpon) was reported to average 57.7 mg/100 g fruit (Lees and Francis, 1972), ranging between 7 and 72 mg/100 g fruit (Sapers et al., 1986) or from 46 to 172 mg/100 grams of fruit (Sapers et al., 1983). Flora (1978) reported that muscadine grapes contained up to 403 mg pigment/100 g grapes. Black raspberries reported by Daravingas and Cain (1965) contained 445 mg/100 g fruit. Bronnum-Hansen and Flink (1985) reported that elderberry (Sambucus nigra L.) contains between 200 to 1000 mg/100 g fruit.

The amount of pigment reported for the members of the Vaccinium genus is considerably lower than the pigment content of the Royal Okanogan Huckleberry, another indication that the berries are not closely related. The relatively high amount of pigment found in the Royal Okanogan

Huckleberry is exceeded by the amount of pigment found in several fruits, however the pigments in those fruits are generally non-acylated mono- and di-glycosides. These types of pigment lack the stability of the acylated pigments (Rommel et al., 1990; Brouillard, 1981) similar to those found in abundance in the Royal Okanogan Huckleberry. Further, the acylated pigments like those found in the Royal Okanogan Huckleberry are more stable to light, heat, and storage than non-acylated pigments (Sapers et al., 1981). This indicates that pigments from the juice of the Royal Okanogan Huckleberry would be resistant to the kinds of degradation that affects other more labile anthocyanin pigments.

HPLC analysis of sugars in single strength Royal Okanogan Huckleberry Juice indicates that there are 2.35 g glucose, 2.88 g fructose and an undetermined amount of sucrose per L. The values obtained for sucrose were unclear due to coelution of several unidentified compounds in the juice. Unsuccessful attempts were made to remove these interfering compounds (filtration through biorex, passing through a sep-pak column). GC analysis of free juice sugars indicated that the only monosaccharides present are glucose and fructose which supports the HPLC data.

The low sugar content of the Royal Okanogan Huckleberry combined with its acid content indicates that the berries would probably need to be sweetened or mixed with

another sweet juice before consumption. The high pH of the juice indicates that in order to preserve the juice or fruit by retorting, the pH would have to be stabilized below 4.6 (Banwart, 1981) in order to be classified as an acid food. This would allow the use of less heat in the processing of the whole berries or juice (i.e. canning). Added sugar used to form a gel (jams, jellies, etc.) also lower the water activity of the food, acting as a preservative, but the pH would again need to be lowered to between 2.0 to 3.5 in order for a pectin gel to form (Whistler and Daniel, 1985).

Garden Huckleberry (S. melanocerasum) data listed in Table 1 was data taken from one processing trial. The pH of the juice, percent yield, and brix values are commensurate with values obtained for juice of the Royal Okanogan Huckleberry. The higher values recorded for titratable acidity and total monomeric anthocyanin content may be due to inherent differences between the two berries or a function of the growing conditions. Differences in the temperature, the amount of light, soil conditions, humidity, etc., play an important part in the development of anthocyanin content (Macheix et al., 1990; Rabino and Mancinelli, 1986). Although the Royal Okanogan Huckleberry and S. melanocerasum were morphologically similar and contained the same pigment compounds, the juice from the latter had an unpleasant smell and the berries were, as Heiser (1987) put it, "practically inedible".

Color and spectral characteristics

Single strength Royal Okanogan Huckleberry juice and juice from S. melanocerasum are both intensely colored. CIE L*a*b* measurements using reflectance, standard illuminant C, and a 5 mm cuvette indicates that the juice is purple black in color. This data is listed in Table 2. Transmission measurements were not practical as the juice was too dark to accurately analyze. Analysis of the juice for total monomeric anthocyanin content required a 1/100 dilution with buffer for the same reason.

Figure 2 demonstrates what happens to the color of Royal Okanogan Huckleberry juice when the pH is adjusted. Although the juice has a more intense color at low or high pH's, the single strength juice at pH 4.4 is extremely dark purple. This is characteristic of its high pigment content as well as to the acylated character of the anthocyanins present in the juice. The high absorbance between pH 4 and 6 may be due to the tannins present in the juice or due to the resistance to hydration of the anthocyanidin portion of the molecule as described by Brouillard (1983).

The juice is more intensely colored at low pH's because the flavium form of the anthocyanins predominates. This charged species is more resistant to hydration and is therefore more stable. At high pH's, the quinoidal form predominates, which again is not likely to react with water

to become hydrated (Brouillard, 1983). The absorbance maxima (see Table 3 and Figure 2) for the juice pigments shift towards longer wavelengths as pH increases indicating a change in the molecular structure. At pH 8 there is a 24 nm increase in wavelength over the maxima at pH 1. This is due to the formation of the quinoidal anhydro base which has one extra double bond in the molecule and would account for some of the shift in absorbance.

Comparison of spectral shifts (Figure 2) to similar data reported for cyanidin-3-5-di-glucoside (Mazza and Brouillard, 1987) and for the di-acylated pigment cyanidin 3,7,3'-triglucoside acylated with caffeic and ferulic acid (Brouillard, 1981) indicates that limited protection of the pyryllium rings of the pigments in the Royal Okanogan Huckleberry is taking place at pH's near neutrality. There is a strong indication that the equilibrium shifts toward the formation of the colorless anhydro base between the pH of 4 and 6. Even though there is evidence that there is color degradation at these pH's, the juice (pH 4.4) has a dark purple color. This phenomena is probably a function of inter- and intra-molecular copigmentation.

Copigmentation, a synergistic interaction between a chromophore and another usually colorless molecule, can occur as a function of hydrophobic interactions between anthocyanins and any number of compounds including polysaccharides, alkaloids, flavanoids, metals, or other

anthocyanin molecules (Mazza and Brouillard, 1990; Osawa, 1982). Difficulty in filtering indicates that there are high molecular weight compounds present in the juice, probably polysaccharides. Perhaps these molecules interact with the anthocyanin compounds present, acting as copigments. High absorbance by unidentified compounds at 280 indicates that other compounds, possibly cinnamic acids, are present in relatively large amounts. These compounds could act as copigments or as pigment stabilizers due to their planar ring structural components. Also, since the Royal Okanogan Huckleberries have tentatively been identified as belonging to the family Solanaceae, a family known for being rich in alkaloids, it is not unthinkable that these compounds act also as copigments.

Copigmentation can occur between an anthocyanin acylated with a cinnamic acid group at the 3 position and another molecule containing an aromatic group by hydrophobic interaction (Hoshino et al., 1980) which stabilizes the anthocyanin. (It has been found that the pigments in Royal Okanogan Huckleberries are mostly acylated with cinnamic acids.) Incubation of unblanched berries with pectinases increases the amount of tannins present in the juice, as indicated in Table 1. This formation of polymers could be a result of endemic enzymes that act to solidify the bonds between anthocyanin molecules and copigments that are naturally present in the juice. Another explanation, which

accounts for the fact that total monomeric anthocyanin content does not change, is that the enzymes present cleave off bits of larger molecules (like polysaccharides) to which anthocyanin molecules have been attached during the maturation of the fruit.

Saito et al. (1985) found that stability of the anthocyanins increased with the degree of acylation with cinnamic and malonic acids as well as with the degree of substitution of hydrogens on the B ring. This means that the delphinidin derivatives found in the Royal Okanogan Huckleberry (delphinidin, petunidin, and malvidin) are more stable than the cyanidin derivatives (cyanidin, pelargonidin, and peonidin). Further, since over 96% of the pigments found in the Royal Okanogan Huckleberry are mono-acylated with caffeic, p-coumaric, or ferulic acid, they should be more resistant to degradation than non-acylated pigments (Sapers et al., 1981). They could also be more stable than pigments with more than one acylating acid (see Teh and Francis's work, 1988).

Another way of depicting the remarkable color change at different pH's is shown in Table 3. This table lists the changes in color and intensity with the change in pH using CIE L*a*b* measurements of reflected light, specular component included. Using a spectrophotometer, data for Figure 2 was collected, the maximum absorption (lambda max), and the amount of absorption at lambda max (Amax) are also

included in Table 3.

Even though the juice was diluted to 1/50 with buffer, the juice was dark in color, as evidenced by the L* values recorded. Little (1975) suggests the use of the fraction b/a to describe color change but the ratios derived for this juice resulted in abrupt changes in value at pH 8 due to the blue-green color. Francis (1975) prefers using the hue angle or $\tan^{-1}$ a/b which presented problems in that the values again make an abrupt switch from negative to positive at pH 8 because the juice became blue-green in color. Sapers et al. (1984) solved the problem of confusing hue angles in the blue regions by normalizing the angles to 360°. This can be visualized by setting (a+, b=0) to 360°, (also equals 0°) and moving counter clockwise through the Hunter color solid perpendicular to the L axis. Under this schemata pure yellow is at 90°, green at 180°, blue at 270°, and red at 360° or 0°. From Table 3 it can be seen that the hue angle steadily progresses towards the blue-green region as pH increases.

The unique color of this juice at different pH's may lend itself to its use as a food colorant. Although blue at pH 8, very few foods are this basic. At pH 1 it is dark red and might make an acceptable substitute for some of the red food colorants currently being used, but again, few foods are this acidic. At the pH of many foods where this berry might be used as a colorant, the color would be purple or reddish blue. This would make it appropriate for the coloring of

juice drinks, berry products or yogurt which have a lower pH and are desirable products when purple in color. Its intense color may also have uses in products using edible packaging (i.e. sausage casings) or stamps of approval that go directly on the product, such as USDA meat inspection stamps.

Characterization of anthocyanin pigments in Royal Okanogan Huckleberry

Figure 3 shows comparative anthocyanin pigment profiles for pigments extracted from cranberry juice, blueberries (both members of the Ericaceae family, genus Vaccinium), blackberry juice (Rosaceae family), eggplant skins, purple peppers, and purple tomatillos (Solanaceae family) and Royal Okanogan Huckleberries. Fruits were examined for similarity in pigment profile based on the belief that the berries were related botanically (according to information supplied by the grower) or because of their intense purple color. Figure 3 demonstrates that the pigment profile from the Royal Okanogan Huckleberry does not overtly resemble that of the cranberry, blueberry, or eggplant and therefore the berries were not believed to be closely related. Pigments from purple tomatillos and purple peppers more closely resembled the pigments of the Royal Okanogan Huckleberry in that the pigments all demonstrated long retention times.

Figure 4 shows the profile of pigments extracted from

Royal Okanogan Huckleberry. Twelve peaks visible at 520 nm were ultimately separated out from the pigment via HPLC using an end capped ODS-2 column. Nine of the peaks demonstrate long retention times characteristic of anthocyanins acylated with a relatively hydrophobic group such as a cinnamic acid (Hong and Wrolstad, 1990; Lea, 1988).

Figure 9 is an HPLC chromatogram of pigments extracted from the Royal Okanogan Huckleberry and then subjected to KOH saponification to remove acylating acids. The chromatogram shows that at 520 nm only three peaks are left after treatment indicating that 9 of the original peaks contain acylating groups. Further, the three resulting peaks have retention times that coincide with those of the first three peaks of the unsaponified pigment extract.

In Figure 10, two chromatograms are superimposed over each other, peaks 1, 2, and 3 of the unsaponified pigments and peaks A, B, and C that are a result of the treatment with KOH. The matching retention times and approximate proportions indicate that peaks 1, 2, and 3 are deacylated pigments that exist as natural components of the pigment extracted from the fruit. The fact that KOH saponification of the pigment extract reduced the total number of peaks from 12 to 3 indicates that the acylated peaks have three possible deacylated structures.

Figure 4 shows the absorbance at 280 nm as well as the absorbance at 520 nm of compounds separated out from Royal

Okanogan Huckleberry pigment extract. The compounds monitored at 520 nm are anthocyanic compounds whereas the peaks monitored at 280 nm are largely unidentified. Interference by compounds that absorbed heavily in the UV region tended to distort the spectra obtained for many of the smaller anthocyanin peaks. This means that it was impossible to confirm matches between peaks with the same retention time. Peaks 1 and A, as well as peaks 3 and C fall into this category where, although they have the same retention time, the spectra are of insufficient quality to confirm a match. Peaks 2 and B were of sufficient size and a spectral match between the two has been confirmed.

Figure 9 is a chromatogram of deacylated pigments seen at 520 nm, but when monitored at 280 nm (Figure 11), the acids cleaved from the pigments can be seen. This chromatogram shows the retention of peaks A, B, and C, as well as the 3 acylating acids identified by both retention time and spectra as caffeic, p-coumaric, and ferulic acid. This is evidence that the 9 acylated peaks present in the pigment are made up of 3 different deacylated pigments and that the difference between the acylated peaks are the different acids shown in this chromatogram.

Also present in the chromatogram (Figure 11) is an unidentified acid that elutes within 0.25 min of the deacylated delphinidin peak. This acid could be an artifact from the compounds present in the berry or it could be an

indication of a second acylating acid. Chromatographic evidence indicates that this peak, as well as caffeic acid is present after the KOH hydrolysis of peak 4 and is visible at 280 nm. Since the retention time is relatively short for an acylated pigment, acylation with 2 cinnamic acids is not suspected. Chlorogenic acid is consistent with this hypothesis since it is made up of caffeic and quinic acid.

Analysis of the anthocyanidins present as part of the pigments of the Royal Okanogan Huckleberry indicated that three different aglycons were present as delphinidin, petunidin, and malvidin. Highbush blueberry and concord grape pigments were used as standards because both were known to be composed of derivatives of the 5 of the 6 most common anthocyanidins, delphinidin, cyanidin, petunidin, peonidin, and malvidin (Hong and Wrolstad, 1990; Ballinger et al., 1979, 1970). Strawberry pigments were also used as standards for cyanidin and pelargonidin, again reported by Hong and Wrolstad (1990).

