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Title: Red Raspberry and Blackberry Juice and Wine: The Effect of Processing and Storage on Color and Appearance

Abstract approved _____

Dr. Ronald E. Wrolstad

Wines were made from thawed, frozen red raspberries and blackberries (Meeker and Evergreen varieties, respectively) by 1. fermentation on the pulp, 2. fermentation of depectinized juice, and 3. fermentation of high temperature short time (HTST) treated depectinized juice. The influence of fining was also investigated. The wines were stored at 2 and 20°C for six months. High performance liquid chromatography (HPLC), spectral and Hunter measurements were made at intermediate processing stages as well as after storage and the results subjected to analysis of variance (ANOVA).

Fermentation was the major processing stage where anthocyanins degraded for all samples with total losses of 50-100% after storage. Cyanidin-3-glucoside was the most reactive pigment in both varieties, disappearing completely during the fermentation of raspberry juice; this

confirms the greater susceptibility of monoglycosides to hydrolysis. Presence of diglucosides explains the greater anthocyanin pigment stability of raspberry compared to blackberry and strawberry wines. Four additional peaks were detected by HPLC which have not been previously reported for 'Evergreen' blackberry. These peaks are believed to be a xylose-cyanidin derivative, two acylated derivatives of cyanidin-3-glucoside or cyanidin-3-rutinoside and cyanidin. Haze and sediment formation was a problem in blackberry wine; for an acceptable product HTST treatment was necessary which indicates presence of native enzymes.

Both raspberry and blackberry wines made from fined HTST treated depectinized juices had the best color stability, color appearance and sensory quality after storage. Blackberry had 53-82% more anthocyanin loss and 2.8 times more haze, 2 times more sediment and 2.6 times more % polymeric color formation than raspberry wine.

Red Raspberry and Blackberry Juice and Wine: The Effect of Processing and Storage on Color and Appearance

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INTRODUCTION

Because of their increasing popularity berry wines have recently become of new interest. In 1981 fruit wine accounted for approximately 30% of Oregon's wine production with berry wines being the most important segment. However, due to problems with: 1. strong haze and sediment formation and 2. color deterioration (color loss, browning) upon storage (shelf life), sales did not increase as expected. Wine made from 'Evergreen' blackberry has been particularly unstable. Reduction of these problems could be of major benefit to Oregon's winemakers since this state is responsible for about 75% of the United States production of blackberries, mainly 'Thornless Evergreen' (Skirvin, 1984). The raspberry variety 'Meeker' was chosen because it is one of Oregon's major cultivars.

'Evergreen', a wild blackberry variety, was not cultivated until recently. Therefore, a HPLC anthocyanin profile has never been published. Such compositional data could be very useful for the determination of adulteration.

With the successful elimination of technological problems, 'Evergreen' blackberry wine could be marketed as a new type of berry wine. Its herbal and nutty flavor and aroma characteristics are not regarded as typical for blackberry.

In addition to their use as dessert wines, fruit wines have a great potential for such uses as additives in cocktails and confections (color and flavor) or colorants. Natural colorants are of particular

significance due to the controversial nature of many synthetic red colorants.

This thesis is divided into three major parts: 1. an extensive literature review which includes discussions on varieties, seasonal influences, composition, measurement of color, maturation, aging and color stability; 2. one paper on the processing and storage effects on color and appearance of red raspberry wine; and 3. a second paper on the color quality of blackberry wine with a major emphasis on the determination of and treatment effects on the anthocyanin profile.

It was the purpose of this study to: 1. develop a processing procedure for good color quality and appearance, 2. simulate commercial conditions in the study of color changes during storage, and 3. as part of the color analyses determine the anthocyanin profile and its changes during processing and storage of both raspberry and blackberry.

LITERATURE REVIEW

A VARIETIES, CULTIVARS, SEASONAL INFLUENCES

1. *Rubus* fruit

Raspberries and blackberries both belong to the extensive *Rubus* genus of *rosaceae.* The blackberry proper in its manifold varieties is botanically 'senior' to the raspberry. (Anonymous, 1965)

The pigment class of anthocyanins is responsible for the bright red color of a wide variety of flowers and fruits including that of *Rubus* fruit (Hong, 1988). All anthocyanins are based on the same chemical structure, the 3,5,7,4' tetrahydroxyflavylium cation, also referred to as aglycone or anthocyanidin (Markakis, 1982) (figure I). The different colors of the anthocyanins are obtained through hydroxylation, methylation and glycosylation (Blom, 1983). Glycosylation (glycosidic bonding of a sugar molecule) usually occurs at the 3-position and less frequently at the 5-position of the aglycone. The attached sugar can be substituted with the same or other sugars or with acids (acylation). (Markakis, 1982)

The aglycones found in the genus *Rubus* are cyanidin (3,5,7,3', 4' pentahydroxyflavylium cation) and pelargonidin (3,5,7,4' tetrahydroxyflavylium cation) and the number of sugars attached (glucose, rhamnose, and xylose) varies from one to three (Jennings, 1980) (figures I&II). If a variety uses a particular sugar all the corresponding combinations of anthocyanins can be expected (Barritt, 1975a).
The combinations found with the aglycone cyanidin are:
a. the monoglycoside cyanidin-3-glucoside (Cy-3-Gl),

b. the diglycosides cyanidin-3-sophoroside (Cy-3-Sop) (2 times glucose), cyanidin-3,5-diglucoside (Cy-3,5-diGl) (2 times glucose), cyanidin-3-rutinoside (Cy-3-Ru) (glucose and rhamnose) and cyanidin-3-sambubioside (Cy-3-Sam) (glucose and xylose), and
c. the triglycosides cyanidin-3-glucosylrutinoside (Cy-3-GlRu) (2 times glucose and rhamnose) and cyanidin-3-xylosyl-rutinoside (Cy-3-XyRu) (glucose, rhamnose and xylose) (Jennings, 1980).

Provided a genotype is able to synthesize pelargonidin it is considered that the sugars contained in the pigments of this aglycone are determined by the same synthesizing systems as those which control their formation in the cyanidins. The enzymes responsible for the transfer of the sugar are not substrate specific. It is probable that seven pelargonidin pigments corresponding the seven cyanidins may occur. (Barritt, 1975a; Jennings, 1980)

Jennings et al. (1980) report that in all *Rubus* fruits the concentrations of the rhamnose pigments, particularly that of Cy-3-Ru, are generally the lowest, even in blackberries which are presumed not to be able to synthesize either sophorose or xylose pigments. This has been attributed to genetic effects (Jennings, 1980). Barritt et al. (1975a&b) and Jennings et al. (1980) report that only two varieties of red raspberry with black raspberry varieties in their ancestries have xylose-containing glycosides. Sapers et al. (1986) later disproved the incapacity of blackberries to produce xylose pigments.

2. Raspberry

The raspberry proper (*Rubus idaeus*) is native to Europe and has

been known for more than 3000 years. Red raspberry (*Rubus idaeus* and subspecies *strigosus*) as well as red raspberry hybrids are all capable of making Cy-3-Sop which is their predominant anthocyanin (Duclos, 1971; Jennings, 1980; Spanos, 1987) and is present exclusively in this variety and its hybrids of the *Rubus* species (Torre, 1977). In addition, Cy-3-Gl was found present in all and Cy-3-GlRu and Cy-3-Ru present in most varieties investigated (Barritt 1975a&b; Duclos, 1971; Torre, 1977; Wrolstad, 1971). Jennings et al. (1980) confirmed the above and reported that Cy-3-GlRu may exceed Cy-3-Gl which usually occurs in larger quantities. The rhamnose-cyanidins Cy-3-Ru and Cy-3-GlRu are present in some varieties including 'Meeker' but not in 'Willamette' (Barritt, 1975a, Torre, 1977; Spanos 1987).

The four related pelargonidin anthocyanins have also been reported as minor pigments (Spanos, 1987; Torre, 1975a&b; Wrolstad, 1971). Barritt and Torre (1975a; 1977) identified trace amounts of Pel-3-Sop, Pel-3-GlRu and Pel-3-Gl in several varieties. Spanos et al. (1987) tentatively identified the same pigments except Pel-3-Gl and found Pel-3-Ru instead. Of the pelargonidin pigments in red raspberries Pel-3-Sop is contained in the largest amounts and occurs in almost all cultivars (Barritt, 1975a; Spanos, 1987).

As determined by HPLC analysis Spanos et al. (1986) could not clarify whether Cy-3,5-diGl is absent or coelutes, possibly with Cy-3-Sop. Barritt et al. (1975a) detected Cy-3,5-diGl in one third of the cultivars they investigated. Jennings et al. (1980) report that this cyanin rarely occurs in red raspberry. The relative proportions of major cyanidin pigments in fruit of 'Willamette' and 'Meeker' do not change

appreciably with increasing fruit maturity (Barritt, 1975a).

3. Blackberry

The blackberry is generally known under the botanical name of *Rubus fructicosus*, though this is, in fact, the designation of only one, even if the most popular of the plants belonging to the *Rubatus* subspecies of blackberry-type *rosaceae* (Anonymous, 1965).

The variety 'Evergreen' (*Rubus laciniatus Willd.*) is a native of Europe and has been found growing wild particularly in the Pacific Northwest of the United States (Waldo, 1957). Oregon is responsible for about 75% of the United States production of blackberries, mainly 'Thornless Evergreen' (Skirvin, 1984). The cultivar 'Thornless Evergreen' was found by chance and is identical with 'Evergreen' except for thorns (Waldo, 1957). Botanically the thornless cultivar is a chimera (a form of mutation) which is a phenomenon in nature where the thorny tissue does not develop (Waldo, 1957). Pigment in the variety 'Evergreen' concentrates in the druplet skin and the berries are low in acid, have a mild flavor and aroma and contain hard seeds (Varseveld, 1980).

Blackberries are presumed only to be able to synthesize the aglycone cyanidin (Jennings, 1980). This was confirmed by Sapers et al. (1986) to whom the spectral properties of the purified compounds found in blackberry extract of various cultivars suggest that all compounds are 3-substituted glycosides of cyanidin. However, Torre et al. (1977) found Pel-3-Gl in one blackberry variety, *Rubus caucasicus* in small amounts.

Cy-3-Gl is common to blackberries usually as their major pigment and Cy-3-Ru as a second pigment in lower concentrations or trace amounts in

all varieties studied (Jennings, 1980; Sapers, 1986; Spanos, 1987; Torre, 1973, 1977). However, in the variety 'Marion' Cy-3-Ru is the major pigment (Spanos, 1987; Torre, 1973). Torre et al. (1977) found five unidentified minor anthocyanin pigments in most varieties studied.

The assumption of Markakis et al. (1982) that blackberries cannot synthesize xylose pigments was contradicted by Jennings et al. (1980) through the detection of small amounts of Cy-3-Sam in the cultivar 'Ashton Cross' (*Rubus bartoni*) which typifies blackberries. This contradiction found further support through a study by Sapers et al. (1986) who identified a cyanidin derivative containing xylose in blackberry extract from six different thornless and thorny blackberry cultivars.

However, the fact that another blackberry variety (*Rubus glaucus*), whose classification is controversial, contains a xylose pigment to Jennings et al. (1980) strongly supports the view that this variety is related to black raspberry (*Rubus occidentalis*). The latter is supported by studies of Torre et al. (1977) who found the xylose containing anthocyanins Cy-3-Sam and Cy-3-XyRu only in black raspberry.

Sapers et al. (1986) in their study of blackberry extract tentatively identified so far unknown anthocyanins, two dicarboxylic acid-acylated derivatives of Cy-3-Gl. Anthocyanins whose sugars are acylated with simple dicarboxylic acids such as malonic or succinic acid have been shown to be extremely unstable and to display complex acid hydrolysis behavior (Sapers, 1986).

Among the 33 mainly thornless blackberry cultivars and selections compared by Sapers et al. (1986) these investigators recognized five

different anthocyanin patterns. One of the patterns which contains all the thorny cultivars is distinctly different from the other four patterns. The patterns could not be supported by differences among cultivars in spectral properties or tristimulus parameters. (Sapers, 1986)

In comparing a series of blackberry samples of several cultivars Sapers et al. (1986) found that with increasing ripeness the anthocyanin patterns are changed in favor of Cy-3-Gl and the xylose containing cyanidin derivative. The two putative acid acylated derivatives of Cy-3-Gl decrease. During ripening the variety 'Thornless Evergreen' is more sensitive to temperature changes than other varieties with respect to the content of anthocyanins and other constituents (Naumann, 1980). Fully ripe fruit show little proportional differences (Sapers, 1986). This supports the small quantitative changes Barritt et al. (1975a) found in the anthocyanin composition in raspberry of increasing ripeness. When juice samples are standardized to compensate for differences in pH and anthocyanin concentrations, ripeness and cultivar effects on juice tristimulus parameters are small (Sapers, 1986).

B COMPOSITION

1. General Properties

a) Red Raspberry

The mean yield of juice produced from seven samples studied by Spanos et al. (1987) was 83.3%. Duclos et al. (1971) report a range from

580-700 mL/kg of fruit depending on ripeness.

Spanos et al. (1987) report a mean °Brix (= weight % soluble solids) value at 7.8 which was lower than reported by Schobinger (1986) in the range from 5.4-14.3 g/100mL of juice. In addition, Spanos et al. (1987) report a mean pH value of 3.1 which was in agreement with previous literature.

The mean values of total (titratable) acidity (TA) and TA expressed as g citrate/100mL (both normalized to 10°Brix) as reported by Spanos et al. (1987) were 1.61 and 2.14, respectively, for the same samples as above. Schobinger (1986) reports TA in the range from 1.4-2.2 g tartrate/100mL of juice.

In 'Meeker' variety the average total yield as reported by Spanos et al. (1987) was 75.3% for juice treated with pectic enzyme. The average values for °Brix, pH, TA (10°Brix), and TA expressed as g citrate/ 100mL (10°Brix) were 8.3, 3.19, 1.61, and 1.94, respectively (Spanos, 1987). Barritt et al. (1975a) report that juice absorbance and pH increase with maturity in fruit of both 'Willamette' and 'Meeker'.

b) Blackberry

In juice from five frozen thornless blackberry varieties juice, yield, pH, TA, and soluble solids were reported in the ranges from 65-77 mL/100g, 3.3-4.4, 0.4-1.3% citric, and 7.5-14.2% at 20°C, respectively (Sapers, 1985). Schobinger (1986) reports TA in the range from 1.4-2.2 g tartaric/100mL and soluble solids in the range from 9.5-12.6 g/100mL of blackberry juice. As determined by Osborn (1964) the content of soluble solids decreased during processing from the original blackberry fruit over

fresh juice to stored juice from 11.71% to 9.8%. The variety 'Evergreen' contains soluble solids in the range from 9.1-14.7% (Varseveld, 1980).

All the red fruit and juice subsamples of 40 frozen thornless blackberry cultivars and selections studied by Sapers et al. (1985) were lower than black subsamples in soluble solids and total anthocyanin contents and higher in TA and anthocyanin recoveries in the pressed juices. This was attributed to the fact that the anthocyanins in the fruit mesocarp which appear in the juice, decrease during ripening. Juice yields were slightly larger with red than with black subsamples. (Sapers, 1985)

2. Anthocyanins, Anthocyanidins

a) Red Raspberry

Total anthocyanin concentrations were reported at a range from 20-60 mg/100g of fruit (Torre, 1977) and 23.8-101 mg/100mL of juice (Spanos, 1987) in a large variety of samples studied. The mean values for the individual anthocyanins as found by Spanos et al. (1987) were Cy-3-Sop 74.2% (range from 42.0-85.3%), Cy-3-Gl 12.2%, Cy-3-GlRu 8.6%, Cy-3-Ru 1.7%, Pel-3-Sop 2.9% and trace levels of Pel-3-Ru and Pel-3-GlRu.

In the cultivar 'Meeker' Torre and Barritt (1977; 1975a) report 18.6 (or 58%) Cy-3-Sop, 10.1 (or 17%) Cy-3-Gl, 8.3 (or 20%) Cy-3-GlRu, and 2.7 (or 5%) Cy-3-Ru in mg/100g of fruit, respectively, and traces (< 2%) of Cy-3,5-diGl, Pel-3-Gl and Pel-3-Sop with a total anthocyanin content of 39.7 mg/100g of fruit. In overripe fruit (pH 3.47) Cy-3-Gl and Cy-3-Ru were increased in favor of Cy-3-Sop and traces of Pel-3-GlRu were also present (Barritt, 1975a). Spanos et al. (1987) found 78.9% Cy-3-Sop,

11.2% Cy-3-Gl, 5.2% Pel-3-Sop, 3.6% Cy-3-GlRu, and 1.1% Cy-3-Ru in the variety 'Meeker'.

HPLC analysis of anthocyanidins gave 96.2% cyanidin and 3.8% pelargonidin in the variety 'Meeker' (Spanos, 1987). The mean of seven varieties studied was 97.4% for cyanidin and 2.6% for pelargonidin. It has to be considered that anthocyanidins are very unstable. (Spanos, 1987)

b) Blackberry

Total anthocyanin contents were reported in the ranges from 83-326 mg/100g of fruit and 62-150 A.U.(Absorbance Units)/g of fruit (or 14.5-40.5%) in a larger number of varieties studied (Sapers, 1985; Torre, 1977).

A range from 32.2-96.8 A.U./g of fruit was found in two frozen thornless blackberry cultivars (Sapers, 1986). In juice from five frozen thornless blackberry varieties the total anthocyanin content was reported in the range from 15-50.5 A.U./mL (Sapers, 1985).

Sapers et al. (1986) isolated the following amounts of anthocyanins in mg with the corresponding HPLC area percentages in their order of elution from combined extracts of various thornless blackberry cultivars: 90.8 (or 82.6%) Cy-3-Gl, <<1 (or 0.59%) Cy-3-Ru, 12.4 (or 7.3%) xylose containing cyanidin derivative, and 1.0 (or 1.8%) and 9.8 (or 6.4%) of two dicarboxylic acid-acylated derivatives of Cy-3-Gl, respectively. The mean HPLC area percentages of one thorny cultivar were: 88.9% Cy-3-Gl, 7.6% Cy-3-Ru, 0.2% xylose cyanidin derivative, and 1.7% of one acid-acylated Cy-3-Gl derivative present only (Sapers, 1986).

In two frozen thornless cultivars ranges from 55.7-73.8% for Cy-3-Gl, 5.7-10.15% for the xylose derivative, and 3.3-5.6% and 10.45-27.8% for the two acid-acylated derivatives, respectively, were reported (Sapers, 1986).

In the cultivar 'Evergreen' Torre et al. (1977) report only Cy-3-Gl at a concentration of 148.7 mg/100g of fruit. In a previous investigation the same authors (1973) found Cy-3-Gl as the major pigment and also trace amounts of Cy-3-Ru.

3. Ash Content

a) Red Raspberry

The total content of ash in raspberries was reported in the range from 0.307-0.5% (Spanos, 1987) with a mean of 0.381% (Spanos, 1987) or a range from 0.3-0.6 g/100mL of juice (Schobinger, 1986). The minerals present were found mainly in the forms K_2O and P_2O_5 (Spanos, 1987). The individual ions in their orders of content were K, phosphate, Ca, Mg, sulfate, Cl, Na, and NO_3 (Schobinger, 1986; Spanos, 1987).

b) Blackberry

Blackberries have the highest content of any berry fruits of iron, magnesium and calcium (Anonymous, 1965). Boland (1968) reports total ash content at a range from 0.39-0.46 g/100mL of blackberry juice consisting mainly of K_2O and P_2O_5. The individual ions in their orders of content were reported as K, phosphate, Mg, Ca, and Na (Schobinger, 1986). Osborn (1964) could not find major differences between the ash contents of blackberry fruit, juice and stored juice.

C MEASUREMENT OF COLOR

1. Spectral Methods

a) Red Raspberry

In juice from the variety 'Meeker' Spanos et al. (1987) report monomeric anthocyanin content (as Cy-3-Gl, normalized to 10°Brix), color density ($A_{420nm}+A_{500nm}$; 10°Brix), polymeric color ($A_{420nm}+A_{500nm}$ bisulfite treated; 10°Brix), % polymeric color (= % tannin, index of polymerization-browning; see also age index, chemical age D I) 5.) and browning index (A_{420nm}) as 68 mg/100mL, 23.6, 1.3, 5.3% and 0.77, respectively.

The mean values of these parameters for seven raspberry varieties studied were 52.0 mg/100mL, 19.9, 1.4, 7.2, and 0.80, respectively. Color density, % polymeric color and browning index show that these juices were high in color and contained low levels of polymeric anthocyanin pigments. (Spanos, 1987)

b) Blackberry

For the anthocyanins identified in combined extracts from various thornless cultivars and selections Sapers et al. (1986) report the following absorbance maxima (visible) and indices of pigment degradation $A_{440nm}/A_{max=513nm,vis}$ (= tint or nuance, also expressed as hue angle; $A_{max,vis}$ anthocyanin degradation; A_{440nm} browning (Bakker, 1986a)): Cy-3-Gl--526nm and 23, Cy-3-Ru--528nm and 0, xylose-cyanidin derivative --527nm and 22, dicarboxylic-acid acylated derivative of Cy-3-Gl--527nm and 22.

In another study the visible absorption spectra of various varieties had their maxima at 510-515nm, with larger max values corresponding to samples with higher pH values (e.g. pH 3.3: 513nm). The color of subsamples had no influence on absorption. (Sapers, 1986)

The index of pigment degradation values were significantly higher for the juice from black compared to red frozen subsamples (more acidic). In addition, there seemed to be a strong negative correlation between this index and the transmission and reflectance Hunter L values, e.g. with increasing Hunter L (lighter) A_{440nm}/A_{513nm} decreased (less degradation). This correlation was due mainly to variations in the visible absorption maximum A_{513nm}. (Sapers, 1985)

c) Other fruit

In strawberry, a pulp fermented sample was found to have the lowest color density (1.8 units) whereas pectinase 'B-20' pre-press enzyme treated juice had the highest (6.6 units) (Flores, 1984). The latter supports the deeply-colored juices with pectin residues and full flavor described in other studies (Dixon, 1985).

Pulp fermented juice samples and all juice samples treated with various pectinases, proteases and cellulases had low polymeric color ranging from 0.200 to 0.620 units and low % polymeric color with the pulp fermented sample having the highest value (16.3%) and a mixture of pectinase 'D5s':protease the lowest (4.1%). With the exception of the treatments with the pectinases 'B-20' and 'VR', there was no increase in the browning indices of the extracted juices. pH was not a factor in color differences. (Flores, 1984)

In the studies of Pilando et al. (1985) comparison of monomeric anthocyanin pigment in strawberry juice with that originally present in fruit revealed a loss in the order of 50%. Pigment analysis of the corresponding wines showed a tremendous decrease in monomeric anthocyanin after fermentation; only 3-9% of the anthocyanin originally present in the juice was retained (Pilando, 1985). Co-precipitation of anthocyanin pigments with yeast probably contributed to pigment loss. Pilando et al. (1985) found further that overripe strawberry fruit with its higher contents of monomeric anthocyanin and total phenolics gave wine with better color than fully ripe fruit.

Somers et al. (1977) report the use of optical absorbance at 280nm as a measure of total phenolics in red and white wine. Somers et al. (1977) report further that a red wine dilution of at least 50:1 is usually necessary before Beer's law becomes operative for anthocyanins, and prefer to use times 101 dilution in 1 M-HCl for their colorimetric measurement.

2. Tristimulus Measurements-Hunter

Bakker et al. (1986a) state that tristimulus measurements adequately correspond to what the eye actually sees and that there is a real effect of Hunter L (lightness index) on hue angle (color tint or nuance). However, tristimulus values give no information about the composition of wine color (Somers, 1977).

As reported by Bakker et al. (1986a) Hunter L can be varied in two ways: 1. by changing the optical path length of the measuring cell at constant pigment concentration and 2. by either changing the pigment concentration at a) constant pH or b) changing the concentration of

colored cationic forms of the anthocyanins (AH^+) by varying pH (Hunter L is largely due to AH^+ ions).

a) Red Raspberry

Spanos et al. (1987) report the following tristimulus parameters (transmission) in juice from 'Meeker': L=23.3, a=50.0 (redness index), b=16.0 (yellowness index), Y=5.4, X=11.8, Z=0.2 providing a hue angle of 72.3, a saturation index (SI) of 52.5 and a hue/SI ratio of 1.4. The respective mean values of seven raspberry varieties studied were L=29.9, a=56.4, b=20.4, Y=9.3, X=18.6, Z=0.3 providing a hue angle of 70.3, a SI of 60.0 and a hue/SI ratio of 1.2.

b) Blackberry

Sapers et al. (1985) report the following ranges of Hunter parameters (reflectance) in fresh fruit from two blackberry varieties: L=13.8-16.6, a=2.2-3.3, and b=(-4.8)-(-3.1). Frozen storage elevated reflectance Hunter a values of black fruit much less than red fruit. Reflectance Hunter b values were greater (less negative) with red than with black or fresh fruit. (Sapers, 1985)

Transmission Hunter L values were about 10% lower (darker) for the juice obtained from black frozen subsamples (mean: 48.8) as compared to juice from red frozen subsamples (mean: 54.7). Smaller although still significant differences in hue angle values were obtained for these juices. (Sapers, 1985)

c) Other fruit

In their studies of freshly made port wines Bakker et al. (1986a) found that with increasing Hunter L, tint values ($A_{440nm}/A_{max=520nm, vis}$) increase. This fact shows that superimposed upon the general influence of Hunter L on hue angle is the effect of self-association of anthocyanins, which affects Hunter L, tint and hue angle. At the pH of wine anthocyanins deviate positively from Beer's law, i.e. color increases more than proportionally with increasing concentrations of AH^+ (decreasing Hunter L). The increase in tint values with increasing Hunter L may thus be due to A_{520nm} being reduced relatively more than A_{420nm}. (Bakker, 1986a)

Dissociation of the pigment complexes at low concentration also effects the hue angle. If self-association is considered as a form of co-pigmentation, the general effect would be blueing with less co-pigmentation caused by increasing Hunter L and vice versa. (Bakker, 1986a)

Bakker et al. (1986a) found further that amongst the tristimulus parameters of freshly-made and aging port wines, there were high correlations between Hunter L or SI and Hunter a.
In individual ports and model anthocyanin solutions Hunter L varied linearly but negatively with hue angle, but in the group of ports examined there was no significant relationship between Hunter L and hue angle because of the additional variable phenolic browning depending upon cultivar. In the model solutions the linear increase of Hunter L was also correlated with an increase in tint which was attributed to dissociation of associated anthocyanin molecules. Hue angle was a more discriminating

parameter for expressing the color nuance of red wines than tint. (Bakker, 1986a)

When comparing tristimulus and spectral measurements of freshly-made and aging port wines there was high correlations between Hunter a and A_{520nm}, and SI and color density, and a high negative correlation between Hunter L and color density. There was little correlation between Hunter b and A_{420nm} which indicates that hue angle did not correspond to tint. (Bakker, 1986a)

In conclusion to their studies of aging port wines Bakker et al. (1986a) found that tristimulus analysis provides a useful technique to monitor color changes in ruby ports, giving also an estimate of the relative extents of aldehyde versus non-aldehyde aging, as reflected in the differences in brownness, when ports were compared at the same lightness value (see also D I) 4.).