Figure 12 is a triple chromatogram of blueberry, strawberry and Royal Okanogan Huckleberry anthocyanidins separated out on the C-18 system. The presence of petunidin, as opposed to cyanidin, was confirmed through comparison of the spectra from 250 to 600 nm to the standards. The chromatogram clearly shows that delphinidin and malvidin are present as part of the pigments present in Royal Okanogan Huckleberries and was also confirmed through spectral

comparisons to the standards. Although there is a small peak present in the Royal Okanogan Huckleberry aglycon extract that approximates the retention time of pelargonidin, the peak has not been identified. The peak appeared in several of the aglycon analyses but was of insufficient quantity to either confirm or deny its identity.

Acid hydrolysis of the pigment extracts was used to separate the sugars from the pigments in order to identify them by GC. Two sugars were present, glucose and rhamnose, in a two to one ratio. This means that of the three deacylated peaks, at least the middle peak, by far the largest, contained one mol of rhamnose and two mols of glucose.

Analysis of peak fractions collected from Royal Okanogan Huckleberry pigment

Fraction separation and collection of the individual peaks was carried out in order to determine the identity of the acylated pigments. (Peaks 1, 2, and 3 were not collected.) Peak purity was checked by injecting collected samples back into the HPLC. All of the collected peaks contained a relatively small amount of the major pigment (peak 8) as a contaminant and small peaks (i.e. 4 and 6) contained a small amount of a larger peak that eluted nearby (i.e. 5). The peak fractions were analyzed to determine the

retention time after alkaline hydrolysis, the identity of the acylating acid, which sugars were attached, and the identity of the anthocyanidin. This data is presented in Table 4 where the peak number given refers to order of retention time (corresponding to Figure 4-520).

The peaks were analyzed as to anthocyanidin content in two ways. The acid hydrolysis method was used where the anthocyanidins were compared to anthocyanidins from acid hydrolysis of blueberry and strawberry pigments both spectrally and by retention time. The other method used was the comparison of deacylated peak retention time and spectrum to that of deacylated whole pigment retention times and spectra. This allowed the deacylated peak to be grouped as coinciding with one of three possible deacylated peaks, thus making it possible to confirm the anthocyanidin content by comparing data obtained for other peaks. This became important when the peak fractions yielded very small amounts of pigment that acid or base hydrolysis diminished to an even smaller amount. For example, peak 4 from Table 4 eluted at a time where peak A should have eluted and the anthocyanidin profile demonstrated that it was either delphinidin or petunidin. From this, the peak was tentatively identified as a delphinidin derivative since peak A was believed to be the delphinidin glycoside.

The predominance of petunidin and p-coumaric acid created problems in data interpretation because they tended

to show up in all of the data analyzed. This is not surprising in light of the fact that petunidin makes up the basis for over 85% of the total pigments present and p-coumaric constitutes a portion of over 80% of the pigments. The pervasive nature of these two compounds is evident in Table 4 where they appear as a possible constituent of each of the various peaks isolated from Royal Okanogan Huckleberry pigments.

Data collected from the alkaline hydrolysis of each of the peaks occasionally yielded mixed results in terms of which acid had been attached. While there was only one distinctive peak for the deacylated pigment corresponding to peaks A, B, or C, often there was more than one acid present in the deacylated pigment extract. Again, this is due to the prevalence of p-coumaric acid in all of the extracts studied. Table 4 shows that all of the peaks tested for acylation were found to contain p-coumaric acid in the extract. Some of the peaks contained other acids as well and it is the appearance of these cinnamic acids that indicated that these acids were probably the ones attached to the anthocyanin peak. Since the peak fractions were collected from a narrow band eluting from the HPLC column, the acids present in the deacylated fractions had to have originated from specific compounds. Although all fractions were contaminated with the major peak, the presence of other cinnamic acids is a strong indication that they were a component of the pigment being analyzed.

When each of the peaks were acid hydrolyzed to identify the anthocyanidins, the sugars were removed for separate analysis and identified by GC. Although the acylated peaks were individually subjected to sugar analysis, only peak 8 yielded conclusive proof of containing 1 mol of rhamnose and two mols of glucose. All other acylated peaks indicated the presence of glucose in small quantities but other sugar(s), if present, were of too small a quantity to identify. This indicates that glucose is the predominant sugar attached to the anthocyanidin, which is supported by the literature (Harborne and Swain, 1979; Francis and Harborne, 1966) and by GC analysis of the whole pigment extract. This hypothesis does not conflict with all of the anthocyanins being of one glycosylated form (3-rutinoside-5-glucoside) since rhamnose would be present at one half that of glucose. Since the glucose peaks for the individual fractions collected were so small, peaks half that height would be impossible for the GC to pick up.

From the data obtained during KOH hydrolysis it has been shown that all of the petunidin derivatives are of the same glycosylated form. Since the mechanism exists to add two mols of glucose and one mol of rhamnose to all of the petunidin derivatives, it is hypothesized that the same mechanism is used to add the same sugars to both delphinidin and malvidin. It is therefore hypothesized that all of the peaks separated from the whole pigment are identical as to

sugar makeup.

Harborne (1958) devised a method of comparing absorbances of anthocyanin pigments at their maxima (lambda max or Amax) to absorbances at 440 nm (A 440) in order to determine whether or not sugars were attached to both the 3 and the 5 position of the anthocyanin. These values are expressed as a percentage of the absorbance at Amax. Values derived for these ratios, using the methods of Harborne, are listed in Table 4 as A440/Amax.

Harborne's results from his 1958 paper indicate that sugar ratios for delphinidin, petunidin, and malvidin mono-glycosides have sugar ratios ranging from 16 to 19% and the di-glycosides from 9 to 12%. The values listed for the acylated peaks in Table 4 range from 9 to 13% indicating that all of the acylated peaks and peak 2 have sugars attached at the 3 and 5 position of the anthocyanin molecule. Since problems existed with coelution of unknown compounds that affect the spectra of peaks 1 and 3, the values obtained for these peaks are of questionable value. Both peaks have high sugar ratios indicating that they seem to be monoglycosides, however the most likely explanation is that the amount of absorbance due to interfering compounds has affected the spectra so that accurate ratios of A440/Amax cannot be determined for these peaks.

Harborne also devised a method of comparing the absorbance of the pigment at the wavelength of maximum

absorption for the attached acid to the Amax of the pigment in order to determine the number of acids attached to the anthocyanidin. Unfortunately, due to the presence of interfering compounds in the Royal Okanogan Huckleberry pigment extract that absorb heavily in the UV region, this type of analysis was impractical (compare figures 4-520 and 4-280).

Spectra from the individual peaks that were separated from the pigments in Royal Okanogan Huckleberries can be seen in Figure 13, where some interesting patterns emerge. Peaks 5, 8, and 10 each have a similar spectra between 250 and 350 nm. This region is where the absorbance of the acylating cinnamic acid is most pronounced and indicates that the acylating acid for these three peaks is identical. This observation is borne out by chemical analysis that indicates that the acid attached is p-coumaric.

Peaks 4, 9, and 11 also have similar profiles between 250 and 340 nm. Peak 9 and 11 were identified as containing ferulic acid but peak 4 has been assigned as having caffeic acid and another unidentified constituent as the acylating group based on chemical analysis. The most likely explanation for this discrepancy is the distortion of peak 4 by the large unidentified peak that absorbs strongly at 280 nm and elutes between peaks 4 and 5 (see Figures 4-520 and 4-280).

Peak 6 is another example of what happens when

interfering compounds are present. The spectra between 250 and 350 nm is distorted to the point where it would be impossible to deduce much based on its spectral characteristics alone. Fortunately, there was enough of this peak collected as a fraction to confirm the presence of ferulic acid as part of the anthocyanin molecule.

Peak 12 doesn't match the spectra of any of the other peaks but evidence suggests that it is attached to 2 molecules of p-coumaric acid. This is consistent with what would be expected of the Royal Okanogan Huckleberry if it is related to S. guineese studied by Francis and Harborne (1966) and also accounts for its long retention time.

Species identification of the Royal Okanogan Huckleberry

Information regarding the genus and species of this berry was anecdotal rather than helpful. It was therefore necessary to put together bits and pieces of information gleaned from the sparse literature regarding huckleberries and from chemical analyses done in the lab.

Morphological characteristics of the Royal Okanogan Huckleberry indicated that it was not related to the either the blueberry or the cranberry. This was supported by anthocyanin profiles made of the two Vacciniums as well as that of the Royal Okanogan Huckleberry. Since the actual plant was not available for study it was necessary to attempt

to identify the berry on the basis of pigment analysis and on basic berry morphology.

The Royal Okanogan Huckleberry is a deep purple color that borders on being black. When cut across the equator it resembles a cherry tomato in that it is divided into sections filled with a jelly-like material containing the lenticular, ovoid seeds which are pointed at one end. There are two major sections (locuses) within the fruit that each have a further 'false' sub-division. This makes it appear that there are actually 4 divisions although scrutiny under a strong magnifying glass shows that this is not the case. Pigment is contained throughout the 1 cm diameter fruit.

Unlike the fruit of the blueberry family the Royal Okanogan Huckleberry is a superior ovary as evidenced by only a slight indentation remaining on the bottom of the berry from the attachment of the style. (The blueberry is an inferior ovary which retains an area at the apex of the fruit where the petals were once attached; Halse, 1991.) In some cases, a small 5 or 3 lobed calyx attached to a stem remained attached to the frozen berries. Information from the supplier of the fruit indicated that Royal Okanogan Huckleberries grew in clusters where several of the berries were attached at the base of their stems in an umbellate fashion, consistent with information regarding some members of the Solanum genus of the Solanaceae family.

A review of the literature indicated that it was

possible that the huckleberry could be defined as a member of the family Solanaceae or nightshade. Since huckleberries from this family are difficult to obtain, it became necessary to plant a garden in order to grow authentic samples of the Solanaceae family thought to be closely related to the Royal Okanogan Huckleberry.

Seeds were germinated indoors early in the spring and then transferred outside once the danger of frost had passed. Seeds from the frozen Royal Okanogan Huckleberries were included in the study but did not germinate. Either the process of freezing and thawing damaged the tissue so that it could not germinate or the cross used to produce the berry (if there was one) rendered the plant incapable of producing viable seeds. Fruit from Solanum burbankii, S. melanocerasum, S. nigrum (not planted but gathered from wild plants found in the area) and Physalis ixocarpa were gathered as they ripened in late summer and early fall.

Fruits of S. melanocerasum were approximately the same size (around 1 cm) as Royal Okanogan Huckleberries and from all outward appearances were the same berry. The author found the aroma and flavor of the two berries to be different. The aroma of both berries was similar to that of a potato but the melanocerasum was slightly more pungent. Neither was particularly objectionable nor were they enticing. The flavor of the raw Royal Okanogan Huckleberry was earthy with a few blueberry notes to it. The flavor of

the melanocerasum was more pronounced in that it had a very distinctive 'perfume' in the mouth. These differences could be due to factors such as soil pH or due to intrinsic differences in the berries even though other evidence suggests that they are very closely related.

Both S. nigrum, and S. burbankii produced small berries (approximately 0.5 cm in diameter) and unlike the Royal Okanogan Huckleberry, contained most of its pigment in the skin. The purple Tomatillos (P. ixocarpa) were 2 - 3 cm in diameter and were encased in a paper like calyx, indicative of the Physalis genus and presented a purple blush on the surface of the fruit where the fruit had burst through the calyx and was exposed to the sun.

The anthocyanins present in the fruit of these plants were extracted with acetone and partitioned with chloroform for comparative purposes. (Only the skin of the tomatillos was used.) Extracts were separated on the ODS-2 HPLC system and Figure 14 shows the results from this analysis. Table 5 lists the area percentage of the peaks (monitored at 520 nm) that match in retention time to that of the Royal Okanogan Huckleberry. Each percentage listed is the percent of the total peak area of the separated extracts monitored at 520 nm for that particular fruit. Where noted, the area percent indicates that a peak matched the peak listed (1 - 12) for Royal Okanogan Huckleberry both spectrally and in retention time. In some cases it was possible to obtain

matching spectra of peaks with matching retention times, and in other cases it was not, especially if the peaks were small and the interference large. Peak 5 matched both spectrally and in retention time for four of the berries. Peaks 8 and 10 matched for all 5 of the berries.

Figures 4 through 8 show chromatograms of the pigment extract from these berries at 280 nm and at 520 nm. These chromatograms show the overwhelming absorbance in portions of the UV region that negated the possibility of obtaining matching spectra for many of the pigment peaks with similar retention times. Figures 6 and 7 are chromatograms of S. nigrum and S. burbankii that show strong similarity in retention time for compounds that absorb at 280 and 520 nm. Matching spectra for many of the peaks monitored at 520 have been obtained for these two species because the 280 profiles are so similar.

The author's hypothesis is that peaks with similar retention times are in fact identical and are indicative of their membership in the Solanaceae family. Without the proof of matching spectra however, this is only a theory. In order to prove this theory, several methods were used to purify these pigments. Ether precipitation of the pure pigment to remove impurities was attempted but selective dissolution of individual peaks resulted. Changes in columns and gradients were also ineffective in getting rid of the interfering compounds.

Judging solely by retention time of the 12 peaks present in the Royal Okanogan Huckleberry these same peaks are present in S. melanocerasum. These two berries share 11 peaks with S. nigrum and 10 peaks with S. burbankii and P. ixocarpa (please se Table 5). Spectral inspection indicates that there is a considerable number of interfering compounds that absorb in the uv region. These compounds make it difficult to confirm the identical nature of the individual pigment peaks. All of the fruits tested contain the major pigment, identified as petunidin 3-(p-coumaroyl rutinoside)5-glucoside, in large amounts.

It is interesting that spectral matches have been confirmed for S. nigrum and S. burbankii for many of the peaks. Not only do these two species have similar absorbance profiles at 520 nm but they also have nearly identical profiles at 280 nm. This would account for the matching spectra of the individual peaks since the interference in the UV region is the same for each of the peaks in question. This would indicate that these two plants are extremely closely related, if not the same plant.[1] The purple tomatillo has the least amount of similarity to the other

[1] These fruits may be of the same species. The plants, flowers, and berry structure look as alike as the chromatograms monitored at 280 and 520 nm. However, it is not the author's intention to enter into the debate that has been raging for nearly a century concerning whether or not S. burbankii is actually just wild nightshade that grows commonly throughout north America. For more information, please see the chapter on Wonderberries by Heiser (1987).

species, as would be expected from the presence of the enveloping calyx and other morphological characteristics, but still demonstrates the presence of several of the pigments characteristic of this group.

From this data it can be deduced that the Royal Okanogan Huckleberry is more closely related to S. melanocerasum than to some of the other members of the Solanum genus. Berry morphology in terms of size, pigment distribution within the fruit, location of seeds, etc., also are similar to that of S. melanocerasum although they smell and taste slightly different to the author. Pigments bear some resemblance to P. ixocarpa in some of the evident peaks which demonstrates how closely related the genuses Physalis and Solanum are to each other. Finally it might be noted that the Wonderberry (S. burbankii) is very closely related to the wild S. nigrum that is widely believed to be poisonous.

Conclusions

From the data it can be concluded that the Royal Okanogan Huckleberry is definitely not a member of the vaccinium genus but rather of the genus solanum. This conclusion has been supported by HPLC data on the pigments present in these genuses and substantiated by comparisons of fruit and seed morphology of both genuses. Placentation pattern and HPLC pigment profiles place the Royal Okanogan

Huckleberry as a variety of Solanum scabrum.

The major pigment found in Royal Okanogan Huckleberries comprises over 70% of the total pigment and has been identified as petunidin-3-(p-coumaroyl-rutinoside)-5 glucoside. The identification of the major pigment is consistent with the findings of Francis and Harborne (1966) and Saito et al. (1965) for S. guineese and S. nigrum var. guineense respectively. Other pigments include delphinidin-3-rutinoside-5-glucosides acylated with p-coumaric, ferulic and an unidentified acid; malvidin-3-rutinoside-5-glucosides acylated with p-coumaric and ferulic acid; and petunidin-3-rutinoside-5-glucoside acylated with 2 p-coumaric acid moieties.

Over 97% of the anthocyanins present in the Royal Okanogan Huckleberry are acylated with cinnamic acids. This indicates that they would have increased stability as a food colorant. The high amount of anthocyanin found in the Royal Okanogan Huckleberry means that purification procedures could be kept to a minimum, which would further reduce the cost of using these pigments as a food colorant.

The juice from the Royal Okanogan Huckleberry is rich in color and has a total monomeric anthocyanin content of over 5 g/L. It has a high pH for a fruit (4.4), a low titratable acidity (0.6 %), and an average brix of 5.3°. The sugars present in the juice are glucose (2.35 g/L) and fructose (2.88 g/L).

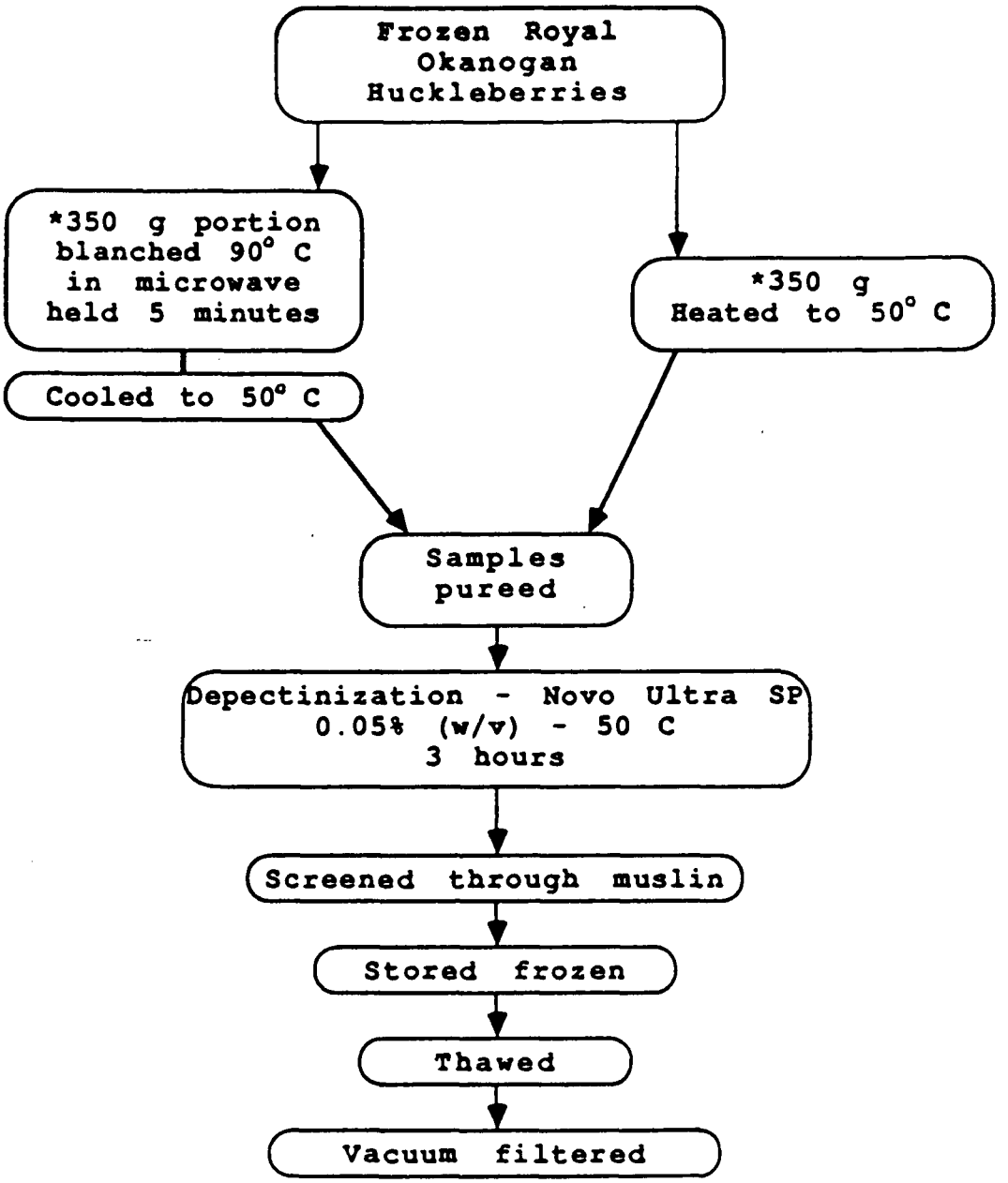
CIE L*a*b* results from acidified juices indicates that the color ranges from red at pH 1 to blue-green at pH 8 making it a likely candidate for use as a food colorant such as was proposed by Francis and Harborne (1966).

A final note

Dr. Richard Halse (1991) of the Department of Botany and Plant Pathology at Oregon State University, confirmed the author's suspicions that the Royal Okanogan Huckleberry was most closely related to the fruit of S. melanocerasum by identifying both as S. scabrum. Comparisons of seed shape and alignment within the fruit as well as the fruit structure were made to samples held in the Botany department at OSU and to placental patterns reported by Nee (1986).

S. melanocerasum has been renamed to S. scabrum (D'Arcy, 1979; Heiser, 1987; Schilling, 1981) but it is reported in this paper as S. melanocerasum because that is what it said on the seed package. Further, since there appears to be great confusion as to the exact classification of this berry, the results have been reported as belonging to melanocerasum in order to avoid confusion.

Figure 1: Flow diagram of process for making Royal Okanogan Huckleberry juice.



* Triplicate samples

Figure 2: Comparative Spectra of pigments from Royal Okanogan Huckleberry juice adjusted to pH 1.17 (a), 2.00 (b), 3.03 (c), 4.05 (d), 4.58 (e), 5.02 (f), 6.03 (g), 7.13 (h) and 8.01 (i).

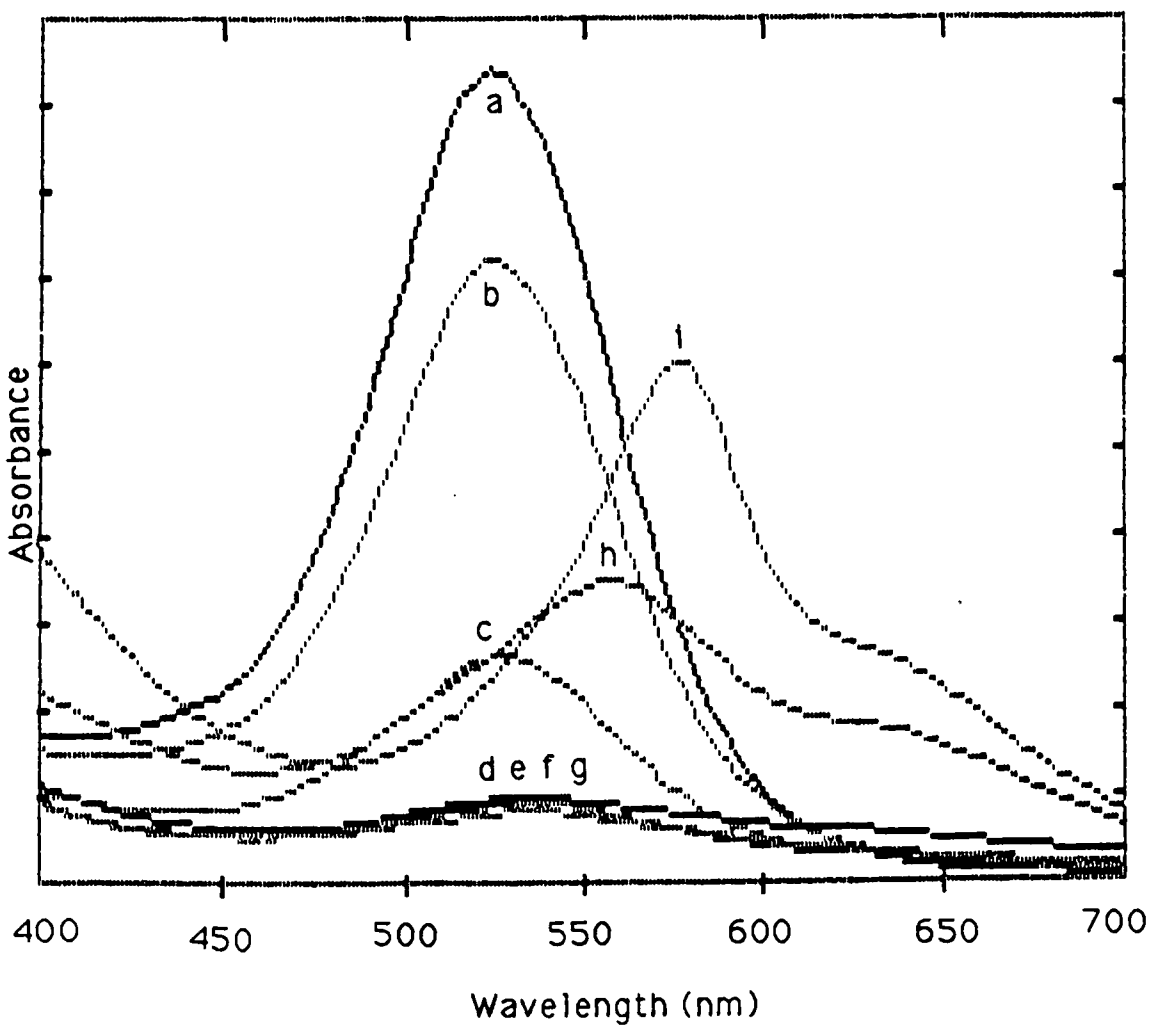


Figure 3: Comparative pigment profiles from various fruits of the Solanaceae, Roseae, and Ericaceae families.