As reported by Abers et al. (1979) in strawberry preserves of the colorimetric measures the hue angle showed the most significant correlation with visual scores.

3. HPLC

a) Red Raspberry

The elution order of the cyanins as determined by Spanos et al. (1987) was Cy-3-Sop, Cy-3-GlRu, Cy-3-Gl, and Cy-3-Ru. This elution order suggests that the hydrophobic CH_3 group of rhamnose causes increased retention of the rutinose glycosides and reverses the general rule that the elution order is tri-, di-, monosaccaride of the same aglycone. Considering the effect of the rhamnose CH_3 group on anthocyanin

retention, the elution order for the pelargonidin pigments should be Pel-3-Sop, Pel-3-GlRu, Pel-3-Gl, and Pel-3-Ru. (Spanos, 1987) Combining the effect of the sugar moiety determined by the number of sugar units and the presence of the CH_3 group, with the effect of the b-ring substitution on the overall polarity of the anthocyanin molecule gave the resulting elution order: Cy-3-Sop, Cy-3-GlRu, Cy-3-Gl, Pel-3-Sop, Cy-3-Ru, Pel-3-GlRu, Pel-3-Gl, and Pel-3-Ru. (Spanos, 1987)

The data of Spanos et al. (1987) showed different mean values for various pigments than previous analyses. Partial hydrolysis or polymerization of anthocyanins during the more rigorous pigment isolation for TLC analysis as well as the quantitation by densitometry could account for these differences (Spanos, 1987).

As determined by Spanos et al. (1987) the variety 'Meeker' showed a complex HPLC profile consisting of four cyanidin pigments with considerable variation between the percentages of cyanidin (for details see B 2.a).

b) Blackberry

Sapers et al. (1986) report the following elution order for blackberry extract from several varieties: Cy-3-Gl, Cy-3-Ru, a xylose-containing cyanidin-derivative, probably a dicarboxylic acid-acylated derivative of Cy-3-Gl, and another derivative of the latter kind. Several unidentified trace components could also be detected (Sapers, 1986).

Sapers et al. (1986) positively identified Cy-3-Gl by co-elution with that standard compound, spectral analysis and acid hydrolysis. Cy-3-Ru was identified by co-elution with that compound extracted from

rhubarb and by acid hydrolysis. Base and acid hydrolysis were used to identify the xylose containing derivative and the fact that it can be expected to elute later than the first two compounds due to its larger hydrophobicity. The dicarboxylic derivatives were identified by controlled acid hydrolysis and further HPLC separation of the obtained products and by base hydrolysis. (Sapers, 1986)

c) Red grapes

Bakker et al. (1986b) found that HPLC peaks of monomeric anthocyanins can be distinguished from polymeric peaks by their discrete peaks without interference. Polymers are seen as diffuse lumps of long retention times. HPLC gives a true measure of the free anthocyanin content of red wines. (Bakker, 1986b)

4. Comparison of HPLC and Spectral Methods

Bakker et al. (1986b) determined that total free anthocyanin (monomeric) contents of red table wines and port wines measured by HPLC were much lower than those estimated previously by the spectral method of Somers and Evans. Consequently, polymeric color derived by the difference between the values of total pigment and monomeric anthocyanin color were higher by HPLC than spectrally. The differences between the methods were greatest in young wines and decreased with aging.

Percent polymeric color/total color ratios of red wine derived by HPLC was 4.8 times greater after racking and 3.2 times greater after bottling than estimated by the spectral method. In port wines the mean value was 3.8 times greater by HPLC than spectrally. (Bakker, 1986b)

These findings indicate that oligomeric pigments ('intermediate pigment forms') formed during red wine aging are partially bleached by the bisulfite used in the spectral method so that the anthocyanin contents calculated on this basis are too high (Somers, 1977, 1986). The greater susceptibility of monomeric anthocyanins to acidification contributes to this effect (Bakker, 1986b).

5. Measurement of Color Degradation

Measuring the hydrolysis of the glycosidic bond by HPLC detection of the increasing and decreasing concentrations of aglycones (anthocyanidins) and anthocyanins, respectively is a rapid and reliable method for following the breakdown of anthocyanins (Blom, 1983).
Blom (1983) hydrolyzed the anthocyanins after their extraction from frozen strawberries with 0.1 N methanol and adjustment to 0.1 N HCl at 80°C in a water bath. He based his HPLC analysis on a gradient elution with water and acetic acid, 7:1, as the initial eluent and used methanol as the gradient builder with an increase from 0-25% in 8 minutes. In result the water soluble glycosides came earlier in the gradient than the water insoluble aglycones and provided distinctive degradation chromatograms over time. (Blom, 1983)

D MATURATION, AGING, COLOR STABILITY

The variable influences on phenolic (i.e. color) composition with related influences on other compositional aspects, are broadly of two sorts, namely exterior or ambient, and those which are intrinsic to the initial wine composition (Somers, 1986).

I) AMBIENT INFLUENCES

1. Processing

a) Thermovinification (HTST)

Sapers et al. (1985) found that heating darkened blackberry juice samples. These samples showed increased indices of degradation (A_{440nm}/A_{513nm}), smaller transmission Hunter L and slightly smaller hue angle values. However, in general, heating effects were very small in comparison to differences between red and black frozen subsamples. (Sapers, 1985)

Heating of berry mash before enzyme treatment to 80-85°C followed by cooling to 50°C best inactivates unwanted enzymes and microorganisms (Schobinger, 1986).

The heating processes used for red grapes today are:

1. simple heating: a) fermentation on pulp at a starting temperature of 20-25°C, b) heating of the mash to 50°C with standing time,
c) heating of the mash to 60-65°C;
2. partial high temperature application: a) to the juice, b) to part of the mash, both of which are later combined with the remaining juice/mash;
3. dipping or flooding process: dipping of a) the grapes, b) the mash; into heated must or combined with flooding;

4. HTST application to the mash at 80°C followed by immediate cooling to 45-50°C. (Maurer, 1973)

In order to obtain red wines with stable color heating of the mash at above 60°C is necessary to denature oxidases whose activation maxima are at approximately 55°C (Maurer, 1973; Wagener, 1981). When lower temperatures are used, very high SO_2 concentrations are needed to inhibit the oxidative enzymes (Wagener, 1981). Oxidases indirectly oxidize pigments in producing insoluble, chocolate colored sediments.

Two parallel processes occur during the heating of red grape mash:
1. a linear increase of color intensity resulting from increased solubility of the pigments due to temperature, and
2. increased oxidation of pigments due to increased activation of the oxidases which decreases rapidly once the denaturation point is passed. (Maurer, 1973)

The loss of color due to oxidation increases with increasing concentrations of oxygen. In the studies by Maurer (1973) the remaining color after five hours aeration of a) mash fermentation versus b) HTST + mash fermentation was 31% versus 82%. Consequently, the color found in the wine is the difference between the dissolved and degraded pigments. Therefore, to avoid oxidation high temperatures have to be reached rapidly. (Maurer, 1973)

In order to minimize the use as well as the loss of energy, the denaturation of pectinases and changes in flavor and quality of the wine, high temperature should only be applied for a very short time. High temperature of about 80°C permeabilizes the cell walls of the grape skins causing the pigments to diffuse more rapidly. (Maurer, 1973)

Heating pre-drained juice instead of mash results in more differences in color intensity and total phenols across all samples (Wagener, 1981). In addition, temperatures of above 60°C increase juice yields, and significantly decrease the time needed for obtaining free run juice (FRJ), clarification and pressing (Maurer, 1973).

Only in ripe fruit or grapes with high pigment contents is HTST application alone sufficient to degrade pectins which make pressing much more difficult due to their high water (juice) binding capacity. Normally additional enzyme treatment is necessary, because the naturally present enzymes (pectinases) are inactivated at above 55°C. Low amounts of added commercial enzymes (mainly pectinases) cause maceration after a few hours in grape varieties with high color intensity. For less ripe fruit or fruit with low color intensity six to twelve hours or sometimes a whole day is needed. Very high amounts of enzyme are recommended for the production of sweet reserves or very moldy fruit. All of these reactions proceed much faster with more enzyme added. (Maurer, 1973)

The major influence on grape wine aroma, a 'cooked' aroma is not due to the presence of hydroxymethylfurfurol (HMF) which was found in juices from other fruit. In red wines the term 'oxidized' is more appropriate as this aroma is due to the oxidation of the mash during or after heating. It can be prevented by rapid heating to high temperatures combined with the retention of oxygen. This treatment seems to have the additional effect of releasing more flavor components in producing fruitier wines with more body. (Maurer, 1973) (on furfural and 5-HMF and their influence on anthocyanin degradation see D I) 5.)

For the heating of red grape mash small tubular heaters with screw

flights were proven to provide best results. The additional use of inert gas is highly recommended. (Maurer, 1973)

b) Enzymes

Juice extraction from berry mash as well as filtration and concentration of the juice are not economical and very difficult without the use of appropriate enzymes (Heatherbell, 1980) since berry fruit has a high content of pectins which have great gelling properties (Schobinger, 1986). Increasing release of FRJ which is due to a decrease in viscosity may increase yield, easier pressing with reduced press time, pressing capacity, and juice quality (Flores, 1984; Schobinger, 1986). Flores et al. (1984) found that partial thawing of fruit for approximately three hours at 20-23^{o}C followed by milling at low speed (180 rpm) with a 3/4" screen size gives optimum release of FRJ in the laboratory.

For the production of strawberry wine Flores et al. (1984) used the pectinases 'Rohapect D5s' (D5L, D--standard pectinases for the clarification of apple juice), 'B-20' (for berry and grape juice to retain color), and 'VR' (Vin Rouge--for good color yield, filterability and savings in clarifying agents), the cellulase '2240', and an experimental fungal protease 'EL57-79' all of which had very similar effects on the release of FRJ (Dixon, 1985). The reaction optima of 'D5s' and 'VR' were at 1250 mg/kg pulp and for 'B-20' and the cellulase at 1000 mg/kg pulp, with the cellulase being much less effective than the other enzymes (Flores, 1984).

In all instances, Flores et al. (1984) found no advantage in the use of excessive enzyme treatment or an enzyme pulp treatment of more than one

hour for a concentration of 250 mg enzyme/kg pulp, as demonstrated by pectinase 'D5s' and reaction conditions standardized to 60 min at 25°C. These conditions also produced a FRJ which gave a negative test for residual pectin. (Flores, 1984)

Pre-press enzyme treatment ('D5s') of the pulp from frozen strawberry was found to increase yields of FRJ from 28% (untreated pulp) up to 56% giving total juice yields of approximately 86% for all treatments (Flores, 1984). Pectinase 'D5s' treatment of fresh strawberries gave approximately 15% increases in both FRJ and total juice yields over that obtained from untreated fruit (Flores, 1984). Yields of kiwi juice from 55-60% were increased up to 84% (total increase up to 42%) by pre-press pectolytic enzyme or press-aid treatment of pulp reducing pressing time from 30 to 15 minutes (Heatherbell, 1980).

Different pectinesterase (PE) activities present in the different enzyme preparations may account for their different behavior in releasing FRJ. 'D5s' has the highest concentration of PE corresponding to the highest yields of FRJ. This is due to increased de-esterification of the pectins in the fruit allowing further pectin degradation by hydrolytic pectinases (polygalacturonases and polymethylgalaturonases) (Flores, 1984).

Due to the action of pectinesterase which is present and needed in commercial pectic enzyme products methanol is produced as a by-product during the degradation of pectin to pectic acid (Maurer, 1973). Even small amounts of methanol have toxic effects leading to permanent damages in the human body such as visual impairment and blindness (Beyer, 1981); Amerine et al. (1980) report the oral lethal dose as 340 mg/kg body

weight.

Methanol contents in enzyme treated red and white wines in the range from 34-104 mg/L (Amerine, 1980), kiwi wine up to 181 mg/L (Heatherbell, 1984), and black currant juice up to 231 mg/L (Food Dictionary, 1981) are reported. Amerine et al. (1980) mention that occasionally methanol is found in excess in fruit wines. In contrast, kiwi wine produced without pectolytic enzymes contained 40-48 mg/L methanol and also retained approximately 15% more ascorbic acid. (Heatherbell, 1980)

Pre-press enzyme treatment of strawberry pulps was found to increase color extraction, most in juices pre-press treated with a mixture of 'D5s':protease (50% over control) followed by 'VR', 'D5s' and 'D5s':cellulase. Increased color extraction in pre-press enzyme treated juices is mainly attributed to pectinases which promote the maceration of fruit skins causing a permeabilization of the cell wall by dissolving protopectin between cells to some extent (Dixon, 1985) and the diffusion of coloring matter. (Flores, 1984)

Both pectolytic enzyme (30 mg/L 'Ultrazym 100' at 45°C for 15 hours) and press-aid (2% cellulose powder) treatments produced kiwi juices of low solids contents (including total phenols). This juice with the highest soluble, and the least settled and suspended solids was thus most readily clarified by filtration, siphoning and centrifuging. (Heatherbell, 1980)

Non-pectin, non-starch polysaccharides present in juices such as cellulose fibrils from cell walls and stone cells and seed or mold extracts (from mold contaminated fruit) are not readily degraded by existing commercial enzymes (Heatherbell, 1984)

Oxidized phenolics are potent inhibitors of pectolytic enzymes. Studies performed in vitro using mitochondrial preparations from various fruits showed that some non-oxidizable polyphenolics have an inhibitory effect on polyphenol-oxidase (PPO) enzyme systems which may be of significance in the control of browning. Hydroquinone, pyrogallol and alpha-naphtol showed about 80% inhibition whereas p-coumaric and ferulic acids exhibited about 30% inhibition. Certain other phenolics did not have any inhibitory or showed even activating effects. Since PPO is a metallo-enzyme it is likely that the inhibition noticed was due to metal chelation. (Prabha, 1986)

c) Fermentation on the pulp

In pulp fermentation it is the alcohol which causes the diffusion of coloring matter not enzymes (Flores, 1984). A large part of the polymers in red and port wine are formed during fermentation on skins (Bakker, 1986).

Methanol contents similar to enzyme treated kiwi wine are obtained when grape wines are prepared by fermentation on the skins. In this case methanol formation is due to naturally present pectinesterase or microbial action. (Heatherbell, 1980).

d) SO_2

Somers et al. (1977) defined 'free SO_2' as both molecular sulfur dioxide (SO_2) and bisulfite (HSO_3^-) as opposed to 'bound SO_2 (to anthocyanins or other phenolics). They report that recent studies of SO_2 toxicity towards yeasts and bacteria indicated that, although

HSO_3^- is by far the more abundant form of SO_2 in the wine pH range, it is molecular SO_2 which is the toxic species.

Brodie (1972, 1973) showed that pelargonidin glycosides, particularly Pel-3-Sam and Pel-3-Ru possess five times more color intensity in the presence of SO_2 than cyanidin glycosides. Sulfur dioxide is the most significant agent which determines the amount of free acetaldehyde in wines (Timberlake, 1976). Somers et al. (1977) report that in red wine acetaldehyde binds much more strongly with SO_2 than do the anthocyanins.

In studies of kiwi wines made without SO_2 it was found that these wines develop excessive brown color and an 'oxidized' flavor during storage despite the presence of high concentrations of ascorbic acid (0.4 g/L). Such changes correlated with the loss of ascorbic acid and were mainly retarded by the presence of free SO_2. Kiwi juice and wine have considerable SO_2 binding power: at bottling, after the addition of 275ppm of SO_2 during processing, 47% was present as SO_2 (11% free, 36% bound), 34% was oxidized to sulfate and 19% was unaccounted for. (Heatherbell, 1980)

e) Pressing and press-aids

With the incorporation of 1-2% press aid, pulped kiwi fruit could be readily pressed with an increase in juice yield of 44%, producing a press cake that was dry, firm and convenient to handle. Cellulose was superior to oak husks, with marginally better juice yields and firmer press cakes. (Heatherbell, 1980)

Schobinger (1986) reports that juice yields can be increased up to

10% when washing the press cake of elderberry with cold water once or twice. In addition, this extraction increases the contents of pigments, sugars and acids significantly (Schobinger, 1986).

f) Clarification and fining

The compounds which are important in juice clarification and post clarification haze and sediment formation are:
1. polysaccharides, 2. proteins, 3. polyphenolics (tannins), 4. polyvalent cations, and 5. lipids (Heatherbell, 1984). The judicious application of fining agents in conjunction with prior enzymatic juice treatment is usually essential for the removal of colloidally dispersed haze forming compounds and particles in the size range from 0.001 to 0.1μ (Heatherbell, 1984) (see D II) 3.)

The fining agents commonly used are:
1) Bentonite (SiO_2^-): provides satisfactory juice clarification and stability in removing proteins and polysaccharides. The use of protease provides a substitute for protein stabilizing by bentonite fining.
2) Gelatin, casein (NH_3^+): gelatin provides satisfactory juice clarification and stability in removing polysaccharides and polyphenolics contributing to residual bitterness and astringency. Only high quality gelatin should be used and over-fining with this agent should be avoided. Casein (preferably sodium caseinate) functions in a similar manner to gelatin but less efficiently; it offers the advantage of producing lighter colored juices by removing browning.
3) Silica sols (SiO_2^- or M^{2+} ions which prevent gelatin from staying in solution): provide good juice clarification and stability in removing

polysaccharides. These agents have the advantage of rapid settling and compacting of lees.

4) Polychlor ATC (PVP), Polyclar AT (PVPP): remove polyphenolics by H-bonding; PVPP removes browning in juices and wines.

5) Carbon (charcoal): deodorizes and tends to remove desirable aroma and taste components.

6) Sparcolloid (mixture of polysaccharides and diatomaceous earth): helpful in assisting the clarification of juices which have proven difficult to clarify. Overall, a convenient and good insurance policy is to combine bentonite, gelatin and silica sol fining as a routine procedure (Heatherbell, 1986).

g) Filtration

Juice sterility can be obtained by high speed centrifuges, filtration with sterile pads and membranes as well as finer grades of diatomaceous earth. However, these processes are usually not practical or economical without incorporating prior juice pre-treatments with enzyme and fining agents (Heatherbell, 1980, 1984). This is necessary to reduce viscosity and remove compounds (see also D II) 3.) which limit filtration rates, block filters, and have a tendency to form hazes and sediments during concentration or storage of clarified juices (Heatherbell, 1984). The filterability of depectinized ('D5s') strawberry juice before pressing increased by approximately 800% over untreated control juice (Flores, 1984).

Ultrafiltration has been successfully applied to the clarification (and preservation) of various juices by selectively removing high

molecular weight cloud-stabilizing compounds such as pectins, starches and proteins (Heatherbell, 1984).

The use of membrane filters (mechanical sieves with a firm pore structure, a pore size of 0.2-3.0 μm, and 100-200 μm thickness) make it possible to retain yeasts and all other bacteria without interference from pressures and pressure differences. In addition, these filters are easy to handle and do not absorb any color. Therefore, it is possible to change the type of beverage filtered quickly. For the filtration of fruit wines in particular, membrane filters are more economical than other kinds of filters. (Seel, 1980)

h) Other processing procedures

In addition to fermentation on the pulp Schobinger (1986) suggests fermentation of appropriately diluted berry juice ameliorated with sugar. For this process enzyme treatment at 40-50^{o}C after pressing followed by cooling to 20^{o}C and centrifuging is recommended. The obtained clear juice is then ameliorated with water and sugar before the addition of yeast. After fermentation is completed and before filtration the wine is sweetened with sugar and finally pasteurized in bottle. (Schobinger, 1986)

2. Freezing

Freezing of blackberries causes mixing of the cell's plasma and vacuolar contents with the result that the anthocyanins are placed in a solution of low pH. Only when blackberries are completely ripe does the pH stay high enough for the anthocyanins to stay blue. Fresh fruits

should have a pH in the range from 3.0-3.5 to retain their black color. (Jennings, 1979)

In studies by Jennings et al. (1979) the pH and TA values of ripening blackberry fruit changed largely after a short freezing period which suggests that the change in color to red occured immediately as a direct consequence of the freeze. In addition, the percentages of red fruit were closely related to the number of freezes applied, an effect which decreased asymptotically with the number of freezes. However, the pH before freezing was not correlated with the percentages of red fruit either for within or between cultivar variation. Similarly, there was no correlation between TA and the percentages of red fruit. (Jennings, 1979) 'Evergreen Thornless' was in the higher range in redness among the 12 ripening varieties studied by Jennings et al. (1979) with 62.4% red fruit after freezing. The color intensity of fully ripe fruit did not decline until after the third month of freezing and then only slightly.

Sapers et al. (1985) found that rapid thawing of frozen blackberry fruit (room temperature) results in less anthocyanin loss than does slow thawing (refrigerator). Juice from slowly thawed samples contained 20-30% less anthocyanin than from rapidly thawed berries. Transmission Hunter L values from slowly thawed fruit were significantly higher (lighter) and hue angles larger (more orange) than from rapidly thawed fruit. This may have been due to increased enzymatic degradation of anthocyanins and sucrose, the latter being due to increased invertase activity during slow thawing. The speed of thawing had no influence on juice absorbance at 440nm. Therefore, it is unlikely that differences in the extent of browning contribute to thawing effects on Hunter L and the hue angle.

(Sapers, 1985)

As a result of their studies with grapes Bakker et al. (1986b) concluded that the extraction of anthocyanins from frozen fruit is probably faster than during the fermentation of fresh fruit due to damage of the cells, which allow the anthocyanins to leach out easier. Flores et al. (1984) found that cell rupture from freezing and thawing increased strawberry juice release by about 20% over fresh fruit.

3. Oxidation

Traditional maturation processes for red wines permit wide variation in the uptake of oxygen before final bottling mainly through racking and pumping operations and ullage (Simpson, 1982; Somers, 1986). Phenolic interactions are initiated and further promoted by the absorption of oxygen (Somers, 1986).

Absorption of oxygen by wine leads to the formation of acetaldehyde from ethanol by coupled autoxidation with phenolic components. Acetaldehyde then induces co-polymerization of anthocyanins in Baeyer condensation reactions with formation of $-CH(CH_3)-$ bridges between phenolic units. Prevalence of the Baeyer reaction, promoted by oxygen uptake does not however constitute evidence that it is fundamentally responsible for pigment phenomena during the evolution of red wines. (Somers, 1986) (see also D I) 4.)

Acetaldehyde is a normal component of new wines and is depleted by interaction with phenolics. In studies by Somers et al. (1986) the patterns of change of two wines with very different phenolic contents were the same even though the initial acetaldehyde levels were quite different

(range from 8-55 mg/L). Consequently, phenolic condensation reactions do not depend on the formation of acetaldehyde by continuing oxidative influences. Acetaldehyde present at completion of primary fermentation is strongly bound to SO_2, whereas any subsequent oxidative influences result in the formation of free acetaldehyde. (Somers, 1986)

4. Maturation

The 'maturation' phase is quite distinct from the 'aging' phase, when the wine is in bottle and well protected from any further contact with air (Somers, 1986) (see D II) 4.).

Bakker et al. (1986b) report that pigment polymerization begins at the moment of crushing the grape. They found that a substantial amount of polymerization (at least 35%) occured during the fermentation of red wines (three days, at 25°C). Right after fortification the total pigment color already contained some contribution from polymeric pigment (22% in port wine) (Bakker, 1986b). In contrast, Somers et al. (1977) report a contribution of polymers of only 0-5% (at 520nm) in newly made wine which increased progressively during conservation and aging.

As derived from spectral data obtained during early maturation by Somers et al. (1986):

a. The reactions fundamentally responsible for progressive formation of polymeric pigments during maturation of red wines are essentially anaerobic (Debicki-Pospisil, 1983). Progressive formation of polymeric pigment occurs under N_2 headspace with much faster reaction rates at 25°C.

b. However, in commercial practice the normal presence of dissolved oxygen

imposes varying and uncertain influence on phenolic composition because of the intervention of acetaldehyde arising from the autoxidation of ethanol (see D I) 3.). Oxidizing enzymes in the pulp could operate as an additional factor during red wine aging (Timberlake, 1976).

c. Most rapid change in phenolic composition occurs during the first few months after vintage.

d. Temperature is the major influence on rates of reactions leading to formation of polymeric pigments.

e. Pasteurization has no influence on the course of phenolic aging reactions.

In conclusion, Somers et al. (1986) found that there is the possibility of more deliberately 'structuring' a red wine during the critical early stage of maturation, i.e. aging may be accelerated in a controlled fashion by bulk storage of a cold-stabilized sterile wine at an elevated temperature under inert gas for several weeks after vintage.

Studies in model wine systems by Timberlake et al. (1976), consisting of alcoholic tartrate buffer containing pure anthocyanins (based on malvidin), phenolic compounds (based on catechins, including procyanidin dimers and trimers), and acetaldehyde, alone and in mixtures, showed that at least six reactions can occur which have implications in wine-making:

1. tranformations of phenolics alone,
2. degradation of anthocyanins alone,

The remaining four reactions can be divided into two types of phenolic condensations which are normally operative during the maturation

of red wines (Somers, 1986):

(I)= 3. Direct condensation or non-aldehyde aging (Bakker, 1986a) between anthocyanins and other flavonoid components (phenolics), and

(II) Baeyer condensation or aldehyde-aging (Bakker, 1986a) by which interaction with acetaldehyde results in the formation of $-CH(CH_3)-$ bridges

4. between anthocyanins alone,

5. between phenolics alone, and

6. between anthocyanins and phenolics. (see also D I) 3.)

Bakker (1986a) found that in port wines non-aldehyde (I) and aldehyde aging (II) are competing with each other. They report further that red wines which show largely non-aldehyde aging are known to increase in brownness on aging, whereas in model solutions aldehyde aging results in an increased violet color. Thus Bakker et al. (1986a) concluded that browning in a port wine will be modified by the extent to which the acetaldehyde aging has occurred. The hue angle in the port will be the net result of both processes (Bakker, 1986a).

Whereas direct condensation (I) to polymeric pigments appears to be a property of the total phenolic extractives in red wines, the extent of Baeyer reactions (II) must depend on the availability of free acetaldehyde (Baranowski, 1983; Somers, 1986). Timberlake et al. (1976) proposed a reaction scheme for the Baeyer condensation (reaction 6) in which the initial reaction product of acetaldehyde and phenolic combines with the anthocyanin at position 8, followed by anhydrobase formation.

Sims et al. (1986) report that anthocyanin-tannin polymerization (direct condensation I) is a normal maturation reaction in most *Vitis*

vinifera wines and helps stabilize the color of these wines. Timberlake et al. (1976) found that in contrast to Baeyer condensation reactions (II) anthocyanins and phenolic compounds alone (I) react very slowly, with the eventual formation of yellow xanthylium salts (one presumed glycosylated, others sugar-free), confirming that condensation occurs via the anthocyanin 4-position and position 8 (or 6) of the phenolic. Hydrolysis of the glucose in the anthocyanin position 5 is not rate determining and can occur more readily than at position 3, which presumably remains in the glycosylated xanthylium salt (Timberlake, 1976). The conversion of monomeric anthocyanins into reddish-brown polymeric pigments by their reaction with other phenolic compounds, probably procyanidins has also been proposed for reaction (I) which according to Timberlake et al. (1976) is the least prominent reaction occuring. Bakker et al. (1986a) also found that port wine low in aldehydes resulted in the brownest port, however that reaction (I) was dominant (during the first six months of storage at least).

This reaction (I) is largely promoted by acetaldehyde (reaction (II), 6) leading to increased color intensity (spectral color) (Sims, 1986). This confirms the findings by Timberlake et al. (1976) that in wine model systems rapid and spectacular color augmentation is due to the formation of highly colored new compounds, detectable by chromatography and believed to consist of anthocyanins and phenolics linked by $-CH_3CH-$ bridges (reaction 6). Bakker et al. (1986a) also reports that port wine with excess acetaldehyde present was more violet (much less brown). Timberlake et al. (1976) found that this color augmentation is unaffected by the nature of the buffer anion (acetate, succinate, malate, tartrate or

citrate) and occurs readily from pH 2.75-4.00 (optimum 3.5). When reacting acetaldehyde with malvidin-3-glucoside (Mv-3-Gl) (reaction 4) after seven months the color was augmented by 60%.

This reaction caused shifts towards violet, with extent of the shift varying with the type of component. The extent of color increase and its stability decreased with malvidin-diglucoside > -acylated diglucoside > -monoglucoside, because of precipitation with the latter. The reactivity of these compounds decreased in reverse order. The rate of reaction 6 appeared to increase with the complexity of the phenolic compound, i.e. epicatechin < procyanidin dimer < procyanidin trimer. (Timberlake, 1976)

Color is enhanced not only by acetaldehyde but also by invert sugar (Timberlake, 1976). However, adding pure aldehyde-free glucose or fructose to these model systems produced no effect comparable to that of acetaldehyde. Timberlake et al. (1976) report further that the complexes formed by copigmentation of anthocyanins with a wide range of flavonoids and other compounds were dissociated by addition of ethanol.

There was little reaction between the anthocyanins and acetaldehyde (reaction 4) except with Mv-3-Gl, which was slowly polymerized. Reaction 5 was complicated by simultaneous disproportionation of the procyanidins. (Timberlake, 1976)

5. Storage, Color Deterioration (Instability)

Pilando et al. (1985) found a progressive decrease of monomeric anthocyanin in strawberry wine through storage, with only 1-2% of the anthocyanin originally present in the juice remaining after six weeks. However, the color of partially aged wine was chiefly polymeric which

increased by 30-50% after fermentation and steadily increased during wine storage. The browning index gradually increased in aging strawberry wines. Hunter L and b values generally increased during storage which showed the bleaching and lightning and yellowing of the wines, respectively. Redness a values showed varying patterns. (Pilando, 1985)

In red wines Somers et al. (1986) found that rapid decrease of total anthocyanins during storage at 25°C was accompanied by the corresponding increase of a percent measure of polymeric pigments (age index) at wine pH. The age index or 'chemical age' is a qualitative measure referring to the progressive displacement of the monomeric anthocyanins in the course of aging reactions by more stable polymeric pigments, which are resistant to decolorization by SO_2 and to changes in pH (Somers, 1977, 1986). This index approached 100% in aged red wines in which the polymeric pigments were almost entirely responsible for wine color. These investigators found that changes in pigment already occured after two weeks of storage. The age index increased to 25% from much lower values typically seen during and immediately after primary fermentation. They report further that during one year the age index increased from 25 to 78% at 25°C, but attained only 40% at 3°C. The rate of change in color composition was greater during the first 100 days of observation. (Somers, 1986)

Somers et al. (1986) also found that wine hue or tint at 25°C changed perceptably in the first months of observation when anthocyanin loss was greatest. Timberlake et al. (1976) report that at pH 3.5 color reached a maximum after 57 days when the color was five times its original. However, despite these large changes in color composition the

wine color density remained virtually constant as reported by Somers et al. (1986) with a slight decrease in E_{520nm} being compensated by a similar increase in E_{420nm}. This confirms the finding by Timberlake et al. (1976) that on storage, mixtures of anthocyanins and phenolics (see D I) 4., reaction I) gradually lost color in the red region (520nm) but increased in the brown region (420-450nm). Similar behavior, however, was shown by anthocyanins alone (which fade) (D I) 4., reaction 2) and phenolics alone (which brown) (D I) 4., reaction 1). Overall it appeared that after seven months of storage a mixture of Mv-3-Gl and epicatechin (reaction I) showed some small net loss of anthocyanin color and an increase in net browning, indicative of interaction, whereas effects were less marked with mixtures with procyanidin dimers and trimers. (Timberlake, 1976)

Sims et al. (1986) report that several factors contribute to color instability of red muscadine wine, including: 1. cultivar (e.g. lack of the necessary tannin species), 2. anthocyanin composition, 3. a low degree of anthocyanin-tannin polymerization (possibly due to insufficient incorporation of diglucoside anthocyanins into these complexes), 4. pH, and 5. processing and storage conditions.

Color deterioration in strawberry preserves was reported to be due to at least three causes: 1. heavy loss of red anthocyanin pigment, > 50% of which could occur without a marked deterioration in color; 2. formation of brown pigments which results in an immediate loss in attractiveness (see also D II) 1.); and 3. discolorization resulting from such factors as heavy metal contamination (Abers, 1979).

Abers et al. (1979) showed that during a 26 week storage period at 21 and 37°C color deterioration occured at a much faster rate in strawberry preserves produced from a variety which contained higher levels of reactive phenolics (leucoanthocyanins, flavanols) which were assumed to play a major role in this process and total phenolics. Somers et al. (1977) also report of the extremely reactive nature of the flavonoid phenolics extracted from skins during vinification of red grapes. In addition, this color deterioration which was due to browning was less pronounced in preserves from another strawberry variety with higher levels of anthocyanin pigment, ascorbic acid, and free amino acids (Abers, 1979). This confirms reports that the rate of ascorbic acid oxidation influences the anthocyanin loss in strawberry products and model systems. The results of the studies by Abers et al. (1979) emphasize the role of brown pigment formation rather than anthocyanin pigment loss as the primary cause of the more rapid color deterioration observed in one of the preserves (see also D II) 1.). General trends for both preserves studied were an increase in color with time. Samples stored at 37°C showed more rapid change in saturation index which decreased slightly by the twenty-sixth week. The decrease could have been caused by development of dark, pigmented compounds which tend to mask color.

Studies by Timberlake et al. (1976) showed that disproportionation of procyanidin dimer in alcoholic tartrate buffer (pH 3.5) into the related trimer and epicatechins, with small amounts of more complex procyanidins was greatly accelerated by light. Exposure to light at these conditions also caused increased production of a yellow-brown chromophore (λmax 450-460nm), which was further accelerated by adding iron salts.

Chromatography of solutions containing the diglucoside or acylated diglucoside of malvidin and (+)-catechin or epigallocatechin (D I) 4., reaction I) revealed discrete yellow spots which turned orange on exposure to ammonia vapor. Their spectral characteristics were in agreement with those expected for xanthylium salts. (Timberlake, 1976).

Sims et al. (1986) report that a red muscadine wine treated with 300 or 600 mg/L acetaldehyde and stored for eight months at 20°C (D I) 4., reaction II) had:

1. less browning; there was very little browning initially as shown by Hunter b values and visual ratings. The browning rate remained lower during eight months which was probably due to increased anthocyanin-tannin polymerization;
2. greater chemical age (anthocyanin-tannin polymerization); after eight months the decrease in phenolics was larger than in untreated wines which could have been due to the formation of phenolic polymers (Baeyer condensation, reaction II) large enough to precipitate;
3. greater color intensity (spectral color) than untreated wines; tannic acid and acetaldehyde combined increased color intensity initially and after eight months as compared to the controls as shown by higher absorbance at 520nm and visual ratings. Acetaldehyde increased the visual intensity to a greater extent than tannic acid. Although acetaldehyde slightly increased the loss of phenolics during eight months, it did not increase the loss of absorbance at 520nm, indicating no greater loss of anthocyanins in wines treated with acetaldehyde. Thus, practices that increase the production of acetaldehyde may enhance the polymerization of anthocyanins with tannins and improve color, provided that oxidation is

not too severe and that anthocyanin-tannin polymers do not become so large as to precipitate. (Sims, 1986) (see also D I) 3. & 4.)

Debicki-Pospisil et al. (1983) report that the very same conditions favoring anthocyanidin degradation during processing and storage) also give rise to the formation of furfural (F) and 5-hydroxymethylfurfural (HMF). Furyl aldehydes can be formed by sugar degradation (F mostly from aldopentoses, HMF from ketohexoses), as well as by the transformation of other compounds, e.g. ascorbic or polyuronic acids. However, it does not necessarily follow that the presence of F or HMF affects pigment degradation.

These investigators found that 0.012 M HMF or F accelerated pigment degradation in blackberry juice as well as a citrate buffer model solution containing Cy-3-Gl. The acceleration was directly temperature-dependent, more pronounced in fruit juice and considerably decreased in nitrogen. At the same molar concentrations, in all instances HMF exhibited a stronger negative influence on pigment retention than F. Cy-3-Gl degradation effect of non-furane aldehydes (0.012 M) was formaldehyde > acetaldehyde > benzaldehyde. Compared to controls, degradation of Cy-3-Gl and cyanidin was always more pronounced on presence of either aldehyde, in both examined systems and at all three temperatures (24, 50, and 70^{o}C). The negative effect of aldehyde was consistently compounded further by the direct effect of storage temperature. In all samples the spectra showed a shift in the λ max for Cy-3-Gl, from the characteristic 510 up to 538nm in the blackberry juice sample with formaldehyde added. (Debicki-Pospisil, 1983).

Cy-3-Gl disappearance followed first order reaction kinetics in the

samples without aldehyde added, in both systems and at all three temperatures (Baranowski, 1983; Tancev, 1974; Tantschev, 1973). Over the examined range of temperatures, a linear Arrhenius plot of the Cy-3-Gl degradation in model and juice systems was obtained. The temperature effect on the rate of pigment loss exceeded the effect of aldehyde presence in all instances except formaldehyde. When either aldehyde was added, the system behavior was found predominantly temperature-dependent at 70°C. At 24 and 50°C, probably oxygen as an additional factor influenced the rate of reaction between the pigment and aldehyde. (Debicki-Pospisil, 1983)

HMF disappearance kinetics followed first order in all model system tests and in juice at 24°C and deviated from the first order in juice at 50 and 70°C. The probable reason was the new simultaneous formation of HMF from the present sugars via nonenzymatic browning reactions. (Debicki-Pospisil, 1983)

6. Microbiological influences

Mold contamination, mainly *Botrytis cinerea*, of overripe strawberry increases juice viscosity, reduces the fermentation rate, and accelerates color degradation. In grapes this mold was found to affect the compositional balance of musts and to produce 'botriticine', an antibiotic that inhibits yeast development. *Botrytis* infection is related to reduced acidity, color intensity, anthocyanins and tannins and increased pH and dry extract. (Pilando, 1985)

Sponholz et al. (1984) supposed that 2-oxo, 5-oxo-gluconic and glucuronic acids are produced by acetic acid bacteria from glucose, since

galactaric acid is produced by oxidation of galacturonic acid by these bacteria. *Botrytis* fungi also produce the intermediate gluconic acid from glucose which is then further oxidized to the 2-oxo and 5-oxo forms. Both acetic acid bacteria and *botrytis* fungi have been found together on moldy berries.

Maurer (1973) suggested that protopectin is probably degraded to pectin by protopectase. Esterified galacturonic acid is formed from pectin by polygalacturonases (polymethyl-galacturonase) and later transformed into galacturonic acid. Pectic acids are also formed from pectin with the help of pectinesterase. A by-product of the latter reaction is methanol. (Maurer, 1973) (see also D I) 1.b)
Sponholz et al. (1984) found that fruit and dessert wines showed very low amounts of galacturonic acid with the exception of blackberry wine which contained 1300 mg/L galacturonic acid. These differences may be due to pectinases contained in fruit or added to the must which degrade galacturonic acid. Glucuronic acid was found in fruit wines in the range from 0-20 mg/L. Other acids formed by acetic acid bacteria were absent or only found in trace amounts. Overall, fruit other than grapes seems to be less susceptible to acetic acid bacteria. (Sponholz, 1984)

Timberlake et al. (1976) report that acetaldehyde is formed in wines by several mechanisms, principally by microbial action during fermentation and more slowly from ethanol by coupled oxidation of certain phenolics. (see also D I) 3.)

II) INTRINSIC INFLUENCES

1. Browning

The browning reaction is the sum of 1. enzymatic browning reactions, mainly due to PPO, and 2. non-enzymatic browning: a) the oxidative browning of phenolics, b) Maillard browning, and c) ascorbic acid browning (Poei-Langston, 1981; Pilando, 1985).

Color deterioration in strawberry is mainly due to the instability of the anthocyanin pigment coupled with enzymatic as well as non-enzymatic browning (Pilando, 1985). Abers et al. (1979) report that the more reactive phenolics (i.e. leucoanthocyanins and catechin) in strawberry preserve may account for a greater suceptibility to polymeric browning. Many of these phenolic compounds can also serve as a substrate for PPO (Abers, 1979). PPO catalyzes browning reactions by acting on d-catechin present in strawberries (Pilando, 1985). Polymerization of catechins, catalyzed by PPO, produces repeated quinone units having a high absorbance in the 400-500nm range (browning) (Pilando, 1985).

In Maillard browning reactions (2.b) reducing sugars could react with amino compounds (Abers, 1979). However, in their study Abers et al. (1979) inferred that the mechanism accounting for the more rapid browning of one type of strawberry preserve as compared to another was different from the classical Maillard reaction. These investigators suggested that anthocyanin degradation and Maillard browning reactions were of less significance in the preserves studied.

Sims et al. (1986) found that diglucoside anthocyanins browned to a greater extent than monoglucoside anthocyanins. This confirmed the finding by Timberlake et al. (1976) that after one year solutions

containing Mv-3,5-diGl and various phenolics (D I) 4., reaction I) exhibited an intense browning (λmax 440-450nm) which appeared much more pronounced than that occuring with Mv-3-Gl because of the comparative lack of red color as a result of the lower pK value of the diglucoside.

2. Complexation

Asen et al. (1972) report that anthocyanin-metal ion-polyphenol complexes may influence anthocyanin color in blackberries for pH > 3.0. However, Sagi et al. (1974) could not detect these complexes in overripe raspberries which had turned purple.

Several polyvalent cations such as Fe, Cu, Al, and Ca are known to exert an effect on haze and sediment formation in wines as they readily form stable complexes with phenolics and can combine with proteins, pectins and starch (Heatherbell, 1984). Coffey et al. (1981) also report that Ca^{2+}, Mg^{2+}, and Zn^{2+} complexes have a negative influence on the stability of color. Cu is of particular interest since it is a cofactor for PPO and can also serve as a catalyst for numerous oxidative reactions (Abers, 1979). Therefore, presence of the above cations in juice products should be avoided by using only stainless steel or non-metallic processing equipment (Heatherbell, 1984).

In contrast to the above it was shown that in wine model systems there was little net effect of ferric chloride on Mv-3-Gl in darkness but that the color was augmented and became violet on exposure to light. The latter effect was attributed to the production of reactive carbonyl compounds by the photochemical decomposition of tartaric acid in the presence of oxygen and iron salts (Timberlake, 1976).

It was also shown that addition of 500ppm Al to strawberry and raspberry jam improved color stability (Abers, 1979). In studies by Coffey et al. (1981) complex formation was indicated by colorimetric values with Cy-3-Gl or raspberry juice and Al^{3+} at pH 2 and 3 and with Sn^{2+} at pH 3 and 4. According to HPLC analyses complex formation occured in raspberry juice samples treated with Sn^{2+} but not in samples of purified Cy-3-Gl (Coffey, 1981).

However, Coffey et al. (1981) note that all these reactions showed a lot of fluctuation as they were time, temperature and pH dependent. pH was a more important source of variation than temperature. Increased temperature seemed to favor the production of complexation or possibly polymerization. There was a trend of increasing degradation over time. The presence of citrate buffers may interfere with complex formation between metals and anthocyanins as citrate is a strong metal chelator. Flavonoids are responsible for many co-pigmented metal-anthocyanin complexes for both Al and Sn. (Coffey, 1981)

Coffey et al. (1981) concluded that these are dynamic processes influenced by the concentrations of the different structural forms of the pigments, intermediates, breakdown products and different buffers. Therefore, the stability conferred by these complexes is not such that a practical application of these results seems imminent (Coffey, 1981).

3. Haze and Sediment Formation, Polymerization

Cloudy or turbid juices are complex colloidal systems containing molecules in true solution as well as in particulate suspension. The compounds which are important in juice clarification and post

clarification haze and sediment formation are: 1. polysaccharides, including pectins, starches and gums (often unidentified such as mold exudates); 2. proteins; 3. polyphenolics; 4. polyvalent cations; and 5. lipids. (Heatherbell, 1984)

Very often these components form insoluble complexes. The latter consist of molecular aggregates and cellular debris and range in size from approximately 0.001μ (1.0mμ) to 1000μ (1.0mm) in diameter (Heatherbell, 1984).

Particles < 100μ are retained in suspension by mutual charge repulsion and by colloidal stabilizing polysaccharides. Larger particles of ca. 100 to 500μ may settle out rapidly. Material > 0.5μ may be removed by centrifugation or filtration. For the removal of colloidally dispersed haze forming compounds and particles in the size range from 0.001 to 0.1μ the application of fining agents in combination with prior enzymatic juice treatment is usually essential. (Heatherbell, 1984)

The polymerization of anthocyanins and reactive phenolics is a major cause of color deterioration in strawberry preserves (Abers, 1979). However, Sims et al. (1986) report that no greater loss of anthocyanins occured in red wines treated with acetaldehyde. Abers et al. (1979) report that it was postulated that the anthocyanins of a new grape wine were incorporated into a polymeric complex of leucoanthocyanin-anthocyanin. Alcohol or sugar acted as dehydrating agents in this reaction (Abers, 1979).

Formation of an insoluble red-brown precipitate was reported as an end product of anthocyanin degradation in strawberry preserves (Abers, 1979). This confirmed the findings by Timberlake et al. (1976) who

proposed the conversion of monomeric anthocyanins into reddish-brown polymeric pigments by their reaction with other phenolic compounds, probably procyanidins in wine model systems. Maurer (1973) reports that oxidases indirectly oxidized pigments in red grape mash in producing insoluble, chocolate colored sediments. Flores et al. (1984) observed that in strawberry juice the use of 2% press aid reduced juice settled solids to < 3% v/v which therefore did not contribute significantly to juice volumes.

In condensation tests performed by Debicki-Pospisil (1983) the reaction product of furfural (F) with cyanidin in methanol appeared as a dark-brown voluminous sediment only after prolonged refrigerated storage. For this reaction to occur addition of Na-acetate was necessary which suggests that cyanidin was active in the reaction as a pseudobase (keto or enol form). Each cyanidin form can enter the reaction with F or HMF, the most reactive form being the cyanidin-anhydrobase (enol form). The mechanism of cyanidin degradation in presence of aldehydes can not be limited to one singular pathway. Debicki-Pospisil (1983) mention three mechanisms of action which were suggested.

In $POCl_3$ the above reaction of Cy-3-Gl with F or HMF occured much faster and resulted in a pinkish-black precipitate which was poorly soluble in methanol or acetone and insoluble in acetic acid. Following partial dissolving in methanol, an increased spectral absorption of the solution was observed at wavelengths of 290, 364, 440 and 538nm. (Debicki-Pospisil, 1983)

Somers et al. (1986) found no essential difference between data sets for cold-stabilized and non-cold stabilized red wines, both of which tend

to throw precipitates.

Unstable proteins (lower molecular weight of ca. 16,000 to 24,000 and of higher iso-electric points with a pI range of 5.2-8.0) are common in white grape juice and wine. In red grape juices these unstable proteins are usually removed by co-precipitation with anthocyanin pigments and polyphenols.

Simpler phenols such as chlorogenic acid have little affinity for proteins but under the influence of heat or oxygen or with aging (storage) of juice, wine or beer, can polymerize to form polyphenols (tannins) which may contribute to turbidity on their own or by complexing proteins.

Protein hazes in particular can occur during juice concentration because of the effect of heat and increase in osmotic pressure. In addition, pH changes due to concentration or blending of juices are responsible for protein phenolic haze formation. (Heatherbell, 1984)

In aged beer, beer proteins (a complex mixture of variable composition) combine with polyphenols to form complexes and these are responsible for the formation of chill haze, the removal of which through filtration increases shelf life (Verzele, 1986). Verzele et al. (1986) report that soluble proteins (e.g. beer protein and bovine serum albumin (BSA)) seem to complex preferentially with the higher molecular weight tannin polygalloyl glucose components. Tannins can be divided into condensed and hydrolyzable tannins. The gallotannins are the most important hydrolyzable tannins in which galloyl groups are bonded to the central polyol nucleus by a hydrolyzable ester or glycosidic bond. (Verzele, 1986)

Hydrolyzable tannins possess anti-oxidation properties and are able to

form soluble and insoluble complexes with proteins. The content of tannic acid (polygalloyl glucose or polygalloyl quinic acid) in a sample is not exactly identical with the tanning capacity (protein precipitating or binding power). It has been stated that tanning capacity is directly related to both 1. molecular weight, with an optimum of ca. 3000, and 2. the number (minimum two) of specific groups in a molecule if they are sterically well separated and each of them is able to bond to proteins. The latter fact may explain the high tanning capacity of some tannins despite their lower molecular weight. The higher polygallol glucoses possess the highest precipitating power for beer protein, BSA and probably for other soluble proteins as well. (Verzele, 1986)

4. Aging

The color of red wine is due to anthocyanins derived from the fruit (monomeric or 'free' anthocyanins) and polymeric pigments formed by anthocyanin condensation with other flavonoid compounds and probably aldehydes during wine aging (Bakker, 1986b). Wine color depends highly on variations in anthocyanin equilibria which were measured to be very large, the main influences upon the positions of the equilibria being wine pH and SO_2 added after fermentation (Somers, 1977). Thus a fault or deficiency in red wine color may well be due to an unfavorably low degree of ionization of anthocyanins rather than a low level of anthocyanins in red wine (Somers, 1977).

Progressive increases in pigment resistance to bleaching by bisulfite and decreases in color gain on polymer acidification are envisaged, as oligomeric and polymeric pigments of increasing complexity

are formed during wine aging. Losses of total pigments and total free anthocyanins were found to be logarithmic with time during port wine aging. The rate constant of anthocyanin loss as determined by HPLC was suggested as a true measure of anthocyanin aging in port wine. Increased color with time (years) was attributed to oxidation of SO_2 and fermentation of ethanal. (Bakker, 1986)

5. Sensory Changes

Somers et al. (1986) report that the composition of red wine color changed continuously during vinification and storage, with associated changes in sensory characteristics of the vintage.

Distinctly different flavor characteristics were produced depending on whether kiwi press juice was clarified by pectolytic enzymes or by settling or centrifuging, before fermentation, or left unclarified. Non-enzyme treated juices had the same unacceptable astringency and bitterness as the sediments from enzyme clarified juice. In grape wines increases of 85-100 mg/L total phenols produced a threshold difference in astringency which together with bitterness was probably also caused by non-phenolic compounds.