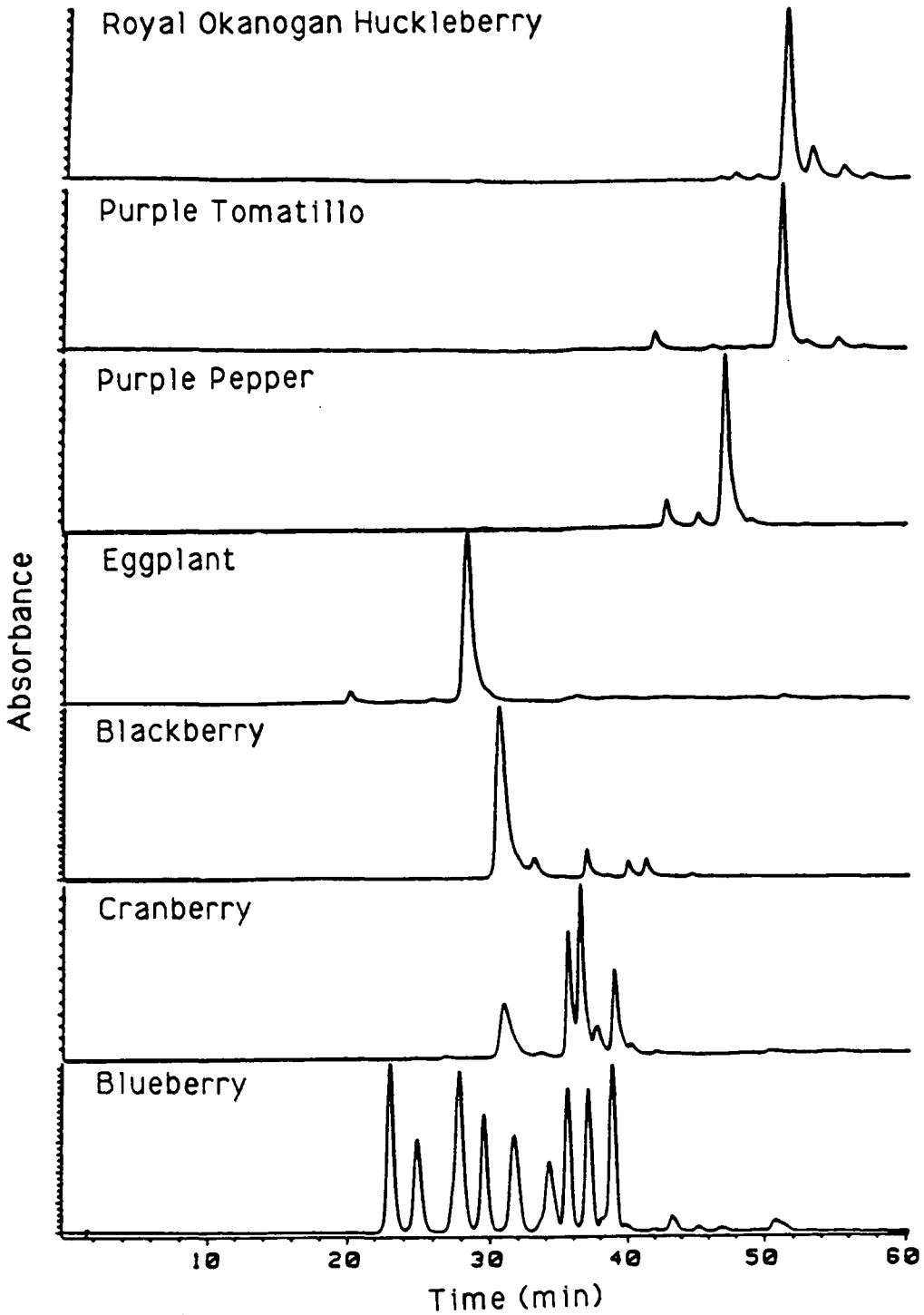


Figure 4: Pigments extracted from Royal Okanogan Huckleberries monitored at 520 and 280 nm.

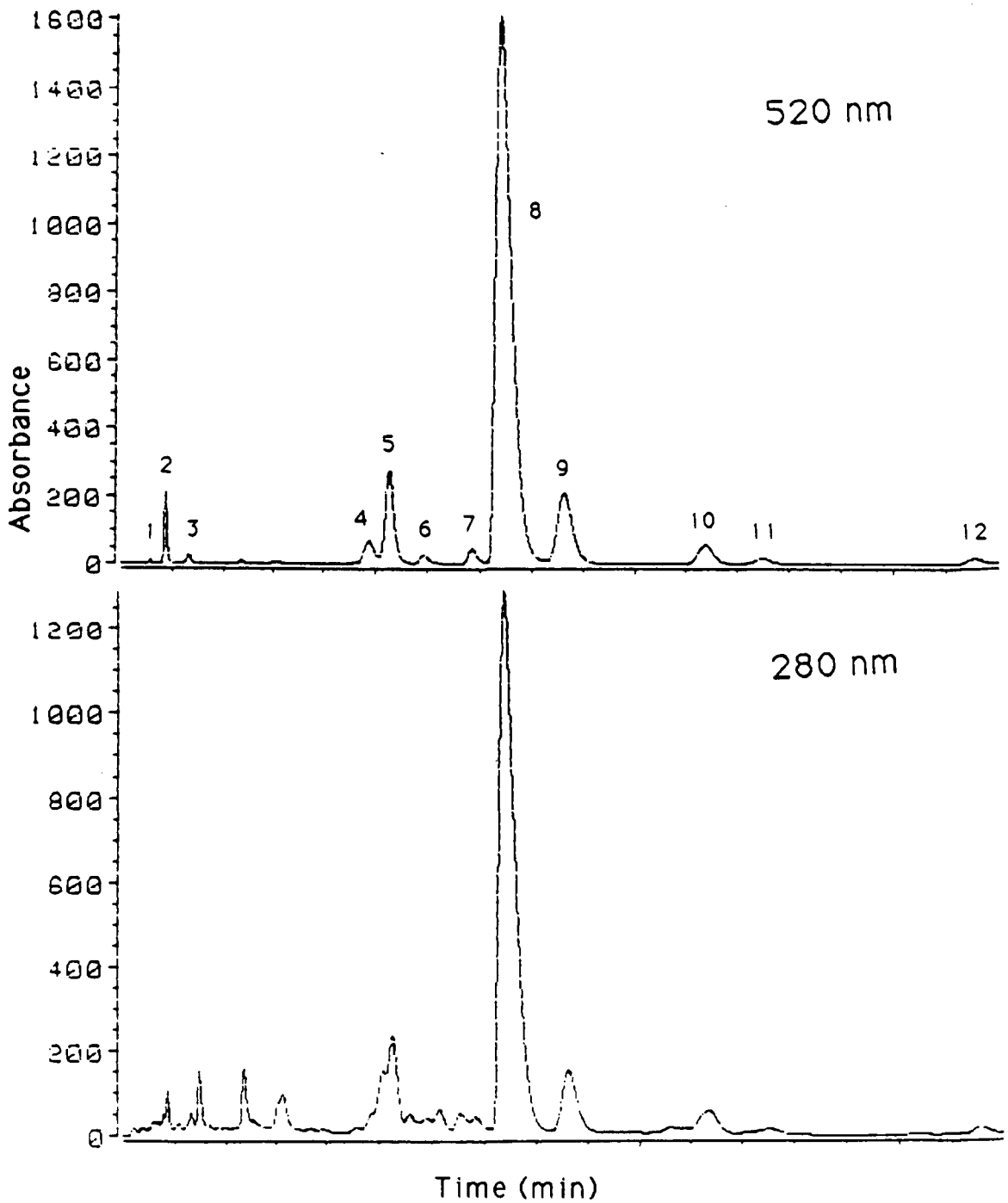


Figure 5: Pigments extracted from Solanum melanocerasum monitored at 520 and 280 nm.

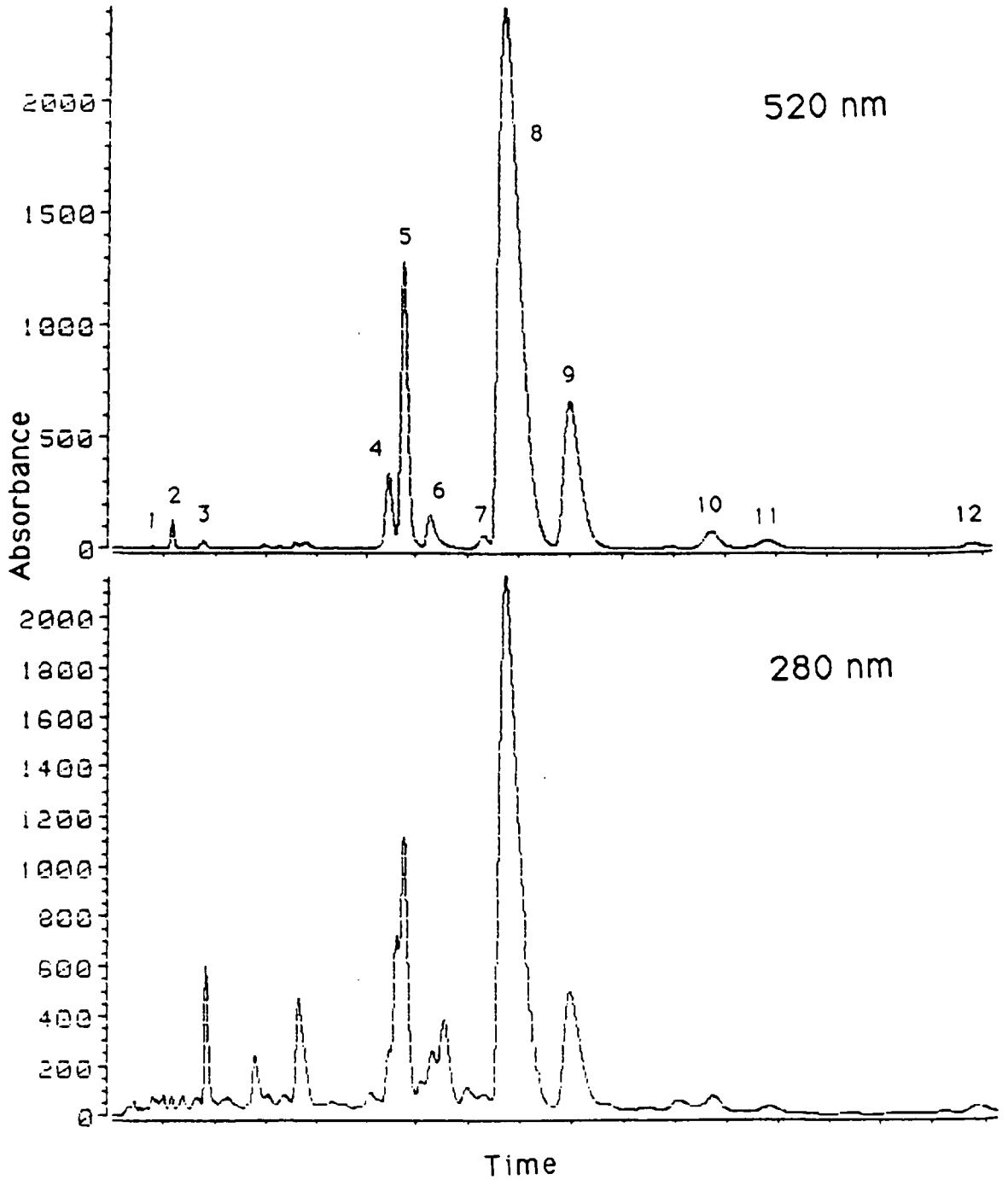


Figure 6: Pigments extracted from Solanum nigrum monitored at 520 and 280 nm.

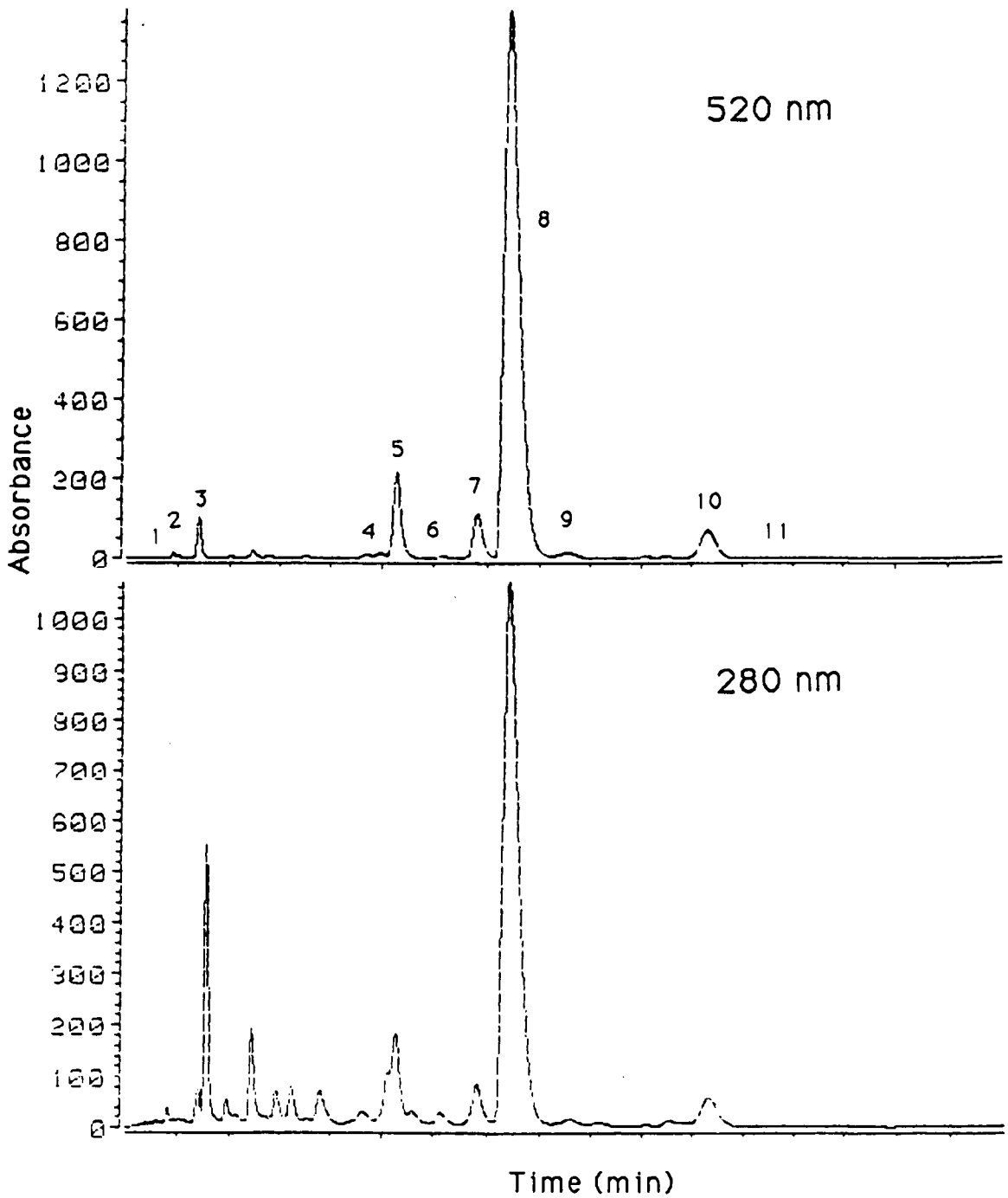


Figure 7: Pigments extracted from Solanum burbankii monitored at 520 and 280 nm.