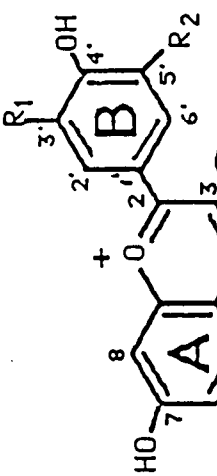
Generally, wines made from juice with a low solids content are usually fresher, cleaner and fruitier than wines made from more turbid juices. It seems likely that enzymes other than pectinases (or nonspecific pectinesterases) are present in commercial enzyme preparations used, and could also be responsible for aroma changes. (Heatherbell, 1980) (see also D I) 1.b)

6. Interrelationships

Pilando et al. (1985) found a correlation between composition, color measurements and sensory scores of strawberry wines. Total phenolics, nonflavonoid phenolics, and soluble solids of the fruit were highly correlated with color density, polymeric color, browning index and Hunter a values of the strawberry wines. Anthocyanin content, TA, pH, and PPO of the fruit were highly related to anthocyanin content of the wine. PPO was highly correlated to overall color and appearance, color intensity, and browning. (Pilando, 1985)

Overall color and appearance, color intensity, and hue measured by sensory analysis were highly correlated to anthocyanin content, flavonols, leucoanthocyanins, TA, and pH. The anthocyanin content of the wine and the Hunter L were found to be important predictors of color since they were both highly correlated to practically all of the sensory indices for color. (Pilando, 1985)

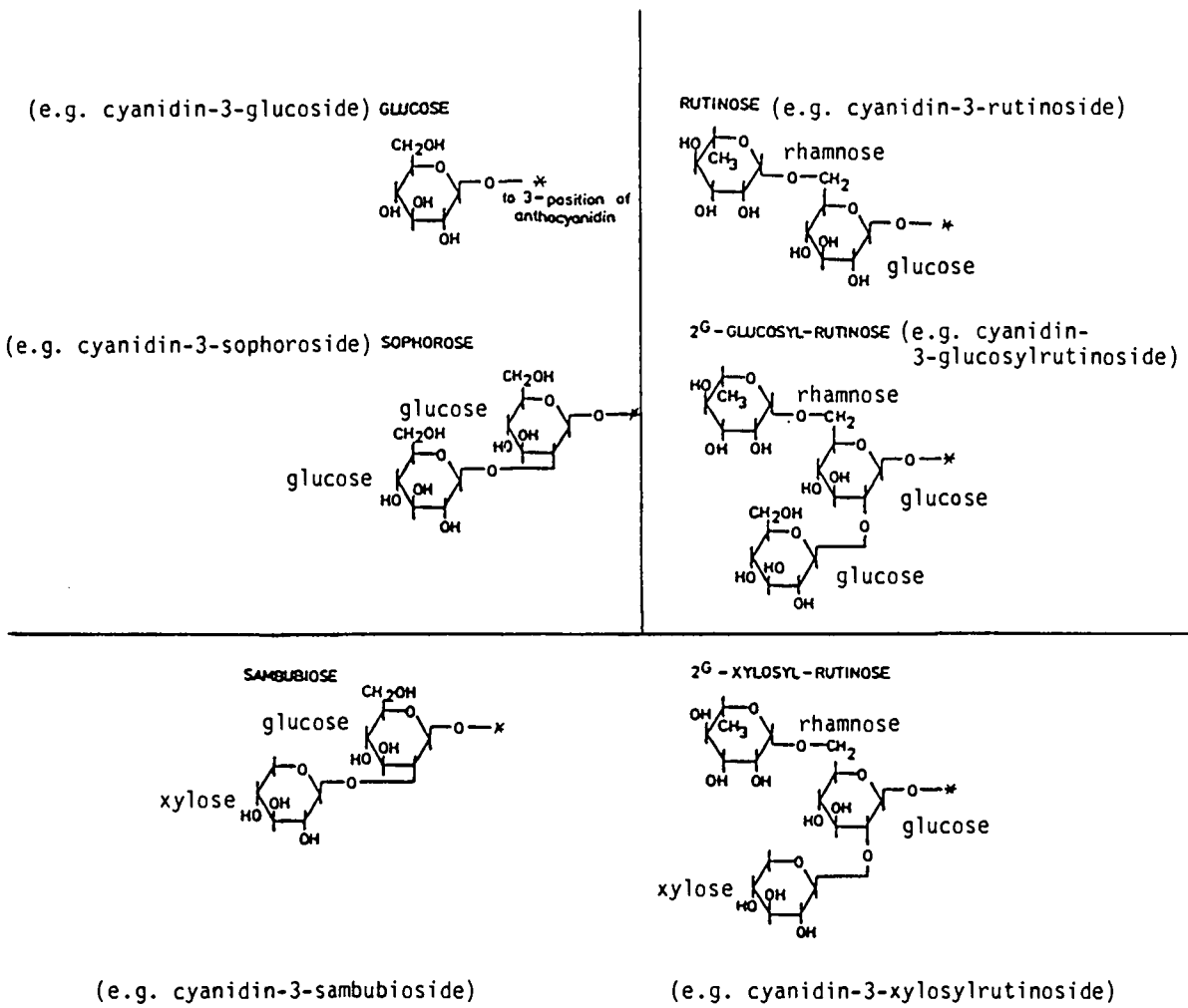
Fig. I-Anthocyanidin structures.



3,5,7,4'-tetrahydroxyflavylium cation

	R_1	R_2
delphinidin	OH	OH
cyanidin	OH	H
petunidin	OCH_3	OH
pelargonidin	H	H
peonidin	OCH_3	H
malvidin	OCH_3	OCH_3

Fig. II-Molecular structure of six sugar residues found in anthocyanidin pigments of *Rubus*.



Title: **RED RASPBERRY JUICE AND WINE: THE EFFECT OF PROCESSING AND STORAGE ON COLOR AND APPEARANCE**

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ABSTRACT

Red raspberry (*Rubus idaeus L.*) wines were made from thawed, frozen fruit (Meeker variety) by fermentation of pulp, depectinized juice, and high temperature short time (HTST) treated depectinized juice. The influence of fining was also investigated. The wines were stored at 2 and 20°C for six months. High performance liquid chromatography (HPLC), spectral and Hunter measurements were made at intermediate processing stages as well as after storage and the results subjected to analysis of variance (ANOVA). Fermentation was the major processing stage where anthocyanins degraded for all samples with total losses of at least 50%. Cyanidin-3-glucoside was the most reactive pigment, disappearing completely during fermentation; this confirms the greater susceptibility of monoglycosides to hydrolysis. Presence of diglucosides explains the greater anthocyanin pigment stability of raspberry compared to blackberry and strawberry wines. HTST application had a beneficial effect through the inactivation of native enzymes. HTST treated depectinized wines with fining had the best color stability, color appearance and sensory quality after storage.

INTRODUCTION

Color loss and deterioration (browning) as well as haze and sediment formation during storage present great problems to the commercial producers of red raspberry (*Rubus idaeus L.*) wine. Such problems make this product unacceptable to the consumer and therefore hardly marketable. An improvement of the color quality, however, could easily increase sales.

Anthocyanins are the major cause for this color instability because of their great susceptibility to degradation and polymerization with the influences of temperature and time (Pilando, 1985). The anthocyanin profile for red raspberry juice of several varieties was determined by HPLC (Spanos, 1987); however, the changes in overall color quality and the anthocyanin profile during processing and storage of raspberry wine have not been studied. Polymers are the major source of color in partially aged strawberry wine (Pilando, 1984). A large part of the polymers in red and port wine are formed during fermentation on skins (Bakker, 1986). Enzyme treatment (mainly pectinases) was shown to inhibit polymerization and increase color extraction and density in strawberry wine (Flores, 1984; Maurer, 1973). Heating of berry mash to 80-85^{o}C followed by cooling to 50^{o}C and enzyme treatment best inactivates unwanted enzymes and microorganisms (Schobinger, 1986) and also releases more flavor compounds (Maurer, 1973). Heat and enzyme treatment were also shown to have a positive influence on total juice yields (Flores, 1984; Maurer, 1973; Schobinger, 1986) which in the case of berry wines is an important factor due to the high cost of the fruit.

It seems likely that the combined application of pasteurization and enzyme treatment improve color stability and quality and possibly also the sensory quality of raspberry wines. Therefore, it was the purpose of this investigation to 1. develop a processing procedure for good color quality and appearance and 2. investigate the influences of storage time and temperature on color changes and stability of the different wines. Three different processing procedures were used to investigate the treatment influences. Detailed analyses were applied to quantify the color changes during processing and storage. HPLC profiles were determined to examine the effects on the individual anthocyanins.

MATERIALS AND METHODS

SOURCE AND TREATMENT OF THE RASPBERRY SAMPLES

Red raspberries (Meeker variety) grown and picked by 'Rainsweet' near Salem, OR in 1986 and commercially block frozen in 13 kg plastic containers by 'Kemico,' in Salem, OR were used in all processing trials. These samples were stored frozen at -12°C until needed.

JUICE AND WINE PROCESSING

The juices and wines were produced following the procedures by Spanos et al. (1987) and Flores et al. (1984), respectively. Three lots of raspberries (ca. 39 kg each) in replicates, were partially thawed at 27°C and ground through a hammermill (Model D Comminuting machine; W.J. Fitzpatrick Co., Chicago, IL) equipped with a 3/4 in. diameter circular pore mesh at a speed of 182 rpm. After addition of 25 ppm SO_2 as a 1% potassium metabisulfite solution, the mashes were:

(1) fermented on the pulp at 25°C by adding 1 g/gal champagne yeast (Scott Laboratories, Inc., San Rafael, CA) to a soluble solids content of ca. 2°Brix,

(2) depectinized by incubating with 100 ppm 'Rohapect D5L' liquid pectic enzyme (Roehm & Haas Co., Philadelphia, PA) at 25-27°C for 5-7 h and a negative alcohol precipitation (1 mL juice plus 5 mL 95% ethanol) used to monitor the completion of depectinization,

(3) pasteurized by high temperature short time (HTST) treatment at 85-90°C for 1 min in a tubular heater with screws (Wingear type, model 200WU; Winsmith Company, Springville, N.Y.), followed by

immediate cooling to 27°C (Maurer, 1973), depectinized with 100 ppm 'Rohapect D5L' and monitored by alcohol precipitation (as in (2)).

After addition of 1% cellulose as press aid all treatments were pressed in a Willmes bag press (60 type, Moffett Co., San Jose, CA) with a final pressure of 4.0 bar. One half of the treatments (2) and (3) was fined with 500 ppm of a 5% bentonite suspension (50 g/L Volclay KWK bentonite, Scott Laboratories, Inc., prepared by slowly adding bentonite to 800 mL of boiling water with stirring), 100 ppm of a 1% gelatin solution (10 g/L gelatin type B, Wineart Co., Portland, OR: gelatin was dissolved in cold distilled water, heated gently to 82°C, cooled to room temperature and brought to volume) and 0.26 mL/L of a 30% 'Clarifying Agent C2' (silica sol) suspension (Roehm & Haas Co., Philadelphia, PA 19105; diluted to 30% with cold distilled water).

After fermentation of the unfined and fined lots (2) and (3) to dryness (0-0.2°Brix) with 1 g/gal champagne yeast (as in (1)) at 25°C in 11.4 L glass jars all the wines were ameliorated to 22°Brix by adding sucrose (dry cane sugar) in three stages (corresponds to 12% v/v alcohol in the finished wines) for further fermentation to dryness (0-0.2°Brix) in 11.4 L glass jars at 25°C. One half of the pulp fermented wine (1) was fined comprehensively as described above after which all the wines were cold stored in 3.8 L glass bottles with screw caps at 2°C for two months. After racking, 25 ppm SO_2 as 1% potassium metabisulfite solution, 180 ppm potassium sorbate and 3% sucrose (dry cane sugar) for sweetness were added.

Samples for analyses were taken after grinding, pressing,

fermentation (before and after amelioration with sugar), filtration and storage. These samples were stored frozen at -12°C in 2 oz. plastic bottles until needed.

CLARIFICATION, BOTTLING AND STORAGE

The ameliorated and stabilized wines were filtered at low pressure using Ertel filter equipment (model E1; Ertel Engineering Company, Kingston, N.Y.) and grade 'SG' filter pads (Scott Laboratories, Inc., San Rafael, CA). The filtered wines were bottled in 750 mL dark green glass bottles and sealed with corks. The bottled wines (three processing trials with replicates) were stored in the dark at 2 and 20°C for 6 months.

COMPOSITIONAL ANALYSES

The following analyses were conducted on all samples at five intermediate processing stages as well as after storage: total acidity (as citric acid with the glass electrode method by Amerine et al. (1980)), pH and °Brix (Abbe refractometer and hydrometer method by Amerine et al. (1980)).

COLOR ANALYSES

From spectral analyses with a Varian DMS 100 UV-visible spectrophotometer interfaced with a Varian DS-15 data station (Varian Instruments Group, Walnut Creek, CA) the following measurements were determined on all samples at five intermediate processing stages and after storage as described by Somers et al. (1977) and Wrolstad (1976):

1. total monomeric anthocyanin pigment (as mg cyanidin-3-glucoside/100 mL

juice, using the extinction coefficient, ϵ = 29600), 2. color density ($A_{420\ nm} + A_{520\ nm}$), 3. % polymeric color (polymeric color/color density X 100) and 4. browning (absorbance at 420 nm after bisulfite bleaching).

Hunter L, a and b color parameters were measured in the transmission mode, spectral component included (arrangement III) using a Hunter CT1100 ColorQUEST C5115 color difference meter (Hunterlab, Hunter Associates Laboratories Inc., Reston, VA) against a white tile. Samples were placed in a 0.5 cm pathlength cell. Percent transmission haze was determined as haze = $Y_{(arrangement\ I)} / Y_{(arrangement\ III)}$ X 100 (arrangement I: light source in the normal, aligned position, specular component excluded).

HPLC ANALYSIS

The individual anthocyanin pigments were separated by HPLC using the procedure of Spanos et al. (1987). The pigments were measured as their percentage of total peak area.

Sample and reagents preparation for HPLC analysis

The juice and wine samples were filtered through 0.45 μm Millipore filters (type HA) and immediately injected onto the HPLC system. Before injection the juice samples were diluted by 1/2 with 0.01% HCl.

For solvent A (15% acetic acid), 150 mL HPLC grade glacial acetic acid were added to 850 mL glass-distilled, deionized water and mixed. The solvents A and B (100% acetonitrile) were filtered through 0.45 μm

Millipore filters, types HA and HV, respectively and degased.

HPLC determination

A Perkin-Elmer 400 high pressure liquid chromatograph (Perkin-Elmer Corp., Analytical Instruments, Norwalk, CT) equipped with a:
1. Varian UV 50 variable wavelength detector (Varian Instrument Group, Walnut Creek, CA) and Perkin-Elmer LCI-100 integrator (until bottling) or
2. Hewlett-Packard 1040A photodiode-array detector and a Hewlett-Packard 9000 computer (Hewlett-Packard Co., Wilsonville, OR) (after bottling), were operated under the following conditions: column, Supelcosil LC-18 (5 um), 250 X 4.6 mm id column (Supelco, Inc., Bellafonte, PA) fitted with ODS-10, 4 cm X 4.6 mm id, Micro-Guard column (Bio-Rad Laboratories); mobile phase--solvent A, 15% acetic acid, solvent B, 100% acetonitrile; flow rate, 1.5 mL/min; elution program, 100% A run isocratically for 5 min followed by a 0-5% linear gradient with B for 10 min and the column equilibrated to the initial conditions for 5 min between injections; detection at 520 nm (visible), 0.1 absorbance unit full scale (AUFS); injection volume, 25 μL.

STATISTICAL ANALYSES

The influences of six processing and two storage treatments (sources) on 21 compositional, HPLC, spectral and Hunter measurements were determined by analysis of variance (ANOVA). These sources were: fermentation of juice and pulp, HTST, enzyme, pressing, fining and storage at 2 and 20^{o}C for six months. The influences of these sources were analyzed at the following sampling stages (with and without fining):

1. ground berries and juice, 2. juice and fermented juice (except pulp treatment), 3. young wine and 4. wine before and after storage.

Significant differences between the source influences on each measurement were determined at the significance levels alpha = 0.05, 0.01 and 0.001, respectively. The individual influences of the components of the sources on each measurement were analyzed by T tests (LSD = least significant difference). In the event of interactions between the sources the LSD between the means of the individual components of the interacting sources were calculated at alpha = 0.05 and 0.01.

In addition, the results of the above compositional, HPLC and color measurements at the above four sampling stages were subjected to multiple correlation analysis. High correlation was assumed for correlation coefficients $|r| \geq 0.85$ (alpha = 0.05).

SENSORY ANALYSIS

The sensory quality (aroma and flavor) of the wines after storage at 2 and 20°C was evaluated by a trained taste panel. These results will be published separately by McDaniel *et al.*, Department of Food Science and Technology, Oregon State University.

RESULTS AND DISCUSSION

JUICE YIELDS AND COMPOSITIONAL MEASUREMENTS

Table 1 lists the sample weights and the yields of free-run juice (FRJ) and press juice for each of the processing procedures. The percent yields of the juices were calculated on a weight basis assuming that one L of juice weighs one kg. While enzyme-treated crushed berries gave higher FRJ yields than pulp fermented crushed berries, the total yields (FRJ and press juice) were essentially the same. Evidently, the gains in percent yield for FRJ with enzyme treatment were compensated for in pressing. High temperature short time pasteurization in combination with enzyme treatment (HTST/enzyme) provided the highest total yield with 8.2% more total yield than the other two types of juice.

Changes of pH, titratable acidity (TA) and percentage weight of soluble solids (^{o}Brix) during processing and storage are listed in table 2 for all three processing procedures. The values reported are overall means for duplicate analyses of two processing trial replicates.

The changes in pH were relatively small for all samples during processing and storage. A similar pattern was evident for all treatments including the fined juices and wines. pH decreased during pressing, increased during fermentation and maturation and decreased again during storage. All of these changes appeared to be more pronounced in the HTST/enzyme-treated juices and wines.

Table 3 lists the significant differences between the source

(treatment) influences on each of 21 compositional and color measurements analyzed by ANOVA at the significance levels alpha = 0.05, 0.01 and 0.001. The decrease in pH after 6 months of storage was significant (alpha=0.001) at both temperatures. The significant influences of the individual components of both the single sources and the interactions on these parameters are listed in table 4. Storage time (2°C, 6 months) had a more significant influence on pH decrease than did increased temperature (20°C).

The changes in TA for all trials showed similar trends during processing and storage (table 2). TA showed a significant increase during pressing which was followed by a decrease during fermentation and maturation and an increase during storage (aging). It shows an opposite pattern of change compared to pH which was confirmed by statistical analysis (table 4). The changes in TA were in accordance with the pH changes.

TA showed a significant decrease (6.6%) (alpha=0.05) (table 3) by the combined effects of enzyme and fermentation as compared to HTST/enzyme-treated juice and fermented juice (table 4). The significant influence of fermentation alone amounted to a decrease of 5.5% in enzyme and HTST/enzyme-treated juices (table 4). Thus it can be concluded that HTST application had a protective effect on TA. This was probably due to the inhibition of enzymic breakdown reactions of organic acids (Maurer, 1973).

In contrast, storage time (2°C) increased TA significantly (alpha=0.01) by ca. 12.2% as compared to unstored wine (table 4). However, the TA

levels of all the unstored and stored wines were still lower than those of the juices (mean of all trials: 1.78g/100mL). TA of the juices is in agreement with the literature (Schobinger, 1986; Spanos, 1987). Unfined HTST/enzyme-treated wine stored at 20°C had the highest TA (1.68g/100mL) and unfined pulp fermented wine stored at 20°C the lowest (1.41g/100mL) of all the wines after storage.

Table 2 also summarizes the °Brix values through fermentation. The crushed fruit showed considerable variation in soluble solids (9.61-11.03g /100mL). As expected fermentation reduced °Brix in all juices (99.5%, alpha=0.001) (tables 3 and 4). Fining resulted in a significant (alpha=0.05) (table 3) decrease (ca. 4.5%) with both enzyme and HTST/enzyme-treated juices. A significant difference (alpha=0.05) (table 3) between the processing procedures in young wine was due to a preserving influence of HTST application (100% more). It is likely that this preserving effect was the result of the inactivation of native degrading enzymes (Maurer, 1973). After fermentation to dryness, HTST/enzyme-treated wine retained the highest amount of soluble solids.

COLOR ANALYSES OF RED RASPBERRY JUICE, YOUNG WINE AND STORED WINE

The color determinations include both spectral (table 5) and Hunter (table 6) measurements. Similar patterns of significant differences (table 3) and interactions (table 4) are shown for both groups. Haze will be discussed in the section on haze and sediments.

SPECTRAL MEASUREMENTS

The changes in total monomeric anthocyanin pigment (as cyanidin-3-glucoside) for all trials showed similar trends during processing and storage (table 5). Anthocyanin content decreased significantly through storage (figure 1). All the fined wines after storage at 2°C and enzyme-treated wine after storage at 20°C had significantly lower contents of anthocyanin than their unfined counterparts.

As pulp fermentation did not decrease anthocyanin content significantly, the highly significant (alpha=0.001) loss (ca. 38.7%) in enzyme and HTST/enzyme-treated juices was due to the combined effects of enzyme treatment and fermentation. This finding was supported further by high positive correlation of °Brix and total anthocyanin in enzyme-treated juice and fermented juice (table 8). In addition, anthocyanin pigment showed a significant (alpha=0.05) decrease through the combined effects of HTST, enzyme and fining in juice (ca. 27.9%) and after storage 20°C (ca. 21.9%). The decrease of ca. 15.1% due to enzyme treatment alone in juice and wine after storage (20°C) was significant (alpha=0.05). HTST application in combination with enzyme decreased anthocyanin more (ca. 16.4%) in juice and stored wine (20°C) than HTST application in combination with fining (ca. 13.7%). (table 4)

Unfined pulp fermented wine after storage at 2°C retained the greatest amount of anthocyanin (32.6mg/100mL) compared to the other wines; it still represents a loss of nearly one half (46.8%) compared to the juices (mean: 61.3mg/100mL). Anthocyanin content of the juices is in agreement with the literature (Spanos, 1987; Torre and Barritt, 1975a, 1977).

Both unfined pulp fermented and HTST/enzyme-treated wines had the lowest anthocyanin contents (21.2mg/100mL) of all the wines after storage at 20°C (figure 1). This loss probably was the result of increased polymerization due to the influence of HTST application (Somers, 1986) and accelerated anthocyanin degradation and polymerization during fermentation and storage at the higher temperature (Flores, 1984, Pilando, 1985). High negative correlations in fermented juices (table 8) further support accelerated anthocyanin polymerization during fermentation.

For all trials the changes in color density ($A_{420nm}+A_{520nm}$) during processing and storage showed similar trends (table 5). Color density decreased until the end of fermentation and increased again during aging (storage) at 2°C. In unfined enzyme-treated wine, color density increased during maturation (figure 2). With the exception of the increases during maturation and aging this pattern of change is very similar to that of anthocyanin. The deviation from this pattern is due to the fact that color density is a function of both anthocyanin concentration and browning. This was confirmed by high positive correlation of color density with both total anthocyanin and browning in juice and fermented juice (table 8). % Polymeric color did not contribute to color density as indicated by high negative correlation of these two parameters in juice and fermented juice (table 8).

Fermentation was a highly significant (alpha=0.001) source of decrease in color density (HTST/enzyme and enzyme-treated juices: ca. 46.5%; all young wines: ca. 34.4%). Equally significant (alpha=0.001) was the increase (ca. 24.6%) due to storage (2°C) which reversed the above

effects of fermentation. In addition, the influence of enzyme treatment decreased (ca. 18.4%) color density significantly (alpha=0.05) in young wine. (table 4)

At the end of storage at either temperature, color density was significantly reduced in all wines compared to the juices (mean: 16.4) (figure 2). Color density of the juices was lower than reported for the variety 'Meeker' by Spanos et al. (1987). Of the wines after storage unfined pulp fermented wine (2°C) had the highest (13.1) and fined enzyme-treated wine (20°C) the lowest (9.6) color density.

Percent polymeric color is a measure of the pigments' resistance to bleaching by bisulfite and reflects the degree of polymerization of the anthocyanins. For all the trials % polymeric color increased during fermentation (most when fermented on pulp), decreased during maturation and then increased again during storage (table 5) (figure 3). In addition, % polymeric color showed a greater increase during storage at higher temperature (20°C).

Fermentation was a highly significant (alpha=0.001) source of % polymeric color increase in pulp fermented (138%) and enzyme-treated juices (with and without HTST) (311%) which is in agreement with reports by Bakker et al. (1986a). This was confirmed by a high negative correlation of °Brix and % polymeric color in fermented juices (table 8). Percent polymeric color also showed a very significant (alpha=0.001) increase with storage; storage time had a greater (ca. 83.1%) influence than temperature (20°C) (ca. 57.2%). Of less significance (alpha=0.05) was the increase in % polymeric color found in the press juice as compared

to the crushed berries (ca. 71.4%) which was probably influenced by fermentation (Bakker, 1986a); fermentation was partially completed in pulp treated juice. This assumption was supported by the fact that during fermentation after pressing, % polymeric color increased by only approximately one half in pulp fermented juices as compared to the two enzyme treatments. (table 4)

Unfined pulp fermented wine after storage at 20°C had the highest % polymeric color (26.5%) of all the wines after storage. Fined pulp fermented wine after storage at 2°C had the lowest % polymeric color (14.6%). In either case low levels of polymers were probably due to their removal (fining) or precipitation after polymerization (Heatherbell, 1984).

The absolute losses of total anthocyanin were small throughout processing and storage. When comparing the contents of monomeric anthocyanin and % polymeric color in press juice (figure 4a) to those for wines after storage at 2 (figure 4b) and 20°C (figure 4c) it is evident that most of the monomeric anthocyanin was polymerized during processing, mainly through fermentation. In press juice, the ratio of % polymeric color to anthocyanin was approximately 1:9 (figure 4a) whereas after storage at 2°C it was about 1:2 (figure 4b) and at 20°C about 1:1 (figure 4c).

The <u>browning index</u> (A_{420nm} after bleaching with bisulfite) showed a low rate of change through processing and storage for all treatments (table 5). The overall amount of browning was high compared to fruit wines of other commodities such as strawberry, particularly after storage

(Flores, 1984; Pilando, 1985). Browning decreased during fermentation and maturation and increased again during storage. In all cases except for unfined HTST/enzyme-treated wine, browning increased more at a storage temperature of 2 than 20°C. This pattern of change is very similar to that of color density which was confirmed by high positive correlation of this parameter and browning during processing (table 8). The values found were in the same order as reported in the literature (Flores, 1984; Pilando, 1985); higher values were probably due to partially bleached oligomeric pigment forms or 'residues' from anthocyanins and polymers (Somers, 1977, 1986).

Since browning only decreased significantly during and after fermentation of enzyme-treated wines, it seems that the combined effects of enzyme and fermentation decreased browning very significantly (alpha=0.001) (ca. 37.8%). This was supported by a high positive correlation of °Brix and browning in enzyme-treated juices and fermented juices (table 8). Browning showed a very significant (alpha=0.001) decrease (ca. 21.6%) by the effect of enzyme treatment alone. In addition, fining reduced browning significantly (alpha=0.05) (ca. 8.1%) in stored wines. (table 4)

Storage at 2°C was the only factor which very significantly (alpha=0.001) increased (ca. 33.1%) browning (table 4). However, even the wines stored at 2°C had lower levels of browning than the juices (mean: 4.6). Unfined pulp fermented wine (2°C) had the highest (4.3) and fined enzyme-treated wine (20°C) the lowest (3.3) degree of browning of the wines after storage.

High positive correlation of total anthocyanin and browning in juice

and fermented juice (table 8) suggests that browning is a function of the concentration of anthocyanins. This explains why pulp fermented wines showed the highest levels of browning. Considerable quantities of anthocyanins and proteins are known to be removed in fined enzyme treated wines (Flores, 1984; Heatherbell, 1984). At such low levels the competition of polymerization with browning reactions (Sims, 1986) may have been the reason for low levels of browning as indicated by high negative correlation of % polymeric color and browning in juice and fermented juice (table 8). In addition, browning did not contribute to increased Hunter b values as indicated by high negative correlation of these parameters in fermented juice (table 8).

HUNTER MEASUREMENTS

The changes in Hunter L (lightness index) and b (yellowness index) values for all trials showed very similar trends during processing and storage (table 6). This was confirmed by high positive correlation of these two parameters (table 8). Hunter L and b reached their highest levels during fermentation after which they decreased until the end of storage but still remained slightly higher than in the juices (mean: L--28.3, b--20.0). This pattern of change was the same in all fined wines on a slightly increased level (figures 5 (L), 7 (b)). In addition, Hunter L and b values increased in unfined enzyme-treated wine during storage at $2^{o}C$ as compared to unstored wine.

Both Hunter L and b values showed highly significant (alpha=0.001) increases by the influences of fining (L: 3.5-6.0%, most in young wine; b: 5.0-5.9%, most in stored wine) and enzyme treatment (L: 3.6-4.8%, during

maturation and storage; b: 3.4-7.9%, most in juice). Also significant were the combined effects of HTST application and fermentation (L: 30.8%, b: 21.1%). (table 4)

HTST application had a very significant (alpha=0.001) stabilizing effect on Hunter L (ca. 6.4% darker) and an inhibitory effect on increased Hunter b (ca. 4.7% less yellow) values as compared to enzyme treatment in juice and fermented juice (table 4). However, these inhibitory effects were subsequently eliminated by fermentation.

Storage at 20°C was the only treatment having a highly significant (alpha=0.001) decreasing (ca. 6.9%) effect on Hunter b values in all wine samples and on Hunter L values in both unfined pulp fermented and HTST/ enzyme-treated wines (ca. 5.7% darker) (table 4). The latter darkening effect did not occur in fined wines after storage at both temperatures. Fined enzyme-treated wines (2°C) had the highest (L--34.4, b--23.3) and unfined HTST/enzyme-treated wines (20°C) the lowest Hunter L and b values (L--28.1, b--19.1) of the wines after storage.

The influences of fining and enzyme treatment changed the composition of the juices such that more total anthocyanin was lost and yellow color was increased in the wines. This was supported by a high negative correlation of both Hunter L and b with total anthocyanin (table 8) which implies that increased Hunter L and b values were due to anthocyanin loss (Pilando, 1985). It seems that HTST application inhibited such effects in preventing anthocyanin degradation (Maurer, 1973). Hunter L and b values were increased during fermentation and decreased during storage (particularly at 20°C) by the presence of % polymeric color as indicated by high positive and negative correlations,

respectively (table 8). Decreased Hunter L and b values during storage may have been due to the greater lengths of the polymers.

The changes of the Hunter a (redness index) values followed the same trends for all treatments during processing and storage (table 6). Hunter a peaked during fermentation after which it decreased until the end of storage with only a slight temporary increase during maturation. All the fined wines followed the same pattern of change on an increased level (figure 6). This pattern of change is very similar to that of Hunter L and b which was confirmed by high positive correlations in the wines after storage (table 8). Bakker et al. (1986a) also found high correlation of Hunter L and a in port wine.

Hunter a increased very significantly (alpha=0.001) by the effects of enzyme treatment (ca. 2.9%) and fining (ca. 2.5%) in stored wines (table 4). The less significant (alpha=0.01) influence of pressing (ca. 9.3%) was probably due to a concentrating effect (table 4). High correlations (table 8) during processing and storage indicate that increases in Hunter a were associated with the removal of anthocyanins and polymers. However, changes in Hunter a could also have been caused by light scattering.

Hunter a was very significantly (alpha=0.001) decreased by storage temperature (20°C) (ca. 5.0%) and also by storage time (ca. 1.6%). Overall, the gain in Hunter a values during processing was subsequently eliminated by the end of storage. Unfined pulp fermented wine (20°C) reached the lowest value (54.7) of all the wines after storage which was even lower than in the juices (mean: 60.6). Fined enzyme-treated wine

after storage at 2°C had the highest Hunter a value (61.3) which was only slightly higher than in the juice.

Table 6 summarizes hue ($\tan^{-1}$ X Hunter a/Hunter b) for all treatments which showed similar trends of change during processing and storage. Overall, the changes in hue were small. Hue decreased most during fermentation after which it increased again during maturation and storage, reaching about the same or slightly lower levels than in the juices. The hue of all fined wines changed in the same way on a lower level. This pattern of change is exactly the opposite of that of Hunter L and b which was confirmed by high negative correlation of hue with these parameters during processing and storage (table 8). Bakker et al. (1986a) report of a linear but negative variation of Hunter L with hue in port wine and model anthocyanin solutions.

Hue showed a highly significant (alpha=0.001) decrease by the effect of fining (ca. 0.7-0.9%) during maturation and storage. Also significant (alpha=0.05) were the combined effects of enzyme treatment and fermentation (ca. 0.6-0.8% decreases). HTST application was the only significant (alpha=0.01) increasing effect (ca. 1.5%) on hue in juice, an effect which was eliminated during fermentation. (table 4)

Decreased hue (lighter, more yellow) was highly influenced by losses of anthocyanins and increased polymerization as indicated by high positive and negative correlations, respectively, in juice and fermented juice (table 8). Fined enzyme-treated wines had the lowest hue (69.3) of the wines after storage. The hue of unfined HTST/enzyme-treated wine stored at 20°C was very similar (70.9) to that of the juices (mean: 71.8).

Therefore, the latter type of wine had the most desirable appearance after storage.

The changes in saturation index ((Hunter a^2 + Hunter b^2)$^{1/2}$) for all trials showed similar trends during processing and storage (table 6). Saturation peaked during fermentation after which it decreased until the end of storage. This pattern is very similar to that of Hunter L and b in unfined wines which was confirmed by high positive correlation of saturation with these parameters (table 8).

All fined wines followed the same pattern of change on a higher level of saturation except for a slight temporary increase during maturation. This pattern of change is the same as that of Hunter a which was confirmed by high positive correlations (table 8). Bakker et al. (1986a) found the same correlation in port wine.

Both pressing of the juices, probably due to a concentrating effect (ca. 7.7%) and fining in stored wines (ca. 2.5%) were highly significant (alpha=0.001) increasing influences on saturation. Less significant (alpha=0.05) was the effect of enzyme treatment in juice and fermented juice (ca. 3.8% increase). Saturation was very significantly (alpha=0.001) decreased by increased storage temperature (20°C) (ca. 4.7%) and also by storage time (2°C) (ca. 2.0%). (table 4)

High negative correlations in the wines after storage suggest that increased Hunter a and saturation values were due to higher levels of anthocyanins in relation to polymers (table 8). The latter was shown to be caused by fining which prevents excessive polymerization. Therefore, lack of fining resulted in more polymerization and precipitation of

polymers which was highly correlated with increased Hunter L and b values (table 8). At the end of storage all the wines except HTST/enzyme-treated wines (2°C) were less saturated than the juices (mean: 63.6); fined enzyme-treated wine (2°C) had the highest (65.6) and unfined pulp fermented wine (20°C) the lowest (58.1) level of saturation.

HAZE AND SEDIMENT FORMATION IN RASPBERRY WINE STORED AT 2 AND 20°C

HAZE

Table 6 lists the changes in % haze during processing and storage for all trials. Haze increased during maturation. However, there was no consistent trend of behavior for all treatments during storage.

Fermentation on pulp was by far the greatest increasing factor on haze in newly fermented juice (ca. 166%) as well as the wines after storage (ca. 51.6%) (alpha=0.05). This was confirmed by high positive correlation of haze and % polymeric color and high negative correlation of haze and °Brix in pulp fermented juice (table 8). The preventive effect of enzyme treatment on polymerization and thus haze formation was shown by high negative correlation of haze and % polymeric color in enzyme-treated juices and fermented juices (table 8). In addition, haze showed significant (alpha=0.05) increases by the influences of storage temperature (20°C) (ca. 60%) and time (2°C) (ca. 20%) in unfined wines. (table 4)

Haze showed the greatest decrease (ca. 58.3%) by the combined effects of storage time, storage temperature and fining (alpha=0.05). This reduction was probably the result of the combined effects of protein removal (fining) and accelerated polymerization leading to precipitation

(Heatherbell, 1984). Precipitation probably occured during maturation as very little sediment was found in fined stored wines. Fining combined with storage time reduced haze more (ca. 37.5%) than fining combined with storage temperature (ca. 20.8%). This suggests that fining either removed haze causing compounds or resulted in more rapid degradation and precipitation. (table 4)

Unfined pulp fermented juices and wines had the highest haze during processing and storage. After storage at 20°C all the unfined wines had significantly more haze than the juices (mean: 4.3% including pulp fermented juice). Unfined pulp fermented wine (20°C) had the highest content (9.8%) of haze of the wines after storage.
All the fined wines except enzyme-treated wine (2°C) had significantly less haze after storage than the juices. Fined HTST/enzyme-treated wine had the least haze (at 20°C: 1.7%) of all the unfined and fined wines before and after storage at both temperatures.

SEDIMENT

The development of sediments as observed visually during six months of storage at 20°C in the dark can be followed in the figure 8. After 19 weeks of observation the level of sediment formation remained constant in all samples.

Fined HTST/enzyme and enzyme-treated wines were the most stable in producing no or very slight sediments after 15 weeks and 12 weeks, respectively, which remained at this low level until after six months of storage. Unfined HTST/enzyme-treated wine behaved in a similar manner except that very slight sediments already occured after 4 weeks in one of

the replicates. Fined pulp fermented wine produced very slight sediments after 4 to 8 and slight sediments after 6 to 12 weeks. By far the greatest amount of sediments was produced by unfined pulp fermented wines with very slight sediments within the first week, slight sediments after 3, medium amounts of sediments after 9 to 13 and strong sediments after 16 to 17 weeks of storage.

In comparing the developments of haze and sediments it can be concluded that wines which produced the least haze also produced the least sediment. This implies that stabilization due to polymer precipitation occured prior to storage, most likely during maturation.

ANTHOCYANIN COMPOSITION OF RASPBERRY JUICE, YOUNG AND STORED WINE

The composition (HPLC area percentages) of anthocyanins found in raspberry juices and wines produced by the different procedures at several stages of processing and storage are listed in table 7. In the crushed raspberries used for all three processing procedures 6 distinctive peaks could be identified by reversed-phase high performance liquid chromatography (HPLC) (figure 9a). As determined by Spanos *et al.* (1987) in the same raspberry variety by the same separation method these peaks represent the following anthocyanins in their order of elution: cyanidin-3-sophoroside (Cy-3-Sop; 70.3%), cyanidin-3-glucosylrutinoside (Cy-3-GlRu; 14.2%), cyanidin-3- glucoside (Cy-3-Gl; 8.60%), pelargonidin-3-sophoroside (Pel-3-Sop; 4.31%), cyanidin-3-rutinoside (Cy-3-Ru; 2.0%) and pelargonidin-3- glucosylrutinoside (Pel-3-GlRu; 0.62%). Except for Cy-3-GlRu which was present at ca. 4 X the quantity these percentages are in agreement with the results by Spanos *et al.* (1987).

Two additional minor peaks appeared on the chromatograms of the crushed berries used for fermentation on pulp (figure 9a). One of the minor peaks (peak 1A) eluted shortly before the major peak (Cy-3-Sop) with an area of 0.08%. This peak particularly increased during fermentation on pulp which was also where the greatest amounts of anthocyanin polymerized. Because of their greater polarities and molecular weights, polymers could be expected to have shorter retention times than the monomeric anthocyanins. Therefore, peak 1A is assumed to represent polymers.

The other minor peak (peak 7) eluted after the sixth peak (Pel-3-GlRu) with an area of 0.36%. This peak was detected mainly at the end of fermentation and during maturation and decreased again largely during storage of all types of wine with fluctuating percentages. Due to its late retention time combined with its spectral maximum at 503nm this peak is believed to represent a pelargonidin derivative, possibly pelargonidin-3-glucoside (Pel-3-Gl) or an acylated pelargonin derivative of one of the six major peaks.

An eighth peak appeared at the end of fermentation which increased during maturation and decreased particularly during storage at $2^{o}C$ for six months but was more stable than peak 7. Because of its late retention time in combination with a spectral maximum at 501nm peak 8 is believed to represent another pelargonidin derivative, possibly pelargonidin-3-rutinoside (Pel-3-Ru) or an acylated pelargonin derivative of one of the six major peaks.

The most striking change which occured during processing was the

complete disappearance of Cy-3-Gl (peak 3) during fermentation (figures 9a&b). Its loss was positively correlated with anthocyanin loss and browning and negatively correlated with % polymeric color in juice and fermented juice (table 8). This implies that it was most actively involved in polymerization and thus the most unstable anthocyanin in raspberry. Cy-3-Gl is the only monoglycoside present in red raspberry which is undoubtedly related to its instability.

Fermentation was a highly significant (alpha=0.001) and the greatest source of reduction for Cy-3-Gl (100%). The degradation of Cy-3-Gl was accelerated by the combined effects of fining and enzyme treatment of juice (enzyme: ca. 44.7%, HTST/enzyme: ca. 18.1%). The latter indicates that HTST application had a stabilizing effect on Cy-3-Gl in juice which subsequently disappeared during fermentation.

For all processing trials Cy-3-Sop (% peak 1) appeared to be the most stable anthocyanin until the end of storage. It showed an apparent increase because of the proportionally greater loss of other anthocyanins during fermentation and storage. Partial hydrolysis of Cy-3-GlRu will form Cy-3-Sop with the loss of rhamnose. This assumption is based on the high negative correlation of Cy-3-Sop with Cy-3-GlRu (peak 2), a correlation which was only found to be high in stored wines but increased during processing.

Fermentation was a very significant (alpha=0.001) source of increase (ca. 5.3-5.5%, including fermentation on pulp) for % peak area. Also significant (alpha=0.01) was storage time ($2^{o}C$) (ca. 2.0% increase). (table 4)

Cy-3-GlRu (% peak 2) was the second most stable anthocyanin which showed slightly bigger differences between the processing procedures and unfined and fined wines than Cy-3-Sop.

Cy-3-GlRu increased (ca. 13.5%) significantly (alpha=0.05) in pulp fermented as compared to HTST/enzyme-treated juice. This indicates that HTST application had a preserving effect on other anthocyanins. However, at the end of storage the increasing influences of storage temperature (20°C) (ca. 10.9%) and enzyme treatment in stored wine (ca. 6.6%) were of more significance (alpha=0.001) than pulp fermentation. Therefore, both types of enzyme-treated wines stored at 20°C had proportionally greater amounts of Cy-3-GlRu compared to the juices and also overall. The latter greatest increase is believed to be relative to the greatest absolute losses of other anthocyanins, particularly Cy-3-Ru. (table 4)

At the end of storage of all wines Pel-3-Sop (% peak 4) was decreased compared to the juices. HTST/enzyme-treated fined wines had the lowest area percentages after storage.

These very significantly (alpha=0.001) decreased levels in HTST compared to enzyme-treated (ca. 5.7%) and pulp fermented (ca. 10.2%) wines after storage were probably relative to the greater losses of other anthocyanins in the wines without HTST stabilization. Pel-3-Sop decreased (ca. 20%) significantly (alpha=0.01) during fermentation in combination with enzyme treatment. (table 4)

Cy-3-Ru (% peak 5) increased during maturation and decreased again

significantly during storage but still remained higher than in the crushed berries as well as the juices. This implies a relative greater stability of Cy-3-Ru compared to other anthocyanins.

Storage time (2°C) was the only significant (alpha=0.05) decreasing (19.7%) effect on Cy-3-Ru. HTST application had a significant (alpha= 0.05) preserving effect in juice and wine with wines after storage at 20°C having ca. 17.4% more Cy-3-Ru than at 2°C. This confirms the above mentioned relative greater stability which was most pronounced in HTST/enzyme-treated wine after storage at 20°C. (table 4)

Pel-3-GlRu (% peak 6) increased proportionally during maturation and storage of all wines which indicates a relative greater stability of this anthocyanin compared to others. After storage at 20°C unfined enzyme treated wine had the highest level of this anthocyanin of all the wines.

Storage time (ca. 12.8%) and temperature (ca. 11.4%) increased Pel-3-GlRu very significantly (alpha=0.001). Its increase after storage (ca. 14.5%) by the combined effects of enzyme treatment and fermentation was of less significance (alpha=0.05). (table 4)

Peak 7 (Pel-3-Gl?) only showed a significant (alpha=0.05) decrease (72.4%) by the influence of storage time. However, comparing the chromatograms it seems that increased storage temperature enhanced the effect of storage time in eliminating this compound almost completely. Such susceptibility to the influence of storage complies with the assumption that peak 7 is a pelargonidin derivative.

Peak 8 (pelargonidin derivative?) decreased (ca. 67.9%) significantly (alpha=0.01) by the influence of storage time (2°C). Since there was no significant difference between the area % of wines before and after storage at 20°C, it can be concluded that increased temperature had no influence on this compound. This explains the greater stability of peak 8 during storage compared to peak 7.

MULTIPLE CORRELATION STUDIES

Table 8 includes the correlations for juice and wine during processing and after storage. While most of the correlations were found during fermentation, these are of less interest than the correlations found in the finished wines (after storage). High correlation was assumed for multiple correlation coefficients $|r| \geq 0.85$ (alpha=0.05).

High positive correlation at three stages of processing was found between 1. Hunter L and b and 2. color density and browning and at two stages between 3. Hunter L and the saturation index and 4. Hunter a and the saturation index. High negative correlation at three stages of processing was found between 5. Hunter L and hue and at two stages between 6. °Brix and % polymeric color and 7. Hunter b and hue.

In wines after storage high positive correlations of the Hunter parameters L, a, b and saturation index show that saturation was the combined effect of Hunter L, a and b and thus increased with them. In addition, this indicates that Hunter a increased with Hunter L and b, an effect which was not significant until storage and possibly due to aging reactions.

As stated earlier these aging reactions involve polymers. In wines after storage increases in % polymeric color were negatively correlated with decreased Hunter L (coefficient: -0.79), a and b (coefficient: -0.82) values. This indicates that during storage (aging) polymers darkened the wine (decreased Hunter L and b) which was exactly the opposite of the effect found during fermentation where polymers increased Hunter L and b. The latter was shown by positive correlation of % polymeric color with Hunter L and b (coefficients: L--0.73, b--0.72), respectively in juice and fermented juice. This implies that during storage polymers thrust redness and yellowness into the background which then reemerged after the precipitation of the polymers. At all stages of processing and storage, polymers never contributed to redness (Hunter a).

CONCLUSIONS

1. Cy-3-Gl was the most reactive of the anthocyanins in red raspberry juice and completely disappeared during fermentation of all treatments. This confirms that monoglycosides (Cy-3-Gl is the only monoglycoside in raspberries) are more susceptible to hydrolysis than di- and triglycosides.

2. Fining was essential to avoid excessive haze formation in wines during storage. Combined with fining, storage time had a greater reducing effect on haze than temperature. After six months of storage HTST application combined with enzyme treatment was necessary to suppress haze. Wines which were least hazy also produced the least sediments. Fined HTST/enzyme-treated wine after storage at 20°C for six months had the least haze (1.7%) and did not show any sedimentation.

3. Fined HTST/enzyme-treated wine after storage at 2°C had the best color stability, color appearance and sensory qualities after six months storage. This implies that inactivation of native enzymes during HTST treatment had a beneficial effect on quality.
   a) color stability: fining and HTST/enzyme-treatment reduced haze and sediments. Storage at 2 is preferable to 20°C for the preservation of anthocyanins. Storage time also reduced haze.
   b) color appearance: fining minimized haze and browning and increased Hunter a and saturation. Storage time increased browning

and decreased haze; therefore, medium storage periods are recommended. HTST/enzyme treatment minimized haze, increases in Hunter L and b values and changes in hue. Storage temperature decreased Hunter L.

c) sensory quality: fining and storage time minimized % polymeric color and thus bitterness and astringency which was supported by preliminary taste tests. Enzyme treatment reduced TA compared to the berries.

It is assumed that the average consumer rather accepts decreased color and increased lightness and acidity (which could be reduced by further amelioration with sugar or dilution with water) than haziness in a wine.

4. The most significant influences on anthocyanin loss in decreasing order were: a) fermentation combined with enzyme treatment, b) HTST/enzyme treatment combined with fining and c) storage temperature ($20^{o}C$). The anthocyanin content in the wines after storage ranged from 21.2 mg/100 mL (unfined HTST/enzyme and pulp treatments, $20^{o}C$) to 32.6 mg/100 mL (unfined pulp treatment, $2^{o}C$); the latter still representing a loss of nearly one half compared to the juices. Most of the anthocyanin pigment (particularly Cy-3-Gl) was polymerized, mainly during fermentation as indicated by high correlations and when comparing the total contents of anthocyanin and % polymeric color in juices and wines after storage.

5. The high negative correlation of the proportions of Cy-3-Sop and Cy-3-GlRu in stored wines implies that Cy-3-Sop was formed from partial hydrolysis of Cy-3-GlRu during storage. This implies that the diglucoside formation is particularly stable in cyanidins.

6. Red raspberry wines have a lower tendency for haze and sediment formation and greater anthocyanin pigment stability when these results are compared to those for strawberry and blackberry wines in our laboratory. This is attributed to the content of diglucosides compared to these other commodities.

Table 1-Raspberry weights, juice volumes and yields of the three treatments

TREATMENT		BERRY WEIGHT (kg)	FRJ (l)	YIELD FRJ (%)	PJ (l)	YIELD PJ (%)	FRJ+PJ (l)	TOTAL YIELD (%)
PULP FERMENTION	replicate 1	37.9	7.6	20.1	19.7	52.0	27.3	72.0
	replicate 2	37.7	7.6	20.2	21.9	58.1	30.0	79.6
	means	37.8	7.6	20.2	20.8	55.1	28.7	75.8
ENZYME	replicate 1	38.4	20.8	54.2	6.8	17.7	27.6	71.9
	replicate 2	37.3	17.8	47.7	11.7	31.4	29.5	79.1
	means	37.9	19.3	51.0	9.3	24.6	28.6	75.5
HTST/ ENZYME	replicate 1	32.8	16.4	50.0	10.1	30.8	26.5	80.8
	replicate 2	36.9	17.6	47.7	12.6	34.1	30.3	82.1
	means	34.9	17.0	48.9	11.4	32.5	28.4	81.5

FRJ free run juice (before pressing); PJ press juice.

Table 2-Compositional analyses of raspberry juice and wine at several stages of processing and storage[a]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	COMPOSITION							
PULP CONTROL	pH	3.41	3.29	3.29[b]	3.30	3.34	3.27	3.31
	TA[d]	1.63	1.69	1.68[b]	1.66	1.46	1.60	1.41
	°BRIX[e]	10.28	1.95	- .50[b]	.00			
PULP FINED	pH			3.29[b]	3.32	3.32	3.26	3.32
	TA			1.68[b]	1.62	1.37	1.53	1.53
	°BRIX			- .50[b]	.00			
ENZYME CONTROL	pH	3.40	3.29	3.31	3.30	3.31	3.25	3.27
	TA	1.73	1.76	1.72	1.66	1.47	1.61	1.61
	°BRIX	11.03	10.49	- .30	.00			
ENZYME FINED	pH		3.36	3.32	3.31	3.32	3.25	3.30
	TA		1.70	1.65	1.63	1.34	1.55	1.51
	°BRIX		10.02	- .30	.00			
HTST/ENZYME CONTROL	pH	3.41	3.26	3.33	3.31	3.34	3.24	3.27
	TA	1.83	1.89	1.80	1.77	1.48	1.66	1.68
	°BRIX	9.61	10.41	.20	.15			
HTST/ENZYME FINED	pH		3.22	3.32	3.30	3.34	3.25	3.28
	TA		1.84	1.74	1.73	1.37	1.63	1.63
	°BRIX		10.01	- .30	.05			

[a]All values reported are means of duplicate analyses and replicates of processing trials; [b]Separate lots, no fining; [c]Crushed berries; [d]Titratable acidity (g citric/100 mL), [e]Weight % soluble solids (measured with Abbe refractometer and hydrometer), methods by Amerine et al. (1980). PRESS JUICE including pulp fermented juice, 50 ppm SO_2 added; FERMENTION after amelioration with sugar during fermentation; YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2 C, dark) for 2 months, 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark.