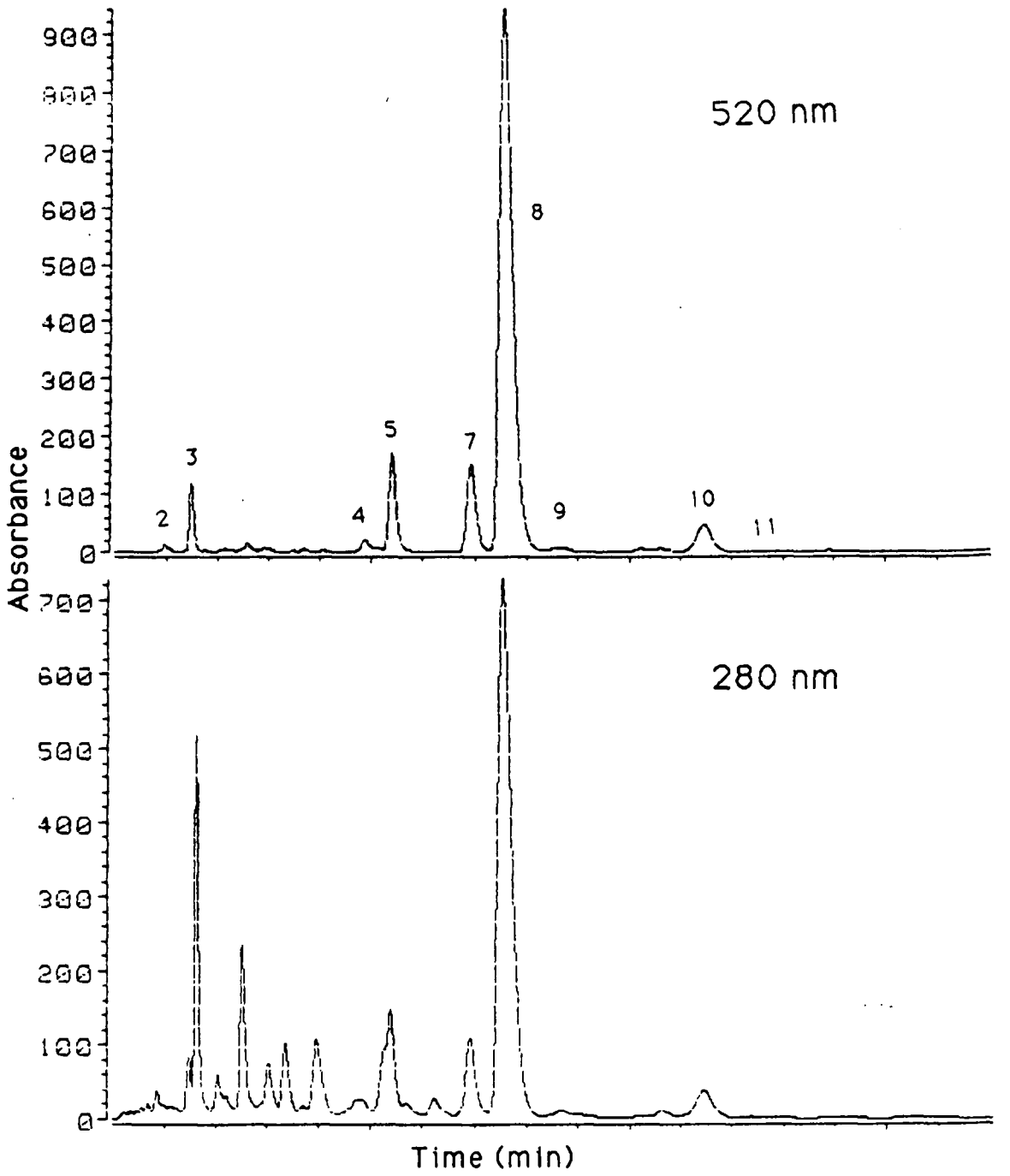


Figure 8: Pigments extracted from Physalis ixocarpa monitored at 520 and 280 nm.

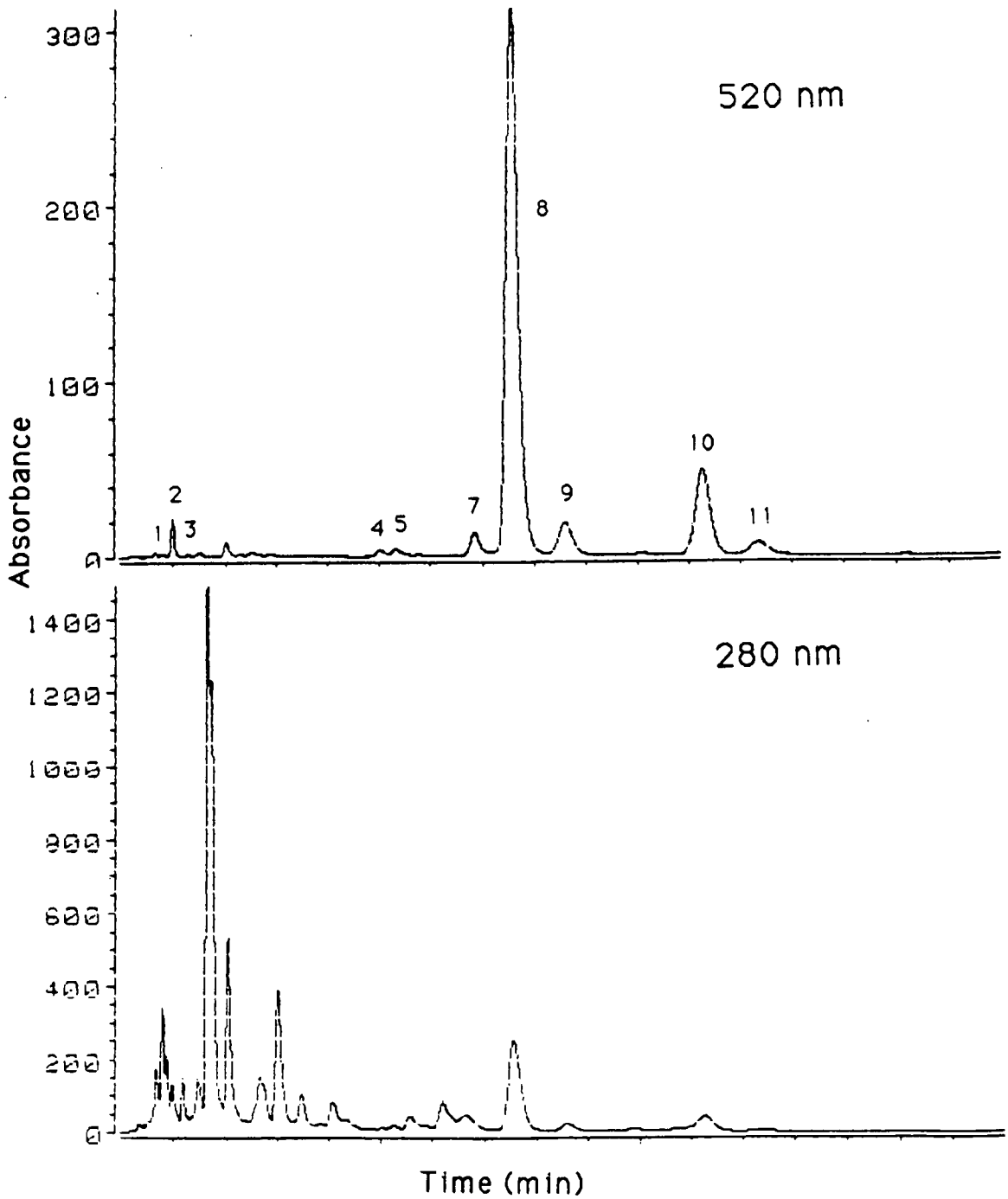


Figure 9: Deacylated pigments from Royal Okanogan Huckleberry monitored at 520 nm.

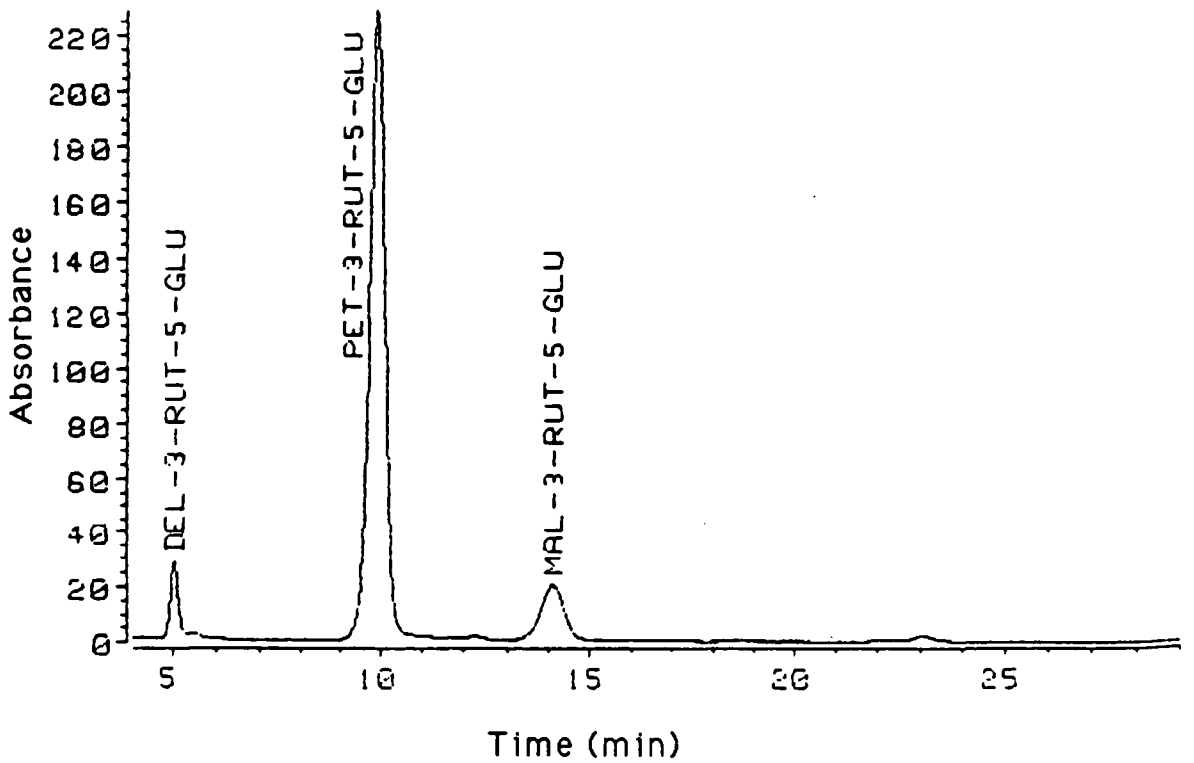


Figure 10: Deacylated peaks from Royal Okanogan Huckleberry juice (A, B, C) showing similar retention time to the first 3 peaks of whole pigment isolate (Corresponds to Figure 4-520).

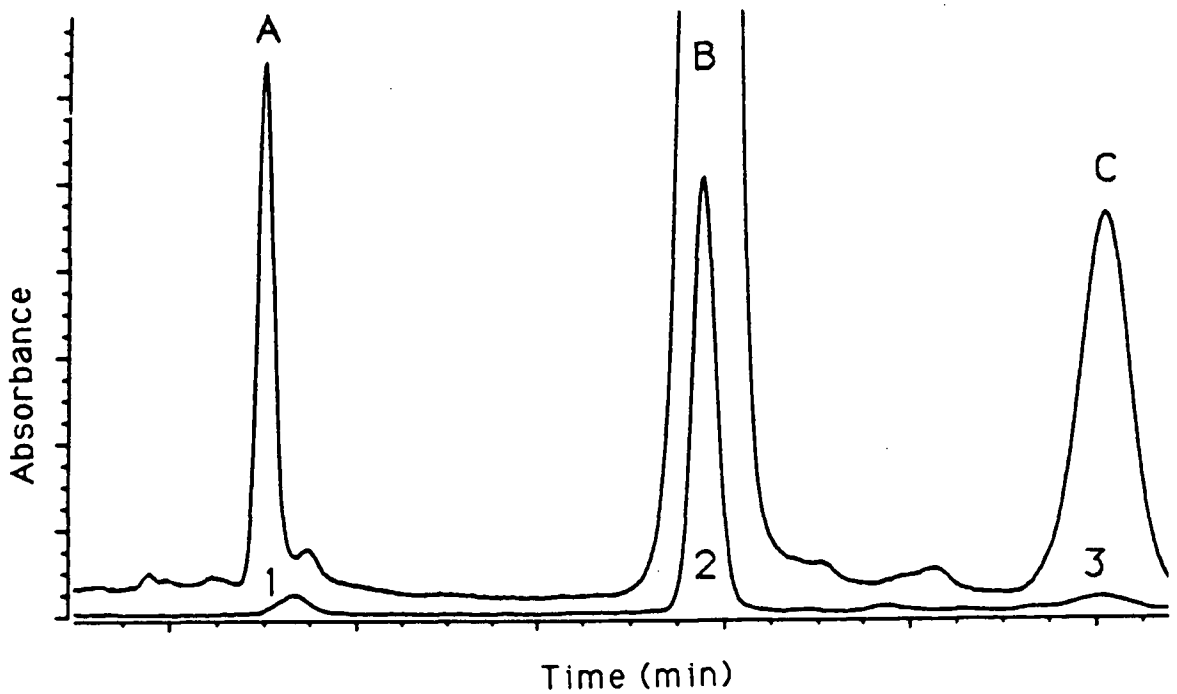


Figure 11: Deacylated Royal Okanogan Huckleberry pigment monitored at 280 nm showing both pigments and acids cleaved by alkaline hydrolysis.

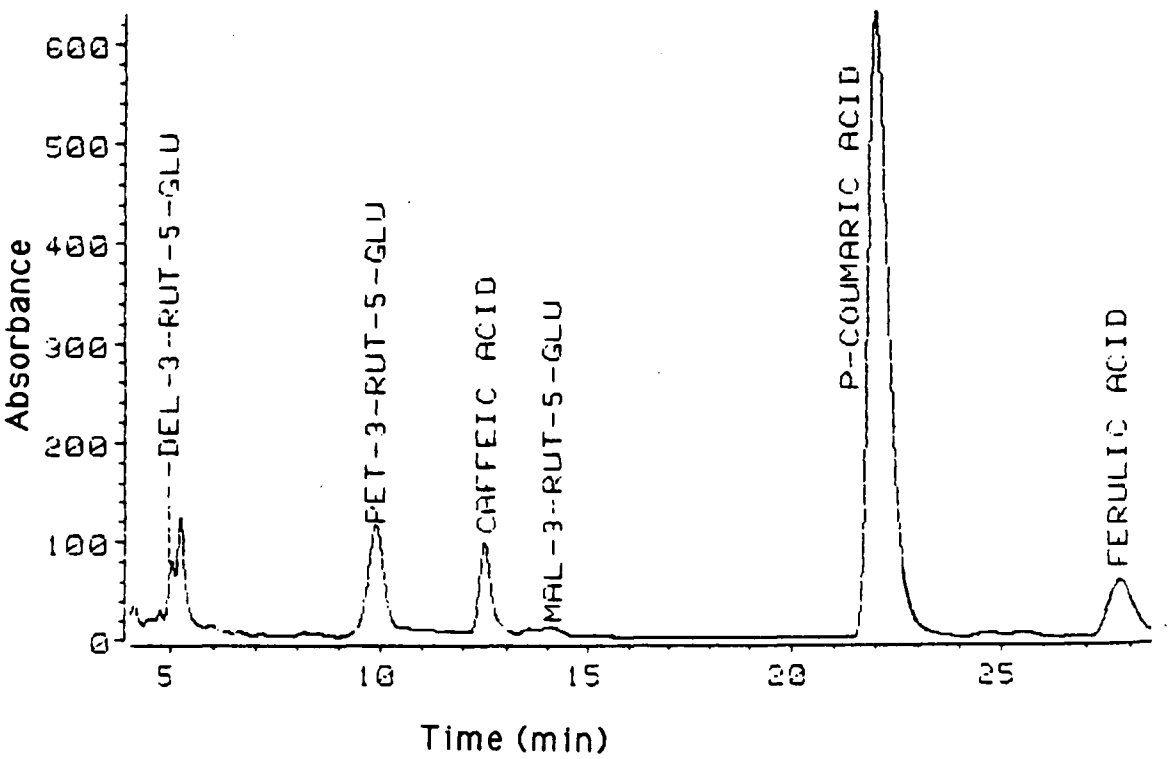


Figure 12: Anthocyanidin profiles from Royal Okanogan Huckleberry, blueberry, and strawberry pigments monitored at 520 nm.

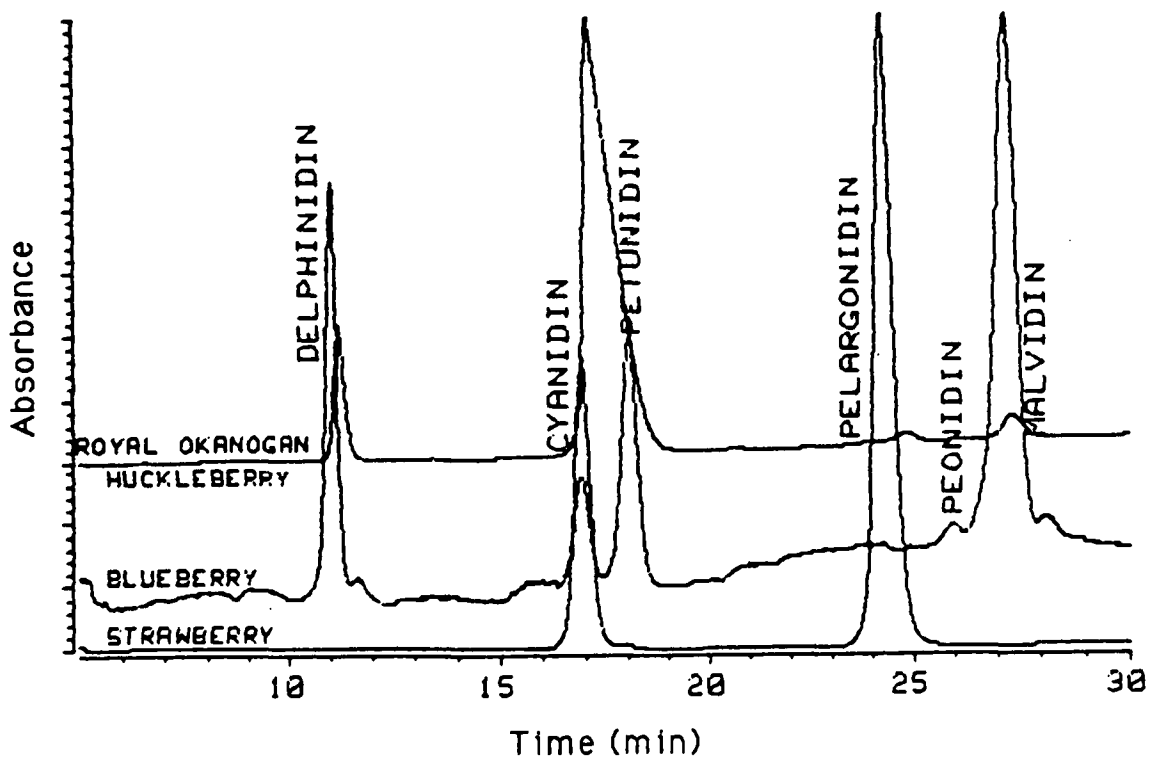


Figure 13: Spectra of each of the twelve peaks separated from Royal Okanogan Huckleberry Juice (Corresponds to Figure 4-520).

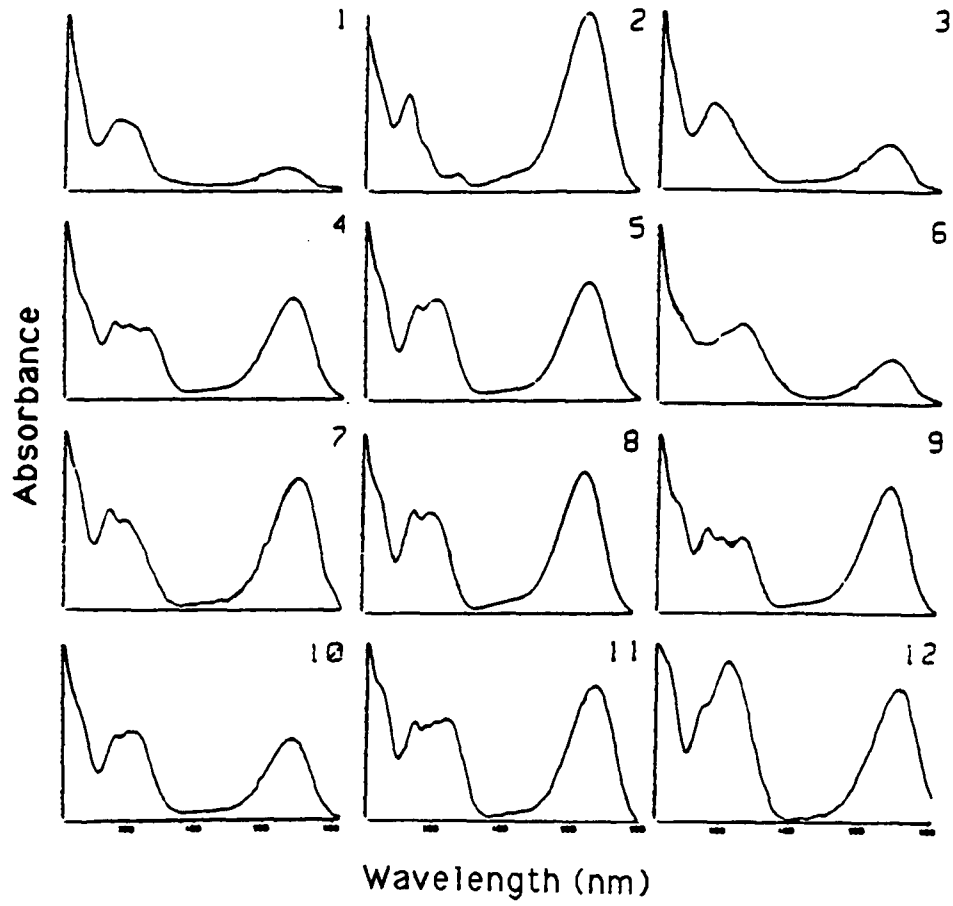


Figure 14: Comparative pigment profiles of various fruits of the Solanaceae family.

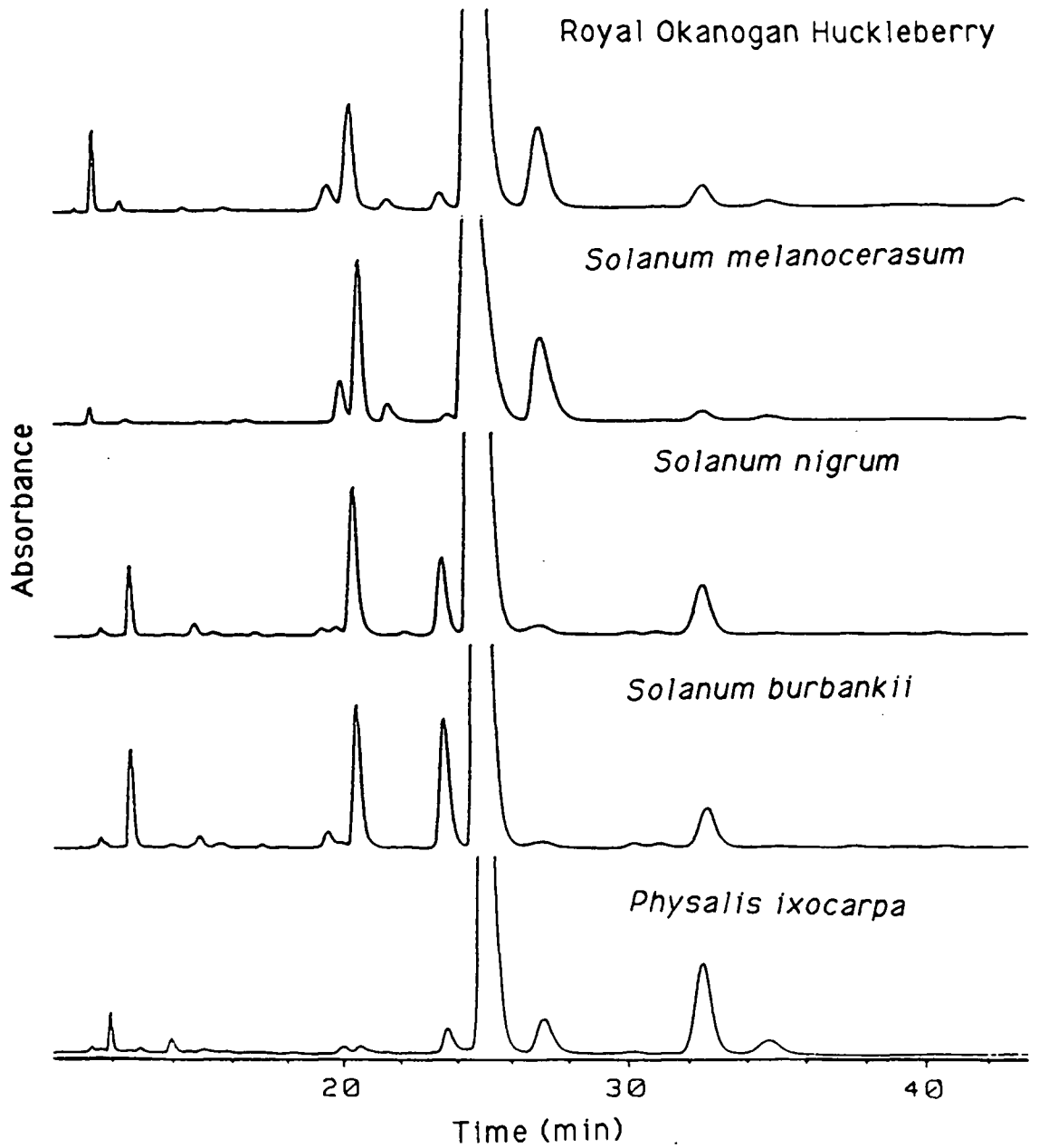


Table 1: Results from four juice processing trials involving Royal Okanogan Huckleberries and one trial involving Solanum melanocerasum.