Table 3a-Raspberry, F values

	SOURCE	DF	pH	T.A.	°BRIX	ACN	C.D.	%P.C.	DI	HUNTER L	HUNTER a	HUNTER b	HUE	SI	%HAZE(TM)
	PROCESS(P)	2	0.28	4.07	592.76 ***	9.67 **	1.58	16.39 ***	1.21	6.97 *	2.87	9.81 **	10.24 **	3.07	27.04 ***
Juice	JUICE (J)	2	2.91	0.29	155.49 ***	21.33 ***	0.15	4.26 *	0.44	9.85 **	15.82 **	15.71 **	2.96	23.78 ***	7.90 *
	P X J	4	1.46	0.09	148.23 ***	4.01 *	0.13	1.87	0.22	2.82	0.08	3.57	2.28	2.58	6.03 *
	MSE(ERROR)	9	0.05	0.01	0.10	11.35	2.66	2.71	0.14	0.95	3.35	0.49	0.19	1.77	0.93
	PROCESS(P)	1	3.99	9.45 *	0.08	0.09	1.52	0.39	3.92	16.39 **	7.08 *	13.01 **	6.69 *	8.81 *	0.14
Juice/	FERMENT(F)	1	1.18	6.54 *	10724 ***	138.69 ***	145.59***	70.86 ***	143.12 ***	198.60 ***	0.61	125.81 ***	200.30 ***	2.23	0.56
ferm.	FINEO (f)	1	0.16	1.43	6.08 *	2.49	0.00	1.87	0.19	13.02 **	1.29	6.68 *	3.13	0.06	1.15
juice,	P X F	1	3.55	0.18	0.54	0.04	0.03	2.49	0.60	13.54 **	6.54 *	9.14 *	5.35 *	1.34	0.02
enz./	P X f	1	2.37	0.00	0.01	0.96	0.48	0.25	0.19	0.87	0.34	0.22	0.67	0.12	0.73
HTST-	F X f	1	0.26	0.05	3.83	0.05	1.43	2.01	1.25	1.57	0.04	1.38	0.17	1.19	0.08
enz.	P X F X f	1	0.94	0.01	0.19	2.94	0.11	0.11	0.07	1.41	0.01	0.56	0.07	0.00	1.73
	MSE	8	0.00	0.01	0.04	16.70	1.65	7.55	0.08	0.60	1.02	0.34	0.14	2.22	0.67
	PROCESS(P)	2	0.04	1.52	8.00 *	0.47	6.51*	2.05	5.19 *	71.75 ***	0.53	83.08 ***	3.77 *	1.21	0.30
Young	FINEO (f)	1	0.05	0.50	2.00	0.66	0.00	2.41	0.00	431.53 ***	4.47	361.02 ***	7.86 *	0.17	1.46
wine	P X f	2	0.21	0.00	2.00	0.13	2.20	0.42	2.08	0.52	0.34	0.72	0.35	0.97	1.20
	MSE	6	0.00	0.01	0.00	23.31	0.90	10.03	0.07	0.03	0.35	0.01	0.09	1.58	1.03
	PROCESS(P)	2	0.82	2.39		0.33	0.00	0.05	0.17	11.27 ***	8.79 **	14.05 ***	7.49 **	11.87 ***	4.15 *
	FINEO (f)	1	0.06	3.07		1.28	1.43	2.27	4.59 *	41.44 ***	19.07 ***	47.80 ***	33.38 ***	16.23 ***	14.82 **
Stored	STOREO (S)	2	10.29 ***	10.25 **		16.75 ***	11.39***	27.47 ***	18.45 ***	16.04 ***	52.93 ***	32.61 ***	3.36	43.48 ***	2.53
wine,	P X f	2	0.18	0.49		0.59	0.23	1.06	0.20	0.10	1.00	0.03	1.05	0.15	2.05
six	P X S	4	0.37	0.71		0.38	0.85	0.17	1.38	2.43	1.02	2.81	2.27	1.76	0.35
months	f X S	2	0.18	0.71		2.25	0.40	0.57	0.30	0.69	1.07	0.93	0.76	1.71	5.07 *
	P X f X S	4	0.05	0.40		0.74	0.26	0.26	0.45	1.81	0.75	0.97	2.11	0.85	0.81
	MSE	18	0.00	0.01		16.36	2.41	12.16	0.17	0.85	1.00	0.29	0.10	1.36	2.66

* significant difference between the sources at alpha=0.05
** significant difference between the sources at alpha=0.01
*** significant difference between the sources at alpha=0.001

Table 3b-Raspberry, F values (continued)

	SOURCE	DF	%PEAK 1	%PEAK 2	%PEAK 3	%PEAK 4	%PEAK 5	%PEAK 6	%PEAK 7	%PEAK 8
	PROCESS(P)	2	0.02	4.72 *	50.67***	0.68	2.14	2.41		
Juice	JUICE (J)	2	8.10 **	0.46	109.46***	1.66	0.74	1.14		
	P X J	4	1.42	1.18	9.56**	0.48	0.51	1.15		
	MSE(ERROR)	9	3.82	0.65	0.20	0.18	0.21	0.09		
	PROCESS(P)	1	0.07	1.22	31.02***	0.33	0.13	1.66		
Juice/	FERMENT(F)	1	14.59 ***	3.36	1317***	12.62 **	1.26	2.15		
ferm.	FINED (f)	1	0.43	0.00	42.28***	0.00	0.69	0.04		
juice,	P X F	1	0.85	0.14	31.02***	0.00	0.03	0.01		
enz./	P X f	1	1.26	1.22	5.47*	1.01	1.63	0.23		
HTST-	F X f	1	2.26	0.84	42.28***	0.95	0.12	1.14		
enz.	P X F X f	1	1.66	0.56	5.47*	0.12	0.02	0.22		
	MSE	8	4.57	1.56	0.12	0.20	0.18	0.50		
	PROCESS(P)	2	0.97	0.16	99999***	1.23	0.83	0.35		
Young	FINED (f)	1	1.67	1.79	99999***	1.49	0.48	0.08		
wine	P X f	2	0.03	0.27	99999***	0.53	0.69	0.43		
	MSE	6	1.80	1.38	0.00	0.06	0.13	0.68		
	PROCESS(P)	2	1.32	6.71 **	1.00	24.80 ***	1.06	4.19 *	1.52	2.580
	FINED (f)	1	0.50	0.00	1.00	0.13	4.35	2.05	2.35	0.310
Stored	STORED (S)	2	9.66 **	24.85 ***	1.00	2.12	11.95 ***	10.69 ***	28.49 ***	6.370 **
wine,	P X f	2	0.05	0.37	1.00	0.95	0.58	0.41	1.95	1.080
six	P X S	4	0.25	0.81	1.00	0.38	3.31 *	2.39	2.52	0.620
months	f X S	2	0.70	0.17	1.00	0.20	0.02	0.39	0.35	0.520
	P X f X S	4	0.21	0.18	1.00	0.26	0.12	1.02	3.64 *	0.300
	MSE	18	1.11	0.51	0.13	0.02	0.04	0.02	0.07	0.302

* significant difference between the sources at alpha=0.05
** significant difference between the sources at alpha=0.01
*** significant difference between the sources at alpha=0.001

Table 4a-Raspberry, significant influences over time[a]

PARAMETER / SOURCE	°BRIX	pH	TA	ACN	C.D.	%PC	BI	H L	H a	H b	HUE	SI	%HAZE
PULP (JUICE)	(↓)[g]					↑[e]		(↑)		↑			↑
PULP (JUI/WI[b])													
PULP (WINE)													
PULP (STORAGE)													↑
HEAT (JUICE)	-[d]			↓*E*f				-		-	↑		
HEAT (JUI/WI[b])			-						↓				
HEAT (WINE)	-												
HEAT (STORAGE)													
ENZ. (JUICE)				↓*F						↑			
ENZ. (JUI/WI[b])			↓*F				(↓*F)				↓*F	↑	
ENZ. (WINE)					↓		↓	↑		↑	↓*F		
ENZ. (STORAGE)								↑	↑	↑	↓*F		
PRESS (JUICE)								↑	↑	(↑)		↑	
FINE (JUICE)	↓			↓*E*H									
FINE (JUI/WI[b])	↓(1)							↑		↑			
FINE (WINE)								↑		↑	↓		
FINE (STORAGE)							↓	↑	↑	↑	↓	↑	↓*T*t
FERM (JUICE)	↓					↑(2)		(↑)		↑(2)			↑(2)
FERM (JUI/WI[b])	↓		↓*E	↓*E	↓	↑	↓*E	↑(3)	↑(3)	↑	↓*E		
FERM (WINE)					↓		↓*E						
2°C (STORAGE)		↓	↑		↑	↑	↑		↓			↓	↑ ↓*t*f
20°C (STORAGE)		↓	↑	↓		↑		↓	↓	↓		↓	↑ ↓*t*f

[a]At alpha ≥ 0.05; [b]Except pulp treatment; [c]Stabilizing effect on other peaks; [d]Preservation; [e]Increase; [g]Decrease; °Brix soluble solids; TA titratable acidity; H L,a,b Hunter L, a and b; SI saturation index; ACN total anthocyanin; C.D. color density; %P.C. % polymeric color; BI browning index; %P1-P8 HPLC area % of peaks 1-8 (Cy-3-Sop, Cy-3-GlRu, Cy-3-Gl, Pel-3-Sop, Cy-3-Ru, Pel-3-GlRu, Pel-3-Gl?, Pel-3-Ru?); (1) juice; (2), (3) pulp, HTST/enzyme treatment; T, t storage temperature, time; f fining; E enzyme; H HTST; F fermentation; (r), (a) relative, absolute change of area % compared to other peaks.

Table 4b-Raspberry, significant influences over time[a] (continued)

PARAMETER / SOURCE	%P1	%P2	%P3	%P4	%P5	%P6	%P7	%P8
PULP (JUICE)		↑(r)	↓(a)					
PULP (JUI/WI[b])								
PULP (WINE)								
PULP (STORAGE)								
HEAT (JUICE)		-[c]			-			
HEAT (JUI/WI[b])			-					
HEAT (WINE)								
HEAT (STORAGE)				↓*E(r)				
ENZ. (JUICE)			↓*f(a)					
ENZ. (JUI/WI[b])				↓*F				
ENZ. (WINE)								
ENZ. (STORAGE)		↑(r)		↓*H(r)		↑*F(r)		
PRESS (JUICE)	(↑(r))							
FINE (JUICE)			↓*E(a)					
FINE (JUI/WI[b])			↓*E(a)					
FINE (WINE)								
FINE (STORAGE)								
FERM (JUICE)	(↑)(r)	↑(2)(r)	↓(2)(a)					
FERM (JUI/WI[b])	↑(r)		↓(a)	↓*E(a)				
FERM (WINE)								
2°C (STORAGE)	↑(r)				↓(a?)	↑(r)	↓(a)	↓(a)
20°C (STORAGE)		↑(r)			-*H*E	↑(r)		-

See previous page for legend.

Table 5-Spectral analyses of raspberry juice and wine at several stages of processing and storage[a]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	COMPOSITION							
PULP CONTROL	ACN[f]	62.9	58.1	41.9[b]	41.1	37.5	32.6	21.4
	C.D.[f]	15.5	15.4	13.5[b]	11.8	9.5	13.1	10.1
	% P.C.[f]	4.5	9.9	11.0[b]	12.8	14.0	16.4	26.5
	BI	4.7	4.4	4.1[b]	3.6	3.1	4.3	3.7
PULP FINED	ACN			42.7[b]	40.1	26.7	30.2	25.5
	C.D.			13.2[b]	10.1	7.8	12.7	10.9
	% P.C.			11.1[b]	11.9	10.8	14.6	20.7
	BI			4.3[b]	3.2	2.4	4.0	3.8
ENZYME CONTROL	ACN	72.0	61.1	35.9	39.7	35.6	30.8	26.2
	C.D.	16.3	16.3	12.3	8.1	11.1	12.4	10.2
	% P.C.	3.2	3.2	11.4	19.3	11.5	15.5	22.5
	BI	4.7	4.4	3.8	2.7	3.5	4.0	3.6
ENZYME FINED	ACN		62.9	34.8	35.4	32.6	28.9	25.2
	C.D.		16.2	11.3	9.1	9.1	11.7	9.6
	% P.C.		3.0	11.1	14.3	13.3	14.8	22.8
	BI		4.4	3.5	2.9	2.9	3.6	3.3
HTST/ENZYME CONTROL	ACN	79.9	66.8	43.3	39.2	33.8	30.2	21.1
	C.D.	16.8	17.9	12.5	9.0	9.2	12.0	11.3
	% P.C.	2.4	4.2	11.6	15.1	12.0	18.2	24.0
	BI	4.8	4.9	3.8	2.9	3.0	3.9	4.1
HTST/ENZYME FINED	ACN		57.6	40.0	37.9	32.4	30.4	23.7
	C.D.		16.4	12.0	9.6	9.1	12.0	10.5
	% P.C.		4.5	11.9	12.4	13.0	14.9	20.1
	BI		4.7	3.7	3.1	2.9	3.8	3.7

[a]All values are reported as means of duplicate analyses and replicates of processing trials; [b]Separate lots, no fining; [c]Crushed berries; [f]Methods by Wrolstad (1974): ACN total monomeric anthocyanin (mg/100 ml), C.D. color density ($A_{420nm} + A_{520nm}$) % P.C. % polymeric color = P.C./C.D. BI browning index (A_{420nm}); PRESS JUICE including pulp fermented juice, 50 ppm SO_2 added; FERMENTION after amelioration with sugar during fermentation; YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2°C, dark) for 2 months, 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark.

Table 6-Hunter analyses of raspberry juice and wine at several stages of processing and storage[a]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	COMPOSITION							
PULP CONTROL	HUNTER L	26.2	29.3	32.8[b]	31.4	31.1	30.3	29.0
	HUNTER a	56.0	61.5	62.1[b]	60.0	59.8	58.0	54.7
	HUNTER b	18.1	21.4	22.6[b]	21.5	21.4	20.7	19.5
	HUE	72.1	70.8	70.1[b]	70.3	70.4	70.4	70.4
	SI	58.9	65.2	64.6[b]	63.8	63.5	61.6	58.1
	%HAZE	3.3	8.5	4.9[b]	3.1	4.8	7.0	9.8
PULP FINED	HUNTER L			33.1[b]	33.4	33.1	32.4	30.4
	HUNTER a			62.3[b]	60.8	61.2	60.0	57.4
	HUNTER b			22.8[b]	22.7	22.7	22.1	20.6
	HUE			69.9[b]	69.6	69.7	69.8	70.3
	SI			66.3[b]	64.9	65.2	62.0	61.0
	%HAZE			3.6[b]	1.6	4.8	2.6	3.5
ENZYME CONTROL	HUNTER L	26.4	29.5	35.8	32.6	30.8	33.4	30.9
	HUNTER a	56.3	61.6	62.9	60.7	60.5	60.8	58.0
	HUNTER b	18.2	20.6	24.4	22.4	21.3	22.7	21.0
	HUE	72.1	71.6	68.8	69.6	70.7	69.5	70.2
	SI	59.2	64.9	67.4	65.9	64.2	64.9	61.7
	%HAZE	3.2	3.5	1.5	2.6	3.5	3.7	6.3
ENZYME FINED	HUNTER L		29.6	36.1	34.6	34.0	34.4	33.2
	HUNTER a		61.5	63.1	61.0	62.0	61.3	58.7
	HUNTER b		20.6	24.6	23.6	23.3	23.3	22.2
	HUE		71.6	68.7	69.3	69.4	69.3	69.3
	SI		65.2	67.8	64.7	66.2	65.6	62.8
	%HAZE		2.8	3.1	3.2	3.5	4.6	2.8
HTST/ENZYME CONTROL	HUNTER L	26.6	25.7	33.3	32.6	32.3	31.3	28.1
	HUNTER a	54.5	58.7	62.4	60.3	61.1	59.4	55.1
	HUNTER b	18.4	18.3	22.9	22.3	22.2	21.4	19.1
	HUE	72.2	72.7	70.1	69.7	70.1	70.2	70.9
	SI	60.4	60.5	66.5	64.3	65.1	63.2	58.3
	%HAZE	3.6	3.3	2.0	3.3	3.7	3.8	6.0
HTST/ENZYME FINED	HUNTER L		27.5	34.9	34.3	32.8	32.9	31.7
	HUNTER a		59.5	62.8	61.3	61.5	60.5	58.1
	HUNTER b		19.0	23.9	23.3	22.5	22.4	21.3
	HUE		72.3	69.2	69.2	69.9	69.8	69.9
	SI		62.4	67.2	63.6	65.5	64.5	61.9
	%HAZE		3.6	3.5	2.1	3.4	1.7	2.8

[a]All values are means of duplicate analyses and replicates of processing trials; [b]Separate lots, no fining; [c]Crushed berries. PRESS JUICE including pulp fermented juice, 50 ppm SO_2 added; FERMENTION after amelioration with sugar during fermentation; YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2°C, dark) for 2 months, 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark; HUNTER L lightness, a redness, b yellowness; HUE = $\tan^{-1}$ X Hunter a/Hunter b; SI saturation index.

Table 7-Anthocyanin composition (%) of raspberry juice and wine at several stages of processing and storage[a]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	ANTHOCYANIN							
PULP CONTROL	PEAK (1A)	.08	.31	.63[b]	.16	.19	.41	.27
	CY-3-SOP (P1)	69.5	74.5	76.8[b]	78.1	74.3	75.1	73.3
	CY-3-GLRU (P2)	15.0	15.0	15.4[b]	14.3	15.9	16.0	17.0
	CY-3-GL (P3)	7.84	4.03	.00[b]	.00	.00	.00	.00
	PEL-3-SOP (P4)	4.51	3.98	3.11[b]	3.37	3.73	3.82	3.70
	CY-3-RU (P5)	2.52	1.94	1.37[b]	2.09	2.98	2.99	2.61
	PEL-3-GLRU (P6)	.47	.83	.62[b]	1.48	1.13	1.43	1.24
	PEAK (7)	.36	.05	.13[b]	.49	1.00	.12	.03
	PEAK (8)	.00	.00	.00[b]	.00	.74	.24	1.19
PULP FINED	PEAK (1A)			.35[b]	.23	.19	.43	.35
	CY-3-SOP (P1)			78.7[b]	76.9	73.9	75.4	74.4
	CY-3-GLRU (P2)			15.3[b]	15.9	15.6	15.9	17.2
	CY-3-GL (P3)			.00[b]	.00	.00	.00	.00
	PEL-3-SOP (P4)			3.21[b]	3.31	3.61	3.76	3.72
	CY-3-RU (P5)			1.52[b]	2.16	2.89	2.77	2.44
	PEL-3-GLRU (P6)			.66[b]	.99	1.04	1.37	1.33
	PEAK (7)			.00[b]	.15	1.20	.15	.00
	PEAK (8)			.00[b]	.00	.80	.19	.56
ENZYME CONTROL	PEAK (1A)	.00	.15	.19	.54	.14	.24	.21
	CY-3-SOP (P1)	70.3	71.6	79.8	77.7	73.7	74.8	72.6
	CY-3-GLRU (P2)	14.0	15.4	14.3	15.2	16.5	16.6	18.4
	CY-3-GL (P3)	8.88	6.89	.00	.00	.00	.00	.00
	PEL-3-SOP (P4)	4.43	4.04	3.20	3.39	3.52	3.52	3.46
	CY-3-RU (P5)	1.63	1.84	3.16	2.16	3.02	2.82	2.58
	PEL-3-GLRU (P6)	.84	1.25	.71	1.26	1.37	1.41	1.73
	PEAK (7)	.00	.03	.00	.00	.95	.12	.36
	PEAK (8)	.00	.00	.00	.00	.68	.42	.65
ENZYME FINED	PEAK (1A)		.00	.52	.55	.29	.27	.12
	CY-3-SOP (P1)		76.5	77.4	76.6	73.8	74.8	72.9
	CY-3-GLRU (P2)		13.6	15.6	15.6	16.8	16.8	18.7
	CY-3-GL (P3)		3.81	.00	.00	.00	.00	.00
	PEL-3-SOP (P4)		3.95	3.35	3.03	3.58	3.61	3.59
	CY-3-RU (P5)		1.44	1.50	1.67	2.85	2.57	2.34
	PEL-3-GLRU (P6)		.61	1.04	1.70	1.32	1.34	1.45
	PEAK (7)		.00	.00	1.09	.16	.16	.00
	PEAK (8)		.00	.00	.00	1.17	.46	.92
HTST/ENZYME CONTROL	PEAK (1A)	.00	.05	.19	.38	.33	.36	.09
	CY-3-SOP (P1)	71.1	73.5	77.5	78.8	74.1	74.5	72.4
	CY-3-GLRU (P2)	13.5	13.3	15.8	14.6	15.6	16.6	18.3
	CY-3-GL (P3)	9.08	8.02	.00	.00	.00	.00	.00
	PEL-3-SOP (P4)	3.98	3.61	3.13	3.12	3.23	3.49	3.31
	CY-3-RU (P5)	1.84	1.50	1.72	1.81	3.00	2.35	2.70
	PEL-3-GLRU (P6)	.55	.49	.95	.77	1.15	1.42	1.57
	PEAK (7)	.00	.00	.00	.43	.76	.86	.00
	PEAK (8)	.00	.00	.00	.21	1.82	.39	.64
HTST/ENZYME FINED	PEAK (1A)		.00	.16	.19	.37	.21	.22
	CY-3-SOP (P1)		73.2	81.0	78.1	73.5	75.4	73.1
	CY-3-GLRU (P2)		13.9	13.2	15.4	15.8	16.0	18.2
	CY-3-GL (P3)		6.57	.00	.00	.00	1.09	.00
	PEL-3-SOP (P4)		4.12	3.10	3.04	3.31	3.41	3.32
	CY-3-RU (P5)		1.70	1.69	1.80	2.90	2.39	2.64
	PEL-3-GLRU (P6)		.52	.64	1.22	1.19	1.15	1.61
	PEAK (7)		.00	.00	.58	1.15	.06	.07
	PEAK (8)		.00	.00	.00	1.42	.43	.90

[a]All values reported are means of duplicate analyses and replicates of processing trials: no fining; [c]Crushed berries. PRESS JUICE including pulp fermented juice. 50 ppm SO_2 added. FERMENTION after amelioration with sugar during fermentation: YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2°C, dark) for 2 months. 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark; P1-P6 peaks 1-6; CY-3-SOP cyanidin-3-sophoroside. CY-3-GLRU cyanidin-3-glucosyl-rutinoside. CY-3-GL cyanidin-3-glucoside. PEL-3-SOP pelargonidin-3-sophoroside. CY-3-RU cyanidin-3-rutinoside. PEL-3-GLRU pelargonidin-3-glucosyl-rutinoside.

[b]Separate lots, no

Table 8-Raspberry, multiple correlation analyses on compositional, spectral and Hunter parameters[a]

	CORRELATION PARAMETERS	JUICE	JUI/FERM JUI ENZ, HTST/ENZ	YOUNG WINE	STORED WINE SIX MONTHS
	BRIX-HU L	===	-0.889	===	===
	BRIX-HU b	===	-0.876	===	===
	BRIX-HUE	===	0.947	===	===
	BRIX-%HAZE	-0.926	===	===	===
	BRIX-ACN	===	0.954	===	===
	BRIX-C.D.	===	0.966	===	===
**	BRIX-%P.C.	-0.877	-0.909	===	===
	BRIX-BI	===	0.956	===	===
	BRIX-%PEAK3	===	0.950	===	===
	HU L-HU a	===	===	===	0.889
***	HU L-HU b	===	0.996	0.993	0.990
***	HU L-HUE	-0.854	-0.974	===	-0.947
**	HU L-SI	0.891	===	===	0.882
	HU L-ACN	===	-0.895	===	===
	HU L-C.D.	===	-0.899	===	===
	HU L-BI	===	-0.907	===	===
	HU L-%PEAK3	===	-0.921	===	===
	HU a-HU b	===	===	===	0.941
**	HU a-SI	0.887	===	===	0.946
	HU a-%P.C.	===	===	===	-0.872
**	HU b-HUE	-0.905	===	===	-0.913
	HU b-SI	0.925	===	===	===
	HU b-ACN	===	-0.871	===	===
	HU b-C.D.	===	-0.885	===	===
	HU b-BI	===	-0.896	===	===
	HU b-%PEAK3	===	-0.909	===	===
	HUE -ACN	===	0.953	===	===
	HUE -C.D.	===	0.966	===	===
	HUE -%P.C.	===	-0.857	===	===
	HUE -BI	===	0.969	===	===
	HUE -%PEAK3	===	0.950	===	===
**	%HAZE-%P.C.	0.887	-0.857	===	===
	ACN -C.D.	===	0.959	===	===
	ACN -%P.C.	===	-0.896	===	===
	ACN -BI	===	0.943	===	===
	ACN -%PEAK3	===	0.911	===	===
	C.D.-%P.C.	===	-0.942	===	===
***	C.D.-BI	0.914	0.995	0.986	===
	C.D.-%PEAK3	===	0.942	===	===
	%P.C.-BI	===	-0.936	===	===
	%P.C.-%PEAK3	===	-0.846	===	===
	BI -%PEAK3	===	0.942	===	===
	%PEAK1-%PEAK2	===	===	===	-0.914

[a] high correlation was assumed for correlation coefficients $\geq$ 0.85 (alpha=0.05) ***, ** high correlation at three and two stages of processing, respectively; BRIX soluble solids content; HU L, a, b HUNTER L (lighness), a (redness), b (yellowness); SI saturation index; %HAZE haze; ACN total anthocyanin; C.D. color density; %P.C. % polymeric color; BI browning; %PEAK1-3 HPLC area %

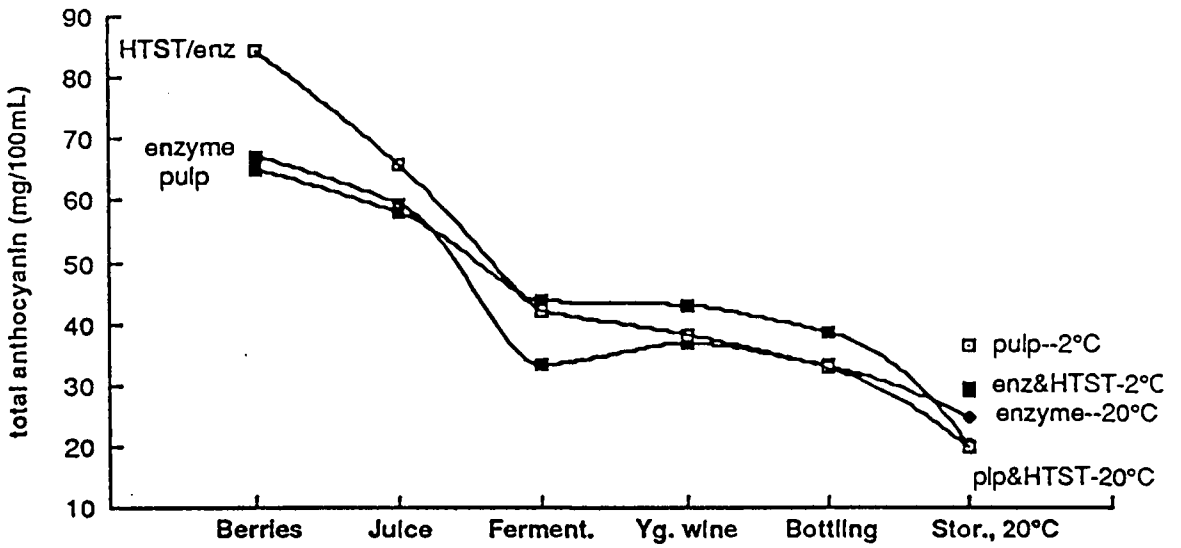

Fig. 1-Changes in total monomeric anthocyanin pigment during processing and storage of red raspberry fruit, juice and wine (replicate 2).