Processing Trial	pH	Yield	Brix	T.A.	T.M.A.	T.A.C	%Poly(d)
Rohapect B1-L							
Blanched (a)	4.4	69%(c)	5.0	0.65	5.2	359	33%
Unblanched (a)	4.4	70%	5.9	0.63	5.2	364	50%
Novo Ultra SP							
Blanched (b)	4.4	60%(c)	5.8	0.59	5.8	348	32%
Unblanched (b)	4.4	66%	5.4	0.55	5.0	330	36%
*S. melano.**	4.3	66%	5.0	0.78	8.1	535	-

(a) - batches made in duplicate

(b) - batches made in triplicate.

(c) - due to filtering loss, one batch was omitted from results.

(d) = % non-monomeric anthocyanin color or % polymeric color.

* - heat treated, single batch, *Solanum melanocerasum*.

Yield = juice yield expressed as % volume / fresh weight.

T.A.- titratable acidity as % citric

T.M.A.- total monomeric anthocyanin content (g/L)

T.A.C.- total anthocyanin content as mg/100g fresh weight.

Table 2: CIE L*a*b* values for single strength juices.

Juice sample	L*	a*	b*
Royal Okanogan Huckleberry (a)	22.66	-0.06	-0.46
S. melanocerasum (b)	22.70	0.07	-0.58

(a) - average of 4 readings taken from each of the 4 processes used to make juice. (Standard error: L*=0.17, a*=0.10, b*=0.11).
(b) - average of duplicate readings of the same sample.

Table 3: Single strength Royal Okanogan Huckleberry juice diluted 1/50 with buffer to determine the change in color with pH.

pH	L*	a*	b*	Hue angle	x λmax	x Amax
1.17	47.5	58.7	-3.7	356	524	2.15
2.00	50.5	56.2	-8.7	351	524	1.65
3.03	64.4	34.4	-9.9	344	526	0.59
4.05	75.6	10.7	-3.3	343	532	0.22
4.58	75.2	8.8	-4.0	336	536	0.21
5.02	76.3	6.4	-2.6	338	536	0.18
6.03	75.4	3.5	-2.7	322	540	0.21
7.13	57.4	11.3	-14.0	309	558	0.79
8.01	50.8	-2.5	-15.4	261	578	1.37

* - CIE measurements usind standard illuminant C, reflectance, specular light included.

x - Spectrophotometric measurements where λmax is the wavelength of maximum absorption in the visible region, Amax is the absorbance at that wavelength.

angle - denotes the angle described by the tan-1 of a*/b* expressed on a 360 degree basis where red=360=0, blue=270 and green=180.

Table 4: Tentative peak assignments for the twelve peaks separated from Royal Okanogan Huckleberry pigment.

PEAK NO.~	DAP	ACDN*	ACID*	A440 λ max	TENTATIVE PEAK ASSIGNMENT
1	A	nt	nt	17	del-3-rut-5-glu
2	B	nt	nt	12	pet-3-rut-5-glu
3	C	nt	nt	15	mal-3-rut-5-glu
4	A	pet(5) del	pc unk	13	del-3-(unk-rut)-5-glu
5	A	del(17) pet	pc	12	del-3-(pc-rut)-5-glu
6	A	del(2.5) pet	pc(2.7) fer	13	del-3-(fer-rut)-5-glu
7	-	pet(8) del	-	13	pet-3-(unk-rut)-5-glu
8	B	pet	pc	10	pet-3-(pc-rut)-5-glu
9	B	pet	fer	11	pet-3-(fer-rut)-5-glu
10	C	mal	pc	11	mal-3-(pc-rut-)-5-glu
11	C	mal(2.5) pet(1.5) del	pc(3) fer	12	mal-3-(fer-rut)-5-glu
12	B	pet	pc	13	pet-3-(di-pc-rut)-5-glu

*- compounds are listed in order of predominance and numbers in parenthesis indicates relative ratio amounts.
nt- no test performed on these compounds.
~ - corresponds to peaks separated out from the Royal Okanogan Huckleberry pigment , see figure 4-520.
DAP - deacylated peak elutes at the same time as one of the three possible deacylated peaks (A, B, or C)
ACDN - anthocyanidin
other abbreviations: rut = rutinoside, glu = glucoside, fer = feruoyl, pc = *p*-coumaroyl, unk = unknown acid pet = petunidin, del = delphinidin, mal = malvidin.

Table 5: Area percentages of peaks separated by HPLC when monitored at 520 nm. (Peaks correspond to Figure 4-520.)

PEAK NO.	R. OKANOGAN HUCKLEBERRY	*SOLANUM MELANO.*	*SOLANUM NIGRUM*	*SOLANUM BURBANK.*	*PHYSALIS IXOCARPA*
1	0.06	0.03	0.03	0.03	0.20
2	1.81 (a)	0.56 (a)	0.22 (b)	0.26 (b)	1.34
3	0.35 (a)	0.22 (a)	1.66 (b)	3.25 (b)	0.25
4	2.03 (a)	3.25 (a)	0.44 (b)	0.21 (b)	0.60
5	7.07 (a)	11.82 (a)	6.68 (a)	7.10 (a)	0.69
6	0.80 (a)	1.87 (a)	0.21	np	np
7	1.26 (a)	0.73 (a)	4.00 (b)	8.12 (b)	2.56 (a)
8	72.23 (a)	63.68 (a)	78.66 (a)	71.44 (a)	70.92 (a)
9	9.52 (a)	13.85 (a)	1.08 (b)	0.85 (b)	5.23
10	2.51 (a)	1.49 (a)	4.07 (a)	4.13 (a)	13.93 (a)
11	0.93 (a)	0.94 (a)	0.12	0.14	2.59 (a)
12	0.71 (a)	0.42 (a)	np	np	np
'others'	0.20	1.14	2.83	4.47	1.69

np - no peak at this retention time.
a, b - has the same retention time and spectral match for a given peak.
'others' - indicates the total percentage of peaks unaccounted for.

Bibliography

Adesina, S.K. and Gbile, Z.O. 1984. Steroidal Constituents of Solanum scabrum subsp. nigericum. Fitoterapia, vol. LV (6):362-363.

Alston, R.E., 1966. Chemotaxonomy or Biochemical Systematics? in Chapt. 3, Comparative Phytochemistry, Swain, T.(ed.), pp 33-56, Academic Press, NY.

Asen, S., Stewart, R.N., and Norris, K.H. 1977. Anthocyanin and pH Involved in the Color of 'Heavenly Blue' Morning Glory. Phytochemistry 16:1118-1119.

Asen, S. 1982. Identification of Flavonoid Chemical Markers in Roses and their High Pressure Liquid Chromatographic Resolution and Quantitation for Cultivar Identification. J. Amer. Soc. Hort. Sci. 107(5):744-750.

Ballinger, W.E. and Maness, E.P. 1970. Anthocyanins in Ripe Fruit of the Highbush Blueberry, Vaccinium corymbosum L., J. Amer. Soc. Hort. Sci. 95(3):283-285.

Ballinger, W.E., Maness, E.P., Galletta, G.J., and Kushman, L.J. 1972. Anthocyanins of Ripe Fruit of a "Pink Fruited" Hybrid of Highbush Blueberries, Vaccinium corymbosum L.. J. Amer. Soc. Hort. Sci. 97(3):381-384.

Ballinger, W.E., Galleta, G.J., and Maness, E.P. 1979. Anthocyanins of Fruits of Vaccinium, sub-genera Cyanococcus and Polycodium. J. Amer. Soc. Hort. Sci. 104(4):554-557.

Banwart, G.W. 1981. Basic Food Microbiology, abridged ed. AVI Publishing, Westport, CT., pp. 87-88.

Bate-Smith, E.C. 1967. Chemistry and Taxonomy of Plants in Chapt. 17 in Chemotaxonomy and Serotaxonomy, Hawkes, J.G. (ed.), pp 193-200, Academic Press, NY.

Belitz, H.D. and Grosch, W. 1986. Food Chemistry, 2nd ed., pp 617, Springer-Verlag, NY.

Bhiravamurty, P.V. and Rethy, P. 1984. Origin and Evolution of Tetraploid Forms within the Solanum nigrum L. Complex. Proc. Indian Acad. Sci. (Plant Sci.), 93(5):553-560.

Bhiravamurty, P.V.and Rethy, P. 1984. Analysis of Phenolic Acids and its Bearing on the Taxonomy of the Solanum nigrum complex. New Botanist 11(2):138-144.

Blakeney, A.B., Harris, P.J., Henry, R.J., and Stone, B.A. 1983. A Simple and Rapid Preparation of Alditol Acetates For Monosaccharide Analysis. Carbohydrate Research 113:291-299.

Bohs, L., 1990. The Systematics of Solanum Section Allophyllum (Solanaceae). Ann. Missouri Bot. Gard. 77:398-409.

Bradley, V., Collins, D.J., Eastwood, F.W., Irvine, M.C., Swan, J.M., and Symon, D.E. 1979. Distribution of Steroidal Alkaloids in Australian Species of Solanum, Chapt. 12 in The Biology and Taxonomy of the Solanaceae. Hawkes, J.G., Lester,R.N., and Skelding, A.D.(eds), pp

203- 210, Academic Press, NY.

Bronnum-Hansen, K. and Flink, J.M. 1985. Anthocyanin Colorants from Elderberry (Sambucus nigra L.).3. Storage Stability of the Freeze Dried Product. J. Food Tech. 20:725-733.

Bronnum-Hansen, K., Jacobsen, F., and Flink, J.M. 1985. Anthocyanin Colorants from Elderberry (Sambucus nigra L.).1. Process Considerations for Production of the Liquid Extract. J. Food Tech. 29:703-711.

Brouillard, R. 1981. Origin of the exceptional Colour Stability of the Zebrina anthocyanin. Phytochemistry 20:143-145.

Brouillard, R. 1983. The In Vivo Expression of Anthocyanin Color In Plants. Phytochemistry 22(6):1311-1323.

Brouillard, R. and Delaporte, B. 1977. Chemistry of Anthocyanin Pigments: Kinetic and Thermodynamic Study of Proton Transfer, Hydration, and Tautomeric Reactions of Malvidin 3-glucoside. J. Am. Chem. Soc. 99(26): 8461-8468.

Brown, E. and Moss, J.P. 1976. The Identification of Potato Varieties from Tuber Characters. J. Nat. Inst. Ag. Bot. 14:49-69.

Carman, A.S., Kuan, S.S., Ware, G.M., Francis, O.J., and Kirschenheuter, G.P. 1986. Rapid High-Performance Liquid Chromatographic Determination of Potato Glycoalkaloids alpha-Solanine and alpha-Chaconine.

J. Agric. Food Chem. 34:279-282.

Chandel, P.S., and Singh, R.P. 1985. Gamma Ray- induced Morphological Abnormalities in Solanum nigrum Complex. Geophytology 15(1):76-81.

Chen, L. and Hrazdina G. 1981. Structural Aspects of Anthocyanin-Flavonoid Complex Formation and Its Role In Plant Color. Phytochemistry 20:297-303.

Coppola, E.D., 1982. Authenticity of Cranberry Products Including Non-Domestic Varieties. Presented at the 2nd annual Fruit Juice Authenticity Workshop, Herndon, VA.

Crawford, D.J. 1978. Flavonoid Chemistry and Angiosperm Evolution. The Botanical Review 44(4):431-456.

Cronquist, A. 1980. Chemistry in Plant Taxonomy, Chapt 1 in Chemosystematics: Principles and Practice, Bisby, F.A., Vaughan, J.G., Wright, C.A. (eds) pp 1-27, Academic Press, NY.

Daniel, J.R. 1989. Sweetners: Theory and Design, Chapter in Frontiers in Carbohydrate Research - 1: Food Applications, Millane, R.P., BeMiller, J.N., Chandrasekaran, R. (eds.), pp 34-65, Elsevier, London.

Daravingas, G. and Cain, R.F. 1965. Changes in the Anthocyanin Pigments of Raspberries During processing and Storage. J. Food Sci. 30:400-405.

Daravingas, G. and Cain, R.F. 1966. The Anthocyanin Pigments of Black Raspberries. J. Food Sci. 31:927-936.

Daravingas, G. and Cain, R.F. 1968. Thermal Degradation of

Black Raspberry Anthocyanin Pigments in Model Systems. J. Food Sci. 33:138-142.

D'Arcy, W.G. 1972. Solanaceae Studies II: Typification Of Subdivisions of Solanum. Ann. Missouri Bot. Gard. 59:262-278.

D'Arcy, W.G. 1979. The Classification of the Solanaceae, Chapt. 1 in The Biology and Taxonomy of the Solanaceae. Hawkes, J.G., Lester, R.N., and Skelding, A.D. (eds.), pp 3-47, Academic Press, NY.

Edmonds, J.M. 1979. Biosystematics of Solanum L., section Solanum (Maurella), Chapt 41 in The Biology and Taxonomy of the Solanaceae. Hawkes, J.G., Lester, R.N., and Skelding, A.D. (eds.), pp 529-548, Academic Press, NY.

Erdtman, H. 1968. The Assessment of Biochemical Techniques in Plant Taxonomy, Chapt. 20 in Chemotaxonomy and Serotaxonomy, Hawkes, J.G. (ed.), pp 235-268, Academic Press, NY.

Flora, L.F., 1978. Influence of leaf, cultivar, and maturity on the anthocyanidin-3,5-diglucosides of Muscadine Grapes. J. Food Sci. 43:1819-1821.

Francis, F.J., Harborne, J.B., and Barker, W.G., 1966. Anthocyanins in The Low Bush Blueberry, Vaccinium angustifolium. J. Food Sci., 31:583-587.

Francis, F.J. 1975. The Origin of $\tan^{-1}$ a/b. J. Food Sci. 40:412.

Francis, F.J. and Harborne, J.B. 1966. Anthocyanins of the

Garden Huckleberry, Solanum Guineese. J. Food Sci. 31:524-528.

Fuleki, T. and Francis, F.J. 1968. Qualitative Methods for Anthocyanins. 2. Determination of Total Anthocyanin and Degradation Index for Cranberry Juice. J. Food Sci. 33:78-83.

Little, A.C. 1975. Off on a Tangent. J. Food Sci. 40:410-411.

Ganapathi, A. and Rao, G.R. 1987. Phylogenetic Relationships in the Evolution of Solanum scabrum. Genome. 29:639-642.

Ganapathi, A. and Rao, G.R. 1987. Cytogenic Relationship between Solanum scabrum Mill. and S. americanum Mill.. Cytologia 52:91-96.

Goodwin, T.W. 1966. The Carotenoids, Chapt. 7 in Comparative Phytochemistry, Swain, T. (ed.), pp 121-137, Academic Press, NY.

Gross, J. 1987. Anthocyanins, Chapt. 2 in Pigments in Fruits, pp 59-85, Academic Press, NY.

Gould, W.A. 1974. Composition of Tomatoes, Chapt. 22 in Tomato Production, Processing, and Quality Evaluation. pp 347-349, AVI Publishing Company, Inc., Westport, CT.

Gould, W.A. 1983. Tomato Production, Processing, and Quality Control. AVI Publishing, Inc., Westport, CT. pp 274-275.

Hale, M.L., Francis, F.J., and Fagerson, I.S. 1986.

Detection of Enocyanin in Cranberry Juice Cocktail by HPLC Anthocyanin Profile. J. Food Sci. 51(6):1511-1513.

Halse, R. 1991. Instructor of Botany and Plant Pathology, Oregon State University, personal communication.

Harborne, J.B. 1958. Spectral Methods of Characterizing Anthocyanins. Biochem. Journal 70:22-28.

Harborne, J.B. 1958. The chromatographic Identification of Anthocyanin Pigments. J. Chromatog. 1:473-488.

Harborne, J.B. 1964. Phenolic Glycosides and their Natural Distribution, Chapt. 4 in Biochemistry of Phenolic Compounds, Harborne, J.B. (ed.), pp 129-170, Academic Press, NY.

Harborne, J.B. 1965. Flavonoids: Distribution and Contribution to Plant Color. Chapt. 9 in Chemistry and Biochemistry of Plant Pigments, Goodwin, T.W. (ed.), pp 247-278, Academic Press, NY.

Harborne, J.B. 1967. The use of Secondary Chemical Characters in the Systematics of Higher Plants. Chapt. 16 in Chemotaxonomy and Serotaxonomy, Hawkes, J.G. (ed.), pp 173-191, Academic Press, NY.

Harborne, J.B. 1975. Biochemical Systematics of Flavonoids, Chapt. 19 in The Flavonoids, Part 2, Harborne, J.B., Mabry, T.J., and Mabry, H. (eds.), pp 1056-1095, Academic Press, NY.

Harborne, J.B. 1977. Flavonoids and the Evolution of the

Angiosperms. Biochemical Systematics and Ecology 5:7-22.

Harborne, J.B. 1986. The natural Distribution in Angiosperms of Anthocyanins Acylated with Aliphatic Dicarboxylic Acids. Phytochem. 25(8):1887-1894.

Harborne, J.B. and Simmonds, N.W. 1964. The Natural Distribution of the Phenolic Aglycones, Chapt. 3 in Biochemistry of Phenolic Compounds, Harborne, J.B. (ed.), pp 77-128, Academic Press, NY.

Harborne, J.B. and Swain, T. 1979. Flavonoids of the Solanaceae Chapt. 18 in The Biology and Taxonomy of the Solanaceae. Hawkes, J.G., Lester, R.N., and Skelding, A.D. (eds), pp 257- 269. Academic Press, NY.

Harborne, J.B. and Turner, B.L. 1984. Taxonomic Principles, Chapt. 2, in Plant Chemosystematics, pp 7-33. Academic Press, NY.

Hawkes, J.G. and Tucker, W.G., 1967. Serological Assessment of Relationships in a Flowering Plant Family (Solanaceae) in Chapt. 8 in Chemotaxonomy and Serotaxonomy, Hawkes, J.G. (ed.), pp 77-88, Academic Press, NY.

Hegnauer, R. 1966. Comparative Phytochemistry of Alkaloids, Chapt. 13 in Comparative Phytochemistry, Swain, T. (ed.), pp 211-230, Academic Press, NY.

Heine, H. 1960. Notes on Solananum. Kew Bull. 14:245-247.

Heiser, C.B., 1987, The Fascinating World of the Nightshades,

Dover Publications, Mineola, NY pp 1-5, 63-106.

Heywood, V.H. 1966. Phytochemistry and Taxonomy. Chapt. 1. in Comparative Phytochemistry, Swain, T. (ed.) pp 1-18, Academic Press, NY.

Heywood, V.H. 1972. The Role of Chemistry in Plant Systematics, in Plenary Lectures Presented at the International Symposium of Chemistry in Evolution and Systematics, pp 355-375.

Hong, V. and Wrolstad, R.E. 1986. Cranberry Juice Composition. J. Assoc. Off. Anal. Chem. 69(2):199-207.

Hong, V. and Wrolstad, R.E. 1990. Use of HPLC Separation / Photodiode Array Detection for Characterization of Anthocyanins. J. of Ag. and Food Chem. 38(3):708-715.

Hong, V. and Wrolstad, R.E. 1990. Characterization of Anthocyanin-Containing Colorants and Fruit Juices by HPLC/Photodiode Array Detection. J. of Ag. and Food Chem. 38(3):698-708.

Hoshino, T. Matsumoto, U. and Goto, T. 1980. The Stabilizing Effect of the Acyl Group on the Co-Pigmentation of Acylated Anthocyanins with C-Glucosylflavones. Phytochemistry 19:663-667.

Hunziker, A.T. 1979. South American Solanaceae: a synoptic survey, Chapt. 2 in The Biology and Taxonomy of the Solanaceae. Hawkes, J.G., Lester, R.N., and Skelding, A.D. (eds), pp 49-86, Academic Press, NY.

Kawai, M., Matsura, T., Kyuno, S., Matsuki, H., Takenaka, M.,

Katsuoka, T., Butsugan, Y. and Saito, K. 1987. A New Physalin from Physalis alkekengi: Structure of Physalin L, Phytochemistry 26(12):3313-3317.

Lea, A.G.H. 1988. HPLC of Natural Foodstuffs. Chapter 8 in HPLC in Food Analysis, pp 277-333. Academic Press, NY.

Lees, D.H., and Francis, F.J. 1972. Effect of Gamma Radiation on Anthocyanin and Flavonol Pigments in Cranberries (Vaccinium macrocarpon Ait.) J. Amer. Soc. Hort. Sci 97(1):128-132.

Liener, I.E. 1969. Miscellaneous Toxic Factors, Chapt 13 in Toxic Constituents of Plant Foodstuffs, Liener, I.E. (ed.), pp 428- 429, Academic Press, NY.

Mabry, T.J., 1972. The Chemistry of Geographical Races, in Plenary lectures presented at the International Symposium of Chemistry in Evolution and Systematics, pp 377-379.

Machiex, J., Fleuriet, A., and Billot, J. 1990. Changes and Metabolism of Phenolic Compounds in Fruits, Chapt. 3 in Fruit Phenolics pp 149-221, CRC Press, Inc., Boca Raton, FL.

Mazza, G. and Brouillard, R. 1987. Color Stability and Structural Transformations of Cyanidin 3,5-Diglucoside and Four 3-deoxyanthocyanins in Aqueous Solutions. J. Agric. Food Chem. 35:422-426.

Mazza, G. and Brouillard, R. 1990. The Mechanism of Co-Pigmentation of Anthocyanins in Aqueous Solutions.

Phytochemistry 29(4):1097-1102.

Medsger, O.P. 1947. Edible Wild Plants The MacMillan Co., NY. pp 68-75.

Nee, M. 1986. Placentation Patterns in Solanaceae. in Solanaceae: Biology and Systematics, D'Arcy, W.G. (ed), pp 169-175, Columbia University Press, NY.

Osawa, Y. 1982. Copigmentation of Anthocyanins. Chapt.2. in Anthocyanins as Food Colors. Markakis, P. (ed.) pp 48-65 Academic Press, NY.

Rabino, I., Mancinelli, A.L., 1986. Light, Temperature, and Anthocyanin Production. Plant Physiology 81:922-924.

Rao, G.R. 1978. Role of Fruit Pigments in Understanding the Inter-relationship and Mechanism of Evolution of Higher Chromosomal Forms of the Species of the Solanum nigrum L. Complex. Acta Botanica Indica 6:41-47.

Roddick, J.G. 1979. Distribution of Steroidal Glycoalkaloids in cells of Solanum and Lycopersicon, Chapt 14 in Biology and Taxonomy of the Solanaceae. Hawkes, J.G., Lester, R.N., and Skelding, A.D. (eds), pp 223-230, Academic Press, NY.

Rommel, A. 1988. Red Raspberry and Blackberry Juice and Wine: The effect of Processing and storage on Color and Appearance. Master of Science Thesis presented March 10, 1988, Dept. of Food Science and Technology, Oregon State University.

Rommel, A.; Heatherbell, D.A.; and Wrolstad, R.E. 1990. J.

Food Sci. 55(4):1011-1017.

Saito, N.; Hotta, R.; Imai, K.; and Hayashi, K. 1965. On Petanin Isolated from the Berries of Solanum nigrum, var. guineense. Proc.Japan Academy, 41(7) 593-598.

Saito, N., Kenzo, A., Honda, T., Timberlake, C.F., Bridle, P. 1985. Acylated Delphinidin Glucosides and Flavonols From Clitoria ternatea. Phytochemistry 24(7):1583-1586.

Sapers, G.M., Taffer, I., and Ross, L.R. 1981. Functional Properties of a Food Colorant Prepared From Red Cabbage. J. Food Sci. 46:105-109.

Sapers, G.M., Phillips, H.M., Rudolf, H.M., and DiVito, A.M. 1983. Cranberry Quality: Selection Procedures for Breeding Programs. J. Amer. Soc. Hort. Sci 108: 241-246.

Sapers, G.M.; Burgher, A.M.; Phillips, J.G.; and Jones, S.B. 1984. Color and Composition of Highbush Blueberry Cultivars. J. Amer. Soc. Hort. Sci. 109(1):105-111.

Sapers, G.M., Graff, G.R., Phillips, J.G., and Deubert, K.H. 1986. Factors Affecting the Anthocyanin Content of Cranberry. J. Amer. Soc. Hort. Sci. 111(4):612-617.

Sapers, G.M.; Jones, S.B.; Kelley, M.J.; Phillips, J.G.; and Stone, E.G. 1986. Breeding Strategies for Increasing the Anthocyanin Content of Cranberries. J. Amer. Soc. Hort. Sci. 111(4):618-622.

Seigel, A.; Markakis, P.; and Bei, C.L. 1971. Stabilization

of Anthocyanins in Frozen Tart Cherries by Blanching. J. Food Sci. 36:962-963.

Schilling, E.E. 1981. Systematics of Solanum section Solanum (Solanaceae) in North America. Syst. Bot. 6(2):172-185.

Schreiber, K. 1979. The Steroidal alkaloids of Solanum, Chapt. 11 in The Biology and Taxonomy of the Solanaceae. Hawkes, J.G., Lester, R.N., and Skelding, A.D. (eds), pp 193-202, Academic Press, NY.

Somers, T.C. 1971. The Polymeric Nature Of Wine Pigments. Phytochemistry 10:2175-2186.

Spanos, G.A. and Wrolstad, R.E. 1987. Anthocyanin Pigment, Nonvolatile Acid, and Sugar Composition of Red Raspberry Juice. J. Assoc. Off. Anal. Chem. 70(6):1036-1046.

Teh, L.S., and Francis, F.J. 1988. Stability of Anthocyanins from Zebrina pendula and Ipomoea tricolor in a model beverage. J. Food Sci. 53(5):1580-1581.

Tetenyi, P. 1970. Infraspecific Differentiation, Chapt. 1 in Infraspecific Chemical Taxa of Medicinal Plants, Tetenyi, P. (ed.), pp 15-72, Chemical Publishing Co., NY.

Tetenyi, P. 1987. A Chemotaxonomic Classification of the Solanaceae. Ann Missouri Bot. Gard. 74:600-608.

Toole, F.H., and Brown, F. 1946. Final Results of the Duyel buried seed Experiment. J. Agric. Res. 72:201-210.

Underhill, J.E., 1989, Northwestern Wild Berries, 2nd ed.,

Hancock House Publishers, LTD. Blain, WA. pp 82-85.

Webster by McKechnie, J.L., 1979, Webster's Unabridged Dictionary, 2nd ed., Simon and Schuster, New York, NY.

Weller, R.I. and Phipps, R.H. 1978/1979. A Review of the Black Nightshade (Solanum nigrum L.). Protection Ecol. 1:121-139.

Whistler, R.L. and Daniel, J.R. 1985. Carbohydrates. Chapt. 3. in Food Chemistry, Fennema, O.R. (ed.), pp 69-139. Marcel Dekker, Inc, NY.

Wrolstad, R.E., Lee, D.D., Poei, M.S. 1980. Effect of Microwave Blanching on the Color and Composition of Strawberry Concentrate. J. Food Sci. 45(6):1573-1577.

Wrolstad, R.E., Skrede, G., Lea, P., and Enersen, G. 1990. Influence Of Sugar on Anthocyanin Pigment Stability in Frozen Strawberries. J. Food Sci. 55(4):1064-1065, 1072.

Young, D.K. 1964. Redwood Empire Wildflower Jewels, Naturegraph Publishers, Healdsburg, CA. pp 27-28.