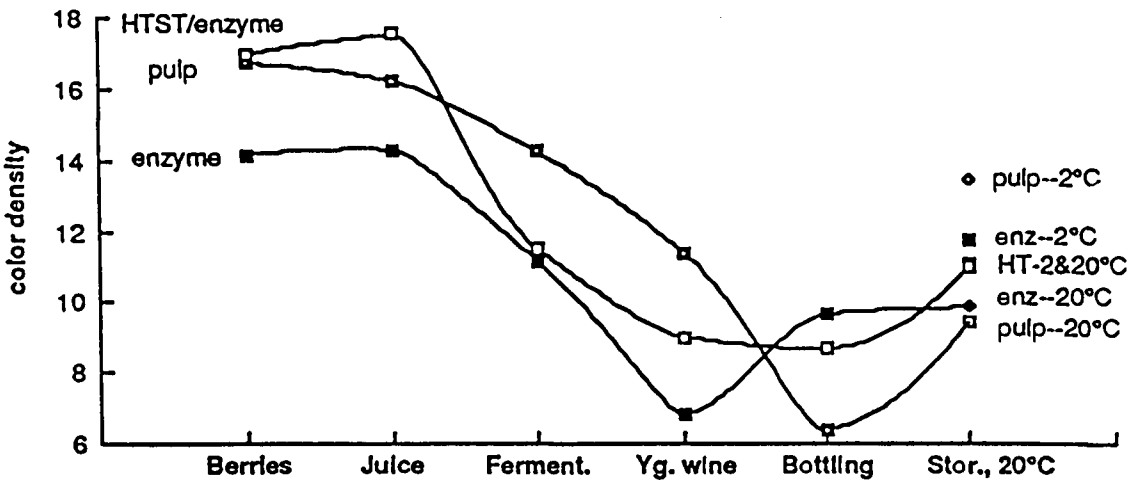

Fig. 2-Changes in color density during processing and storage of red raspberry fruit, juice and wine (replicate 2).

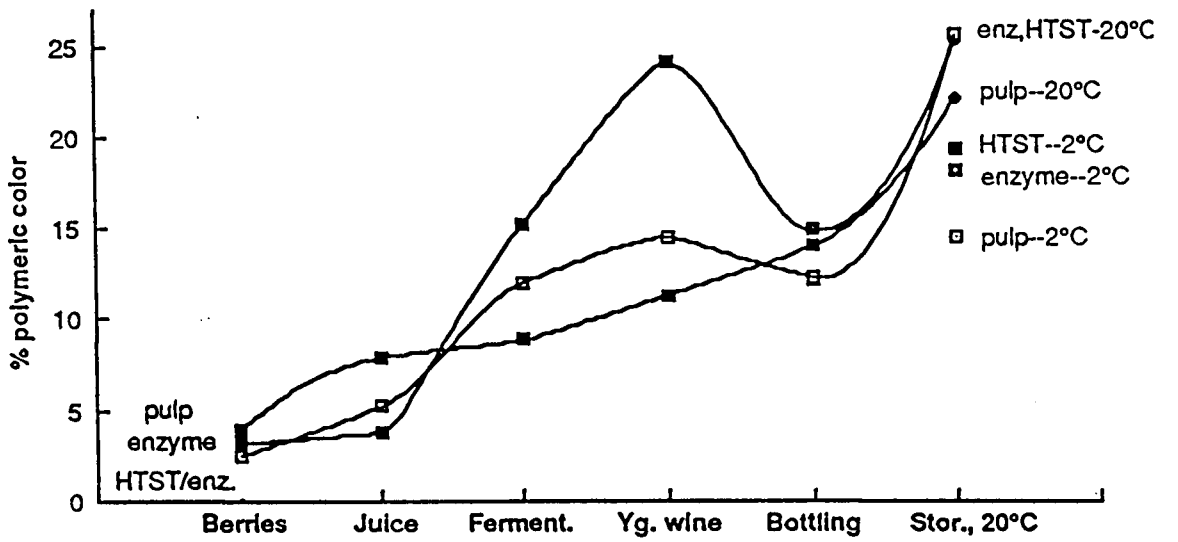

Fig. 3-Changes in % polymeric color during processing and storage of red raspberry fruit, juice and wine (replicate 2).

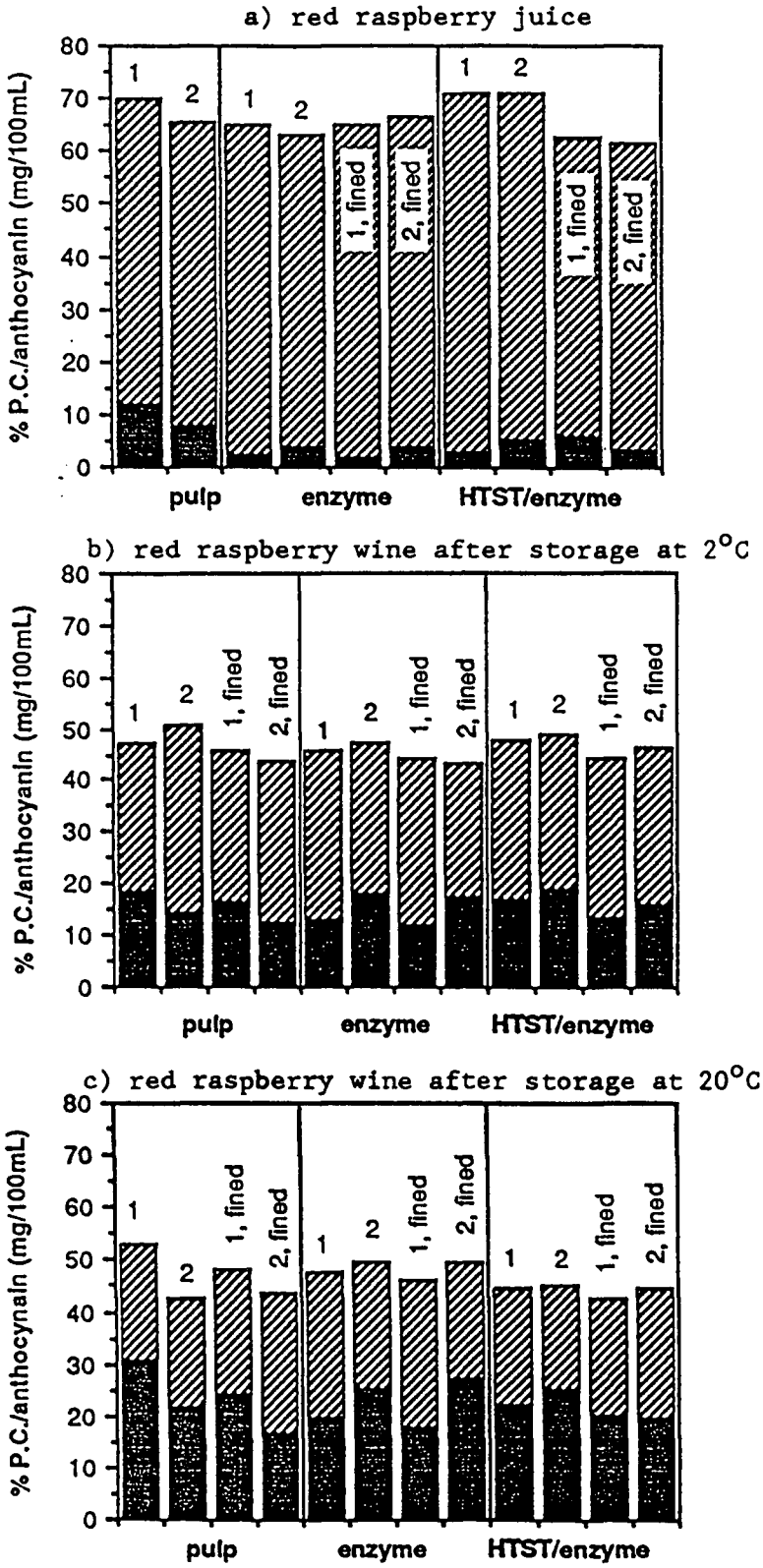

Fig. 4-Anthocyanin to % polymeric color ratios of red raspberry juice and wine after storage (in processing trial replicates, with and without fining).

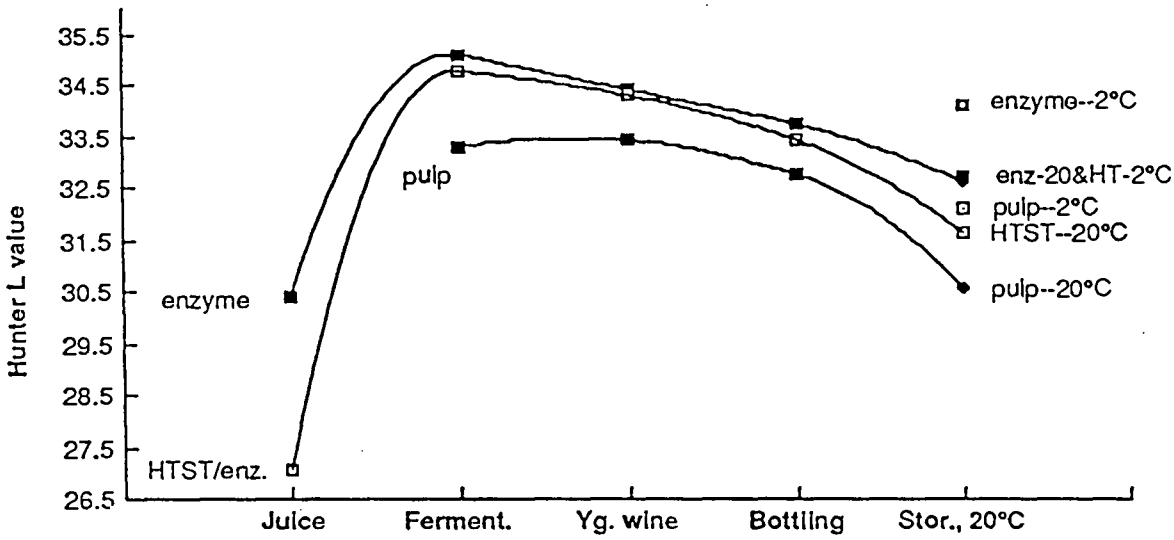

Fig. 5-Changes in Hunter "L" values during processing and storage of fined red raspberry juice and wine (replicate 1).

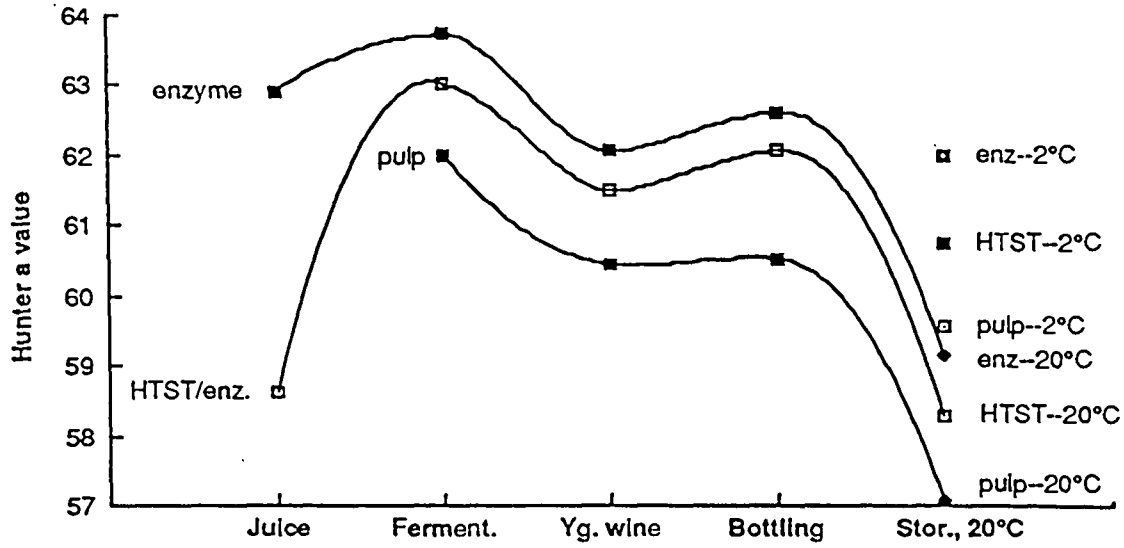

Fig. 6-Changes in Hunter "a" values during processing and storage of fined red raspberry juice and wine (replicate 1).

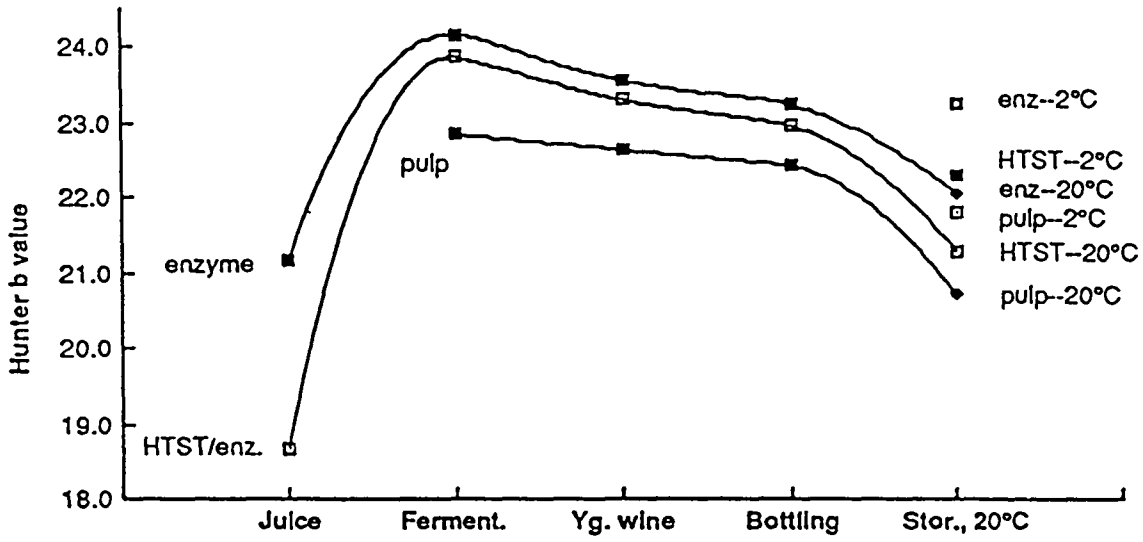

Fig. 7-Changes in Hunter "b" values during processing and storage of fined red raspberry juice and wine (replicate 1).

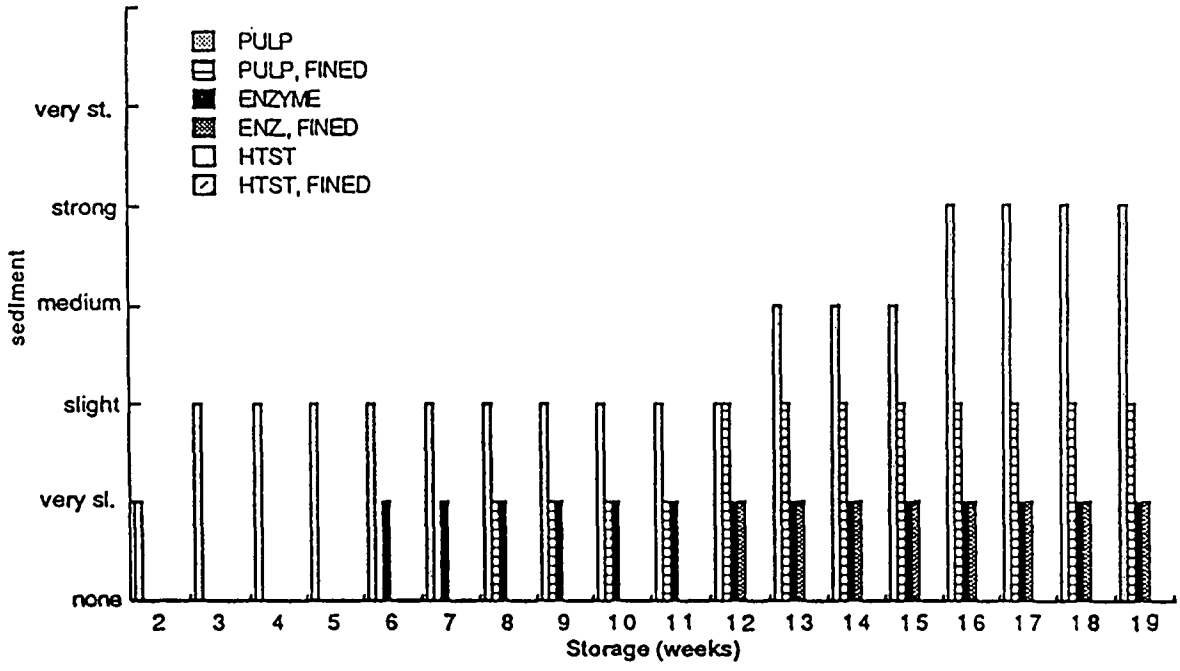

Fig. 8-Sediment formation of red raspberry wine during storage at 20°C (replicate 1).

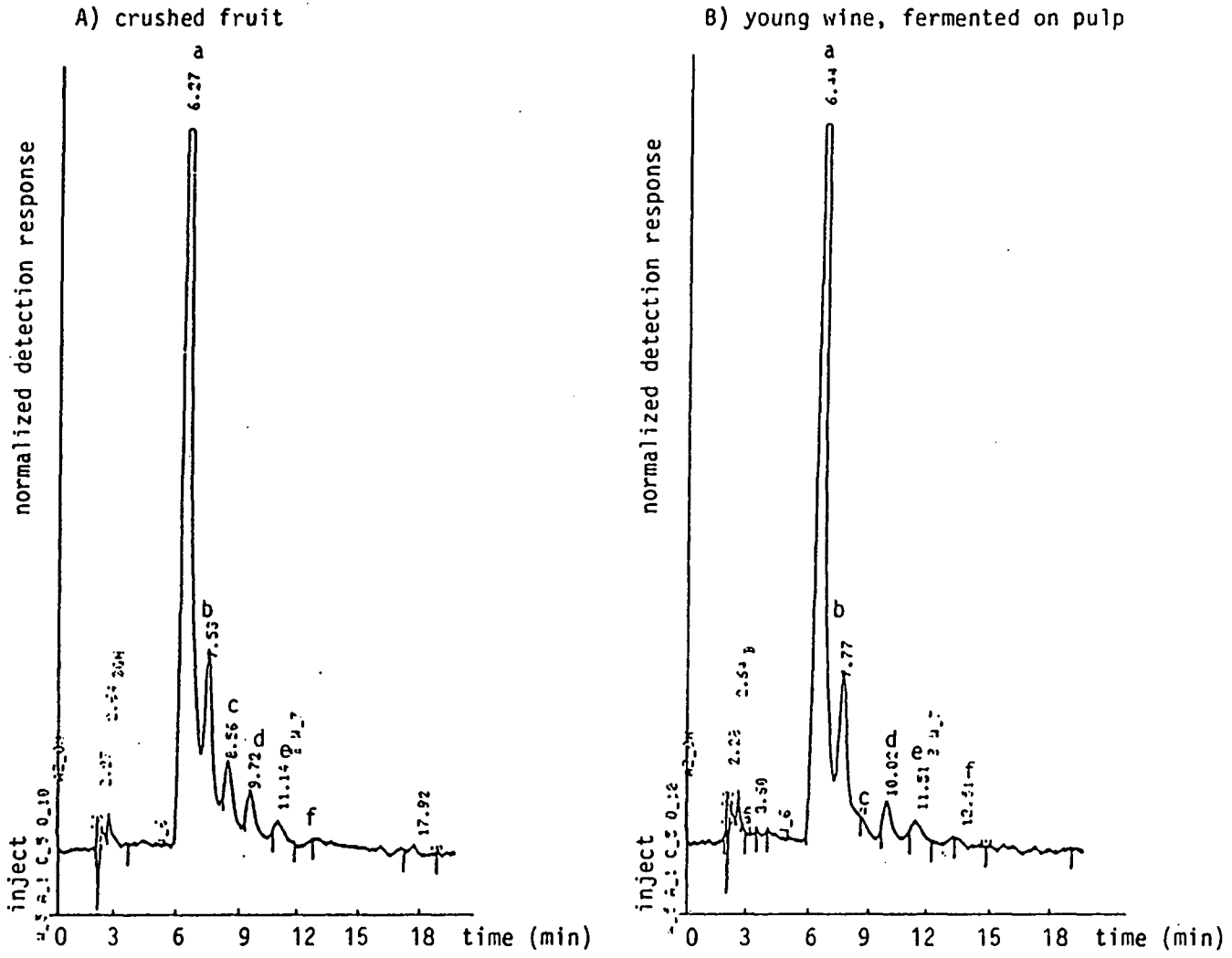

Fig. 9-HPLC chromatograms of the anthocyanins in red raspberry fruit (A) and young wine (B) (replicate 1). Relative peak areas calculated with detection at 520nm in parentheses for (A) and (B), respectively: peak identification, a. cyanidin-3-sophoroside (71, 80%); b. cyanidin-3-glucosylrutinoside (15, 14%); c. cyanidin-3-glucoside (7, 0%); d. pelargonidin-3-sophoroside (4, 3%); e. cyanidin-3-rutinoside (2, 2%); f. pelargonidin-3-glucosylrutinoside (1, 1%).

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Title: **BLACKBERRY JUICE AND WINE: THE EFFECT OF PROCESSING AND STORAGE ON COLOR AND APPEARANCE**

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ABSTRACT

Blackberry wines were made from thawed, frozen fruit (Evergreen variety, *Rubus laciniatus Willd.*) by fermentation of pulp, depectinized juice and high temperature short time (HTST) treated depectinized juice. The influence of fining was also investigated. The wines were stored at 2 and 20°C for six months. Four additional peaks were detected by high performance liquid chromatography (HPLC) which have not been previously reported for 'Evergreen' blackberry. These peaks are believed to be a xylose-cyanidin derivative, two acylated derivatives of cyanidin-3-glucoside or cyanidin-3-rutinoside and cyanidin. The great reactivity of cyanidin-3-glucoside during fermentation (polymerization) found for red raspberry was confirmed. The absence of diglucosides probably explains the great anthocyanin loss after storage (85-100%). Pulp fermentation was responsible for haze. Sediment formation was a problem. Only HTST treated depectinized wine with fining gave an acceptable product.

INTRODUCTION

Blackberry wine has not been produced commercially from the variety 'Evergreen' (*Rubus laciniatus Willd.*) because of insurmountable problems with haze and sediment formation and color loss. Such a product has great potential for a new type of berry wine because of the distinct aroma of 'Evergreen' which is not typical for and therefore not ususally associated with blackberry.

As in strawberry, instability of the anthocyanins is the major cause for the above problems (Pilando, 1985). Anthocyanin polymerization is responsible for both color deterioration (Bakker et al., 1986) and color itself (Pilando et al., 1985). Enzyme treatment (Flores et al., 1984; Maurer, 1974) and pasteurization (Maurer, 1974; Schobinger, 1986) can be expected to have a positive influence on color extraction (density), inactivation of unwanted enzymes, flavor and total juice yield. Heating was found to have a darkening effect on blackberry juice (Sapers et al., 1985). The anthocyanin profile of 'Evergreen' blackberry has never been investigated by HPLC. HPLC analyses of a large number of other blackberry varieties revealed three hitherto unknown peaks (Sapers et al., 1986); they were tentatively identified as a xylose cyanidin and two acid acylated cyanidin derivatives.

It was the purpose of this investigation to 1. develop a processing procedure for wines with acceptable color quality and appearance and 2. characterize anthocyanin pigments by HPLC as well as determine their changes during processing and storage. Such a profile could also be helpful as a reference for adulteration studies. Three different

processing procedures were used to investigate the treatment influences. Detailed analyses were applied to quantify the color changes during processing and storage.

MATERIALS AND METHODS

SOURCE AND TREATMENT OF THE BLACKBERRY SAMPLES

Blackberries (Evergreen variety) grown and picked by 'Rainsweet' near Salem, OR in 1986 and commercially block frozen in 13 kg plastic containers by 'Kemico,' in Salem, OR were used in all processing trials. These samples were stored frozen at -12°C until needed.

JUICE AND WINE PROCESSING

The juices and wines were produced following the procedures by Spanos et al. (1987) and Flores et al. (1984), respectively. Three lots of blackberries (ca. 39 kg each) in replicates, were partially thawed at 27°C and ground through a hammermill (Model D Comminuting machine; W.J. Fitzpatrick Co., Chicago, IL) equipped with a 3/4 in. diameter circular pore mesh at a speed of 182 rpm. After addition of 25 ppm SO_2 as a 1% potassium metabisulfite solution, the mashes were:

(1) fermented on the pulp at 25°C by adding 1 g/gal champagne yeast (Scott Laboratories, Inc., San Rafael, CA) to a soluble solids content of ca. 2°Brix,

(2) depectinized by incubating with 100 ppm 'Rohapect D5L' liquid pectic enzyme (Roehm & Haas Co., Philadelphia, PA) at 25-27°C for 5-7 h and a negative alcohol precipitation (1 mL juice plus 5 mL 95% ethanol) used to monitor the completion of depectinization,

(3) pasteurized by high temperature short time (HTST) treatment at 85-90°C for 1 min in a tubular heater with screws (Wingear type, model 200WU; Winsmith Company, Springville, N.Y.), followed by

immediate cooling to 27°C (Maurer, 1973), depectinized with 100 ppm 'Rohapect D5L' and monitored by alcohol precipitation (as in (2)).

After addition of 1% cellulose as press aid all treatments were pressed in a Willmes bag press (60 type, Moffett Co., San Jose, CA) with a final pressure of 4.0 bar. One half of the treatments (2) and (3) was fined with 500 ppm of a 5% bentonite suspension (50 g/L Volclay KWK bentonite, Scott Laboratories, Inc., prepared by slowly adding bentonite to 800 mL of boiling water with stirring), 100 ppm of a 1% gelatin solution (10 g/L gelatin type B, Wineart Co., Portland, OR: gelatin was dissolved in cold distilled water, heated gently to 82°C, cooled to room temperature and brought to volume) and 0.26 mL/L of a 30% 'Clarifying Agent C2' (silica sol) suspension (Roehm & Haas Co., Philadelphia, PA 19105; diluted to 30% with cold distilled water)..

After fermentation of the unfined and fined lots (2) and (3) to dryness (0-0.2°Brix) with 1 g/gal champagne yeast (as in (1)) at 25°C in 11.4 L glass jars all the wines were ameliorated to 22°Brix by adding sucrose (dry cane sugar) in three stages (corresponds to 12% v/v alcohol in the finished wines) for further fermentation to dryness (0-0.2°Brix) in 11.4 L glass jars at 25°C. One half of the pulp fermented wine (1) was fined comprehensively as described above after which all the wines were cold stored in 3.8 L glass bottles with screw caps at 2°C for two months. After racking, 25 ppm SO_2 as 1% potassium metabisulfite solution, 180 ppm potassium sorbate and 3% sucrose (dry cane sugar) for sweetness were added.

Samples for analyses were taken after grinding, pressing,

fermentation (before and after amelioration with sugar), filtration and storage. These samples were stored frozen at -12°C in 2 oz. plastic bottles until needed.

CLARIFICATION, BOTTLING AND STORAGE

The ameliorated and stabilized wines were filtered at low pressure using Ertel filter equipment (model E1; Ertel Engineering Company, Kingston, N.Y.) and grade 'SG' filter pads (Scott Laboratories, Inc., San Rafael, CA). The filtered wines were bottled in 750 mL dark green glass bottles and sealed with corks. The bottled wines (three processing trials with replicates) were stored in the dark at 2 and 20°C for 6 months.

COMPOSITIONAL ANALYSES

The following analyses were conducted on all samples at five intermediate processing stages as well as after storage: total acidity (as citric acid with the glass electrode method by Amerine et al. (1980)), pH and °Brix (Abbe refractometer and hydrometer method by Amerine et al. (1980)).

COLOR ANALYSES

From spectral analyses with a Varian DMS 100 UV-visible spectrophotometer interfaced with a Varian DS-15 data station (Varian Instruments Group, Walnut Creek, CA) the following measurements were determined on all samples at five intermediate processing stages and after storage as described by Somers et al. (1977) and Wrolstad (1976):

1. total monomeric anthocyanin pigment (as mg cyanidin-3-glucoside/100 mL

juice, using the extinction coefficient, ϵ = 29600), 2. color density ($A_{420\ nm} + A_{520\ nm}$), 3. % polymeric color (polymeric color/color density X 100) and 4. browning (absorbance at 420 nm after bisulfite bleaching).

Hunter L, a and b color parameters were measured in the transmission mode, spectral component included (arrangement III) using a Hunter CT1100 ColorQUEST C5115 color difference meter (Hunterlab, Hunter Associates Laboratories Inc., Reston, VA) against a white tile. Samples were placed in a 0.5 cm pathlength cell. Percent transmission haze was determined as haze = $Y_{(arrangement\ I)} / Y_{(arrangement\ III)}$ X 100 (arrangement I: light source in the normal, aligned position, specular component excluded).

HPLC ANALYSIS

The individual anthocyanin pigments were separated by HPLC using the procedure of Spanos et al. (1987). The pigments were measured as their percentage of total peak area.

Sample and reagents preparation for HPLC analysis

The juice and wine samples were filtered through 0.45 μm Millipore filters (type HA) and immediately injected onto the HPLC system. Before injection the juice samples were diluted by 1/2 with 0.01% HCl.

For solvent A (15% acetic acid), 150 mL HPLC grade glacial acetic acid were added to 850 mL glass-distilled, deionized water and mixed. The solvents A and B (100% acetonitrile) were filtered through 0.45 μm

Millipore filters, types HA and HV, respectively and degased.

HPLC determination

A Perkin-Elmer 400 high pressure liquid chromatograph (Perkin-Elmer Corp., Analytical Instruments, Norwalk, CT) equipped with a:
1. Varian UV 50 variable wavelength detector (Varian Instrument Group, Walnut Creek, CA) and Perkin-Elmer LCI-100 integrator (until bottling) or
2. Hewlett-Packard 1040A photodiode-array detector and a Hewlett-Packard 9000 computer (Hewlett-Packard Co., Wilsonville, OR) (after bottling), were operated under the following conditions: column, Supelcosil LC-18 (5 um), 250 X 4.6 mm id column (Supelco, Inc., Bellafonte, PA) fitted with ODS-10, 4 cm X 4.6 mm id, Micro-Guard column (Bio-Rad Laboratories); mobile phase--solvent A, 15% acetic acid, solvent B, 100% acetonitrile; flow rate, 1.5 mL/min; elution program, 100% A run isocratically for 10 min followed by a 0-15% linear gradient with B for 10 min and the column equilibrated to the initial conditions for 5 min between injections; detection at 520 nm (visible), 0.1 absorbance unit full scale (AUFS); injection volume, 25 μL.

STATISTICAL ANALYSES

The influences of six processing and two storage treatments (sources) on 20 compositional, HPLC, spectral and Hunter measurements were determined by analysis of variance (ANOVA). These sources were: fermentation of juice and pulp, HTST, enzyme, pressing, fining and storage at 2 and 20^{o}C for six months. The influences of these sources were analyzed at the following sampling stages (with and without fining):

1. ground berries and juice, 2. juice and fermented juice (except pulp treatment), 3. young wine and 4. wine before and after storage.

Significant differences between the source influences on each measurement were determined at the significance levels alpha = 0.05, 0.01 and 0.001, respectively. The individual influences of the components of the sources on each measurement were analyzed by T tests (LSD = least significant difference). In the event of interactions between the sources the LSD between the means of the individual components of the interacting sources were calculated at alpha = 0.05 and 0.01.

In addition, the results of the above compositional, HPLC and color measurements at the above four sampling stages were subjected to multiple correlation analysis. High correlation was assumed for correlation coefficients $|r| \geq 0.85$ (alpha = 0.05).

SENSORY ANALYSIS

The sensory quality (aroma and flavor) of the wines after storage at 2 and 20°C was evaluated by a trained taste panel. These results will be published separately by McDaniel et al., Department of Food Science and Technology, Oregon State University.

RESULTS AND DISCUSSION

JUICE YIELDS AND COMPOSITIONAL MEASUREMENTS

Table 9 lists the sample weights and the yields of free-run juice (FRJ) and press juice for each of the processing procedures. The percent yields of the juices were calculated on a weight basis assuming that one L of juice weighs one kg. High temperature short time pasteurization in combination with enzyme (HTST/enzyme) treated juice gave the highest and enzyme treatment the second highest yields of total and FRJ. Pulp fermented mash gave the lowest yields of FRJ (ca. one half of HTST/enzyme) and total juice despite its by far highest yield of press juice. HTST/enzyme treatment provided ca. 8.4% more total yield than pulp fermented and ca. 4.3% more than enzyme-treated juices, the latter of which gave ca. 3.9% more total yield than pulp fermented juice. The total yields obtained are in agreement with reports by Sapers et al. (1985) for juice from frozen thornless blackberries.

Table 10 lists the changes of pH, titratable acidity (TA) and percentage weight soluble solids (^{o}Brix) during processing and storage for all three processing trials. The values reported are overall means for duplicate analyses of two processing replicates. The changes in pH were small during processing and storage and showed similar trends for all trials. pH decreased during pressing and increased again during fermentation and storage at both temperatures.

The significant differences between the source (treatment) influences on each of 20 compositional and color measurements analyzed by

ANOVA at the significance levels alpha = 0.05, 0.01 and 0.001 are listed in table 11. Table 12 lists the significant influences of the individual components of both the single sources and the interactions on these measurements. Enzyme treatment had a highly significant influence on pH (alpha=0.001) (ca. 1.9% increase). The combined effects of HTST application and fermentation were also significant (alpha=0.05) (ca. 0.82% increase).

The changes in TA were similar for all trials during processing and storage (table 10). TA increased during pressing and decreased until the end of fermentation. With the exception of unfined HTST/enzyme-treated wine, TA decreased further during maturation after which it increased again until the end of storage. This pattern of change is the opposite of that for pH until bottling. A high negative correlation of pH and TA in the wines (table 16) confirms the opposite behavior of these two measurements.

HTST application had a significant (alpha=0.01) influence on TA retention (ca. 9.2% higher) in enzyme-treated juice and fermented juice. While pressing had a highly significant (alpha=0.001) increasing influence (ca. 26.9%) on TA in juice, this was undoubtedly a concentration effect. Pulp fermentation did not significantly influence TA, while the combined effects of enzyme treatment and fermentation resulted in a highly significant decrease (alpha=0.001) (ca. 11.3%); this was shown by the fact that pulp fermented young wine had significantly (alpha=0.01) higher (ca. 16.9%) TA values than the two types of enzyme-treated wine. Enzyme treatment by itself also had a significant (alpha=0.05) influence in juice

(ca. 4.3% decrease) and stored wine (alpha=0.01; ca. 15.3% decrease). (table 12)

The TA values of all the stored wines were lower than in the young wines (mean: 1.24 g/100 mL). Of the stored wines, unfined, pulp-fermented (2°C) and unfined, HTST/enzyme-treated (20°C) wines had the highest TA (1.28 g/100 mL) and fined, enzyme-treated wine (2°C) the lowest (0.98 g/100 mL).

The °Brix values through fermentation are listed in table 10. The soluble solids contents of the unfined press juices are in the higher range of previous reports for blackberries including 'Evergreen' variety (Osborn, 1964; Sapers, 1985; Schobinger, 1986; Varseveld, 1980). Fermentation had an obvious and highly significant (alpha=0.001) influence on °Brix in HTST/enzyme and enzyme-treated (ca. 98.3% decrease) and pulp fermented juices (ca. 84.3% decrease). Enzymatic depectinization resulted in a significant (alpha=0.05) increase in °Brix of press juice compared to the crushed berries (ca. 15.4%).

COLOR ANALYSES OF BLACKBERRY JUICE, YOUNG WINE AND STORED WINE

Tables 13 and 14 list the spectral and Hunter data, respectively, for the samples throughout processing and storage. The F values are listed in table 11 and the significant treatment effects of the single sources and interactions in table 12. Haze will be discussed in the section on haze and sediment.

SPECTRAL MEASUREMENTS

The changes in total monomeric anthocyanin pigment showed similar trends for all trials during processing and storage (table 13). With the exception of the concentration effect in pressing, anthocyanin decreased during processing and storage; storage temperature (20°C) had a more significant decreasing effect than storage time (2°C) (figure 10). This pattern of change was the same for all the fined wines except pulp fermented wine (20°C) on a slightly lower level.

HTST-treated fruit and juice retained more anthocyanin. This positive influence continued through processing and was highly significant (alpha=0.001) after storage with HTST-treated wines having ca. 111% more anthocyanin pigment than the other treatments. HTST application and enzyme treatment had a significant (alpha=0.05) influence on anthocyanin retention (ca. 88.0% more) with HTST application by itself being significant (alpha=0.05) for the crushed berries (ca. 55.7% more retained).

Storage temperature (20°C) had a highly significant (alpha=0.001) effect on anthocyanin loss (ca. 53.4%). Enzyme treatment and fermentation combined had a greater influence (alpha=0.01; enzyme: ca. 83.6%, HTST/enzyme: ca. 78.8% decrease) than fermentation by itself. The combined influences of fining and enzyme treatment on anthocyanin loss were of less significance (alpha=0.05) (juice and fermented juice: ca. 17.6%, young wine: ca. 28.2%).

In press juice, the anthocyanin content ranged from 18.3 mg/100 mL (pulp, fermented) to 63.8 mg/100 mL (unfined HTST/enzyme) (figure 10). After storage unfined HTST/enzyme-treated wine (2°C) had the highest

content (9.4 mg/100 mL). No monomeric anthocyanin was detected in unfined, pulp fermented wine after storage at 20°C. Compared to the juices these represent losses of 85.3% to 100%, respectively. The apparent increase of anthocyanin pigment in press juice was due to the release of anthocyanins into the liquid phase; enzyme and HTST treatment resulted in greater anthocyanin yields.

The changes in color density ($A_{420nm} + A_{520nm}$) during processing and storage were similar for all trials (table 13). Color density increased during pressing (concentration) and decreased during fermentation after which it increased again slightly during maturation and particularly during storage. It increased more during storage at 20 than at 2°C in all cases except for enzyme-treated wines (figure 11). This pattern of change was the same for the fined wines, however, they exhibited a slightly reduced level. With the exception of the increase during storage this pattern of change is the same as that for anthocyanin. Monomeric anthocyanin will contribute to color density; there is a high positive correlation of these two parameters during processing. The increase of color density during storage can be ascribed to the contribution of % polymeric color; there is a high negative correlation of these two parameters in juice and fermented juice and a high positive correlation in young and stored wines. (table 16)

The combined effects of HTST application and pressing increased color density very significantly (alpha=0.001) (ca. 102%). HTST application by itself was highly significant (alpha=0.001) (ca. 40.7% increase) for the crushed berries. (table 12)

Color density showed a highly significant (alpha=0.001) decrease with the combined effects of enzyme treatment and fermentation (enzyme: ca. 74.1%, HTST/enzyme: ca. 67.4%). Of high significance (alpha=0.001) were also the combined effects of enzyme treatment and fining in the juices (ca. 20.8-21.3%): this effect was eliminated by fermentation. The decrease with fining by itself was highly significant (alpha=0.001) in the wines after storage (ca. 11.3%) (young wine: alpha=0.05, ca. 17.0%).

HTST had a very significant (alpha=0.001) preserving effect on color density throughout processing and storage. The two types of enzyme-treated juices had ca. 6.7% increased retention over fermented juices. HTST/enzyme-treated young and stored wines had ca. 22.8% and 32.5% more color density than pulp fermented young and stored wines and ca. 101% and 94.1% more than enzyme-treated young and stored wines, respectively. Pulp fermented wines had ca. 63.6% and 46.5% more color density than enzyme-treated young and stored wines, respectively.

Color density of the wines before and after storage was significantly lower than in the juices (mean: 10.6). Unfined HTST/enzyme-treated wine after storage at 20°C had the highest color density (8.7) and fined enzyme-treated wine after storage at 20°C the lowest (2.2) (figure 11).

Percent polymeric color is a measure of the pigments' resistance to bleaching by bisulfite and reflects the degree of polymerization of the anthocyanins. The changes in % polymeric color showed similar trends for all trials during processing and storage (table 13). Percent polymeric color decreased during pressing except for pulp fermented crushed berries

and increased during fermention and storage with greater increases at 20 than at 2°C (figure 12). This pattern of change is similar for fined, pulp-fermented and HTST/enzyme-treated wines, with a slightly lower level. It is opposite to the pattern for anthocyanin pigment content except for the maturation stage of pulp and enzyme-treated wines. This pattern is also opposite to that for color density, except for the stage of storage at 20°C.

These patterns and a high negative correlation of % polymeric color with total anthocyanin, color density, browning, hue angle and °Brix in juice and fermented juice, respectively (table 16), indicate that during fermentation most of the anthocyanin was polymerized. It also reveals that at the fermentation stage, % polymeric color contributed little to color density. A high positive correlation of % polymeric color and color density in young wines (table 16) shows that polymers contributed to color density after fermentation and also during storage at 20°C. Sediment formation occured during maturation; it is evident that both anthocyanins and polymers were lost with sedimentation. During maturation % polymeric color decreased in pulp fermented and unfined enzyme-treated wines while it increased in fined enzyme-treated and unfined HTST/enzyme-treated wines.

Several factors had significant effects on increase of % polymeric color, of highest significance (alpha=0.001) being fermentation (pulp: ca. 29.0%, others: ca. 126%). The combined effects of enzyme treatment, fining and storage temperature (20°C) increased % polymeric color almost as much as fermentation (alpha=0.01, ca. 121%). Both storage temperature (pulp: 61.9%, enzyme: 64.3%, HTST/enzyme: 45.2% increase) and time (pulp:

45.2%, enzyme: 38.1%, HTST/enzyme: 26.2% increase) significantly (alpha=0.01) affected % polymeric color. Temperature had a greater influence than storage time (pulp: 16.7%, enzyme: 26.2%, HTST/enzyme: 19.0% increase). (table 12)

The % polymeric color contents of the wines before and after storage were considerably higher than those of the juices (pulp: 33.1%, mean--enzyme and HTST/enzyme: 18.1%). Due to the significant inhibitory effect of HTST application on % polymeric color development (30.8-34.8% less than the other stored wines), pasteurized wines had the lowest overall levels of % polymeric color after storage (fined, 2°C: ca. 49.4%) (figure 12). Fined enzyme-treated wine after storage at 20°C had the highest content of polymeric color (100%).

HTST/enzyme-treated juices had by far the highest amounts of anthocyanin overall as well as the highest ratio of anthocyanin to % polymeric color, 3:1 (figure 13a). Enzyme-treated juices were second with a ratio of 2:1 and pulp fermented juice had the least anthocyanin compared to % polymeric color with a ratio of 1:2. HTST/enzyme-treated wines retained this greater proportion of anthocyanin compared to polymeric color even after storage at both temperatures (figures 13b&c). When comparing the total amounts of anthocyanins and polymers in pulp fermented and enzyme-treated wines after storage, it appears that almost all anthocyanin was polymerized.

Changes in the <u>browning index</u> (A_{420nm} after bleaching with bisulfite) during processing and storage are listed in table 13. The overall amount of browning was comparable to that for raspberry juice and

wine (Rommel, 1988) and high compared to strawberry wine (Flores, 1984; Pilando, 1985). The browning index increased during pressing except for pulp fermented juice. It decreased for all trials during fermentation, remained essentially the same during maturation and increased during storage; browning increased more at 20 than at 2°C. This pattern was the same for fined (except enzyme treatment, 20°C) and unfined wines, with fined wines having lower browning indices.

This pattern is the same as that for color density which is reasonable to expect as browning, like anthocyanin is a component of color density (Wrolstad, 1976); there was a high positive correlation of these parameters and anthocyanin during processing and storage (table 16). Sapers et al. (1981) also found occurence of browning reactions in parallel with anthocyanin degradation during storage of red cabbage colorant solutions. A high negative correlation in juice and fermented juice suggests that increased % polymeric color coincided with decreased browning; after storage these two measurements were highly positively correlated (table 16). Wrolstad (1976) reports that in addition to monomeric anthocyanins, polymerized anthocyanins and brown pigments arising from enzymic and non-enzymic browning contribute to color.

Browning showed a significant (alpha=0.01) increase in juice (ca. 56.9%) by the combined effects of HTST application and enzyme treatment. Other (alpha=0.05) significant influences were HTST by itself (ca. 44.6%), and storage at 20°C for all wines (ca. 29.6% increase).
Enzyme treatment had a highly significant (alpha=0.001) decreasing influence on browning in the wines during storage (ca. 38.9%). Also significant (alpha=0.01) were the combined effects of enzyme treatment and

fermentation in reducing browning by ca. 59.4%. This confirms the very significantly (alpha=0.001) lower levels of browning (ca. 43.3%, 76.3%) found in enzyme compared to HTST/enzyme-treated fermented juices and young wines, respectively, and in enzyme-treated compared to pulp fermented young wines (ca. 53.3%); these levels were in the same order as those before fermentation. A possible explanation for this decrease could be the low anthocyanin content and color density and higher sedimentation observed in enzyme-treated juices during fermentation and after maturation (racking). Another significant (alpha=0.01) influence on reduced browning was the combined effects of enzyme treatment and fining in juice (ca. 20.6%). Fining by itself was significant (alpha=0.05) in stored wines (ca. 11.3% decrease). (table 12)

With the exception of pulp fermented wines after storage at 20°C, browning in the wines after storage was lower than for the corresponding juices (range from 3.3-6.8). This could have been due to the loss (by sedimentation) of polymers which contributed to browning. Unfined HTST/enzyme-treated wine (20°C) had the highest (4.8) and fined enzyme-treated wine (20°C) the lowest (1.4) level of browning.

HUNTER MEASUREMENTS

The changes in Hunter L (lightness index) and b (yellowness index) were similar for all trials during processing and storage (table 13) which was confirmed by a high positive correlation (table 16). Hunter L and b values increased during fermentation after which they decreased or remained stable during maturation and storage at 2°C. During storage at 20°C, L and b decreased in pulp fermented and increased in enzyme-

treated wines. This pattern of change was the same for the L and b values of the fined juices and wines with higher values (figures 14 and 16, respectively).

The combined effects of enzyme treatment and fermentation had a very significant (alpha=0.001) effect on increased L and b values (enzyme: L-161%, b-139%; HTST/enzyme: L-120%, b-104%; fermentation by itself (pulp): L-105%, b-100%). Other highly significant (alpha=0.001) effects were the combined influences of enzyme treatment and fining in juice (enzyme: L-53.7%, b-58.4%; HTST/enzyme: L-49.7%, b-49.4% increase) and juice and fermented juice (L increase ca. 15.5%). Of high significance (alpha=0.001) was also the increase of Hunter L by the effect of enzyme treatment by itself during storage (ca. 52.4%). Fining by itself increased L significantly (alpha=0.05) in young (ca. 12.8%) and stored (ca. 10.4%) wines. (table 12)

A high negative correlation of Hunter L with anthocyanin, color density, % polymeric color, browning and hue angle, respectively, during processing (table 16) indicates that the increased lightness was the result of sedimentation and anthocyanin loss. This confirms reports by Hong (1988).

Highly significant (alpha=0.001) retention in L values in fined (ca. 7.4% darker) and fermented enzyme-treated (ca. 40.8% darker) juices resulted from HTST application. This was confirmed by the fact that enzyme-treated young wine was ca. 61.7% lighter than HTST/enzyme-treated and ca. 32.1% lighter than pulp fermented young wine, respectively; the latter had ca. 22.4% higher L values than HTST/enzyme-treated young wine. (table 12)

Hunter b values decreased significantly (alpha=0.01) through the

combined effects of enzyme treatment, fining and fermentation (ca. 38.0% decrease) and also HTST application. In contrast, after storage pulp fermented compared to enzyme-treated wine showed a significant (alpha=0.05) decrease of b (ca. 24.2%) (table 12).

After storage the L and b values of the enzyme and HTST/enzyme-treated wines and the L values of the fined pulp fermented wine were considerably higher than in the corresponding juices (mean--enzyme, HTST/enzyme: L-17.0, b-11.7; pulp: L-26.0). Pulp fermented wines had the same or lower levels of Hunter L and b than the fermented juices (L--24.3-26.0; b-16.5). Fined and unfined enzyme-treated wines (20°C) had the highest L and b values (52.2 and 22.8, respectively). Unfined HTST/enzyme-treated wine (2°C) and fined pulp fermented wine (20°C) had the lowest L and b values (23.9 and 12.7, respectively). (figures 14 and 16, respectively)

The changes in Hunter a (redness index) which were similar for all processing trials are listed in table 14. Hunter a had a maximum after pressing or fermentation and decreased during maturation and storage. This pattern was similar for the a values of the fined pulp and enzyme treatments with lower and for the fined HTST/enzyme treatment with higher values (figure 15). The above pattern is also very similar to that for anthocyanins indicating that Hunter a was largely influenced by the concentration of anthocyanins; this is supported by a high negative correlation of a and L in young wine and a correlation of L increase with anthocyanin loss during fermentation and in young wines (table 16). Hong (1988) reports that Hunter L and a values were highly correlated to anthocyanin concentration in cranberry juice cocktail. Hunter a was also

highly positively correlated with % polymeric color and browning in young wine (table 16) which may indicate that polymers and brown compounds were associated with redness.

The combined effects of HTST application and fermentation increased Hunter a most significantly (alpha=0.001) (ca. 44.7%); additional significant influences (alpha=0.05) were fermentation on pulp (ca. 91.6% increase) and the combined effects of enzyme and fining in juice (ca. 58.9% increase). In contrast, Hunter a showed a highly significant (alpha=0.001) decrease (ca. 18.5%) by the combined effects of enzyme treatment, fining and fermentation. Also significant (alpha=0.01) was the influence of storage temperature (20°C) (ca. 18.9%). (table 12)

Of high significance (alpha=0.001) was HTST application which prevented the increase in a values in enzyme-treated juice (ca. 7.0%) and also (alpha=0.01) the decrease in young and stored wines. The a values of HTST/enzyme-treated young wines were ca. 29.3% higher than in enzyme-treated and ca. 8.9% higher than in pulp fermented young wines (alpha=0.001). After storage HTST/enzyme-treated wines had ca. 20.3% higher a values than wines of both other treatments (alpha=0.01).

After storage both pulp fermented and enzyme-treated wines had lower Hunter a values than the corresponding juices (mean: 49.8) while HTST/enzyme-treated wine had higher values (mean of the juices: 38.2). Fined HTST/enzyme-treated wine (2°C) had the highest (50.0) and fined enzyme-treated wine (20°C) the lowest (30.1) levels of a (figure 15).

Changes in hue angle ($\tan^{-1}$ X a/b) showed similar trends for all trials during processing and storage (table 14). With the exception of a

concentration effect in pressing, hue angle decreased during fermentation, remained stable during maturation and decreased further during storage at 20°C; it increased again during storage at 2°C. This pattern was the same for most fined wines with lower values.

With the exception of the increase during storage at 2°C, the above pattern is very similar to that for anthocyanin which indicates that hue angle is largely a function of the concentration of anthocyanins. This was supported by a high positive correlation of anthocyanin, color density, browning and hue angle during processing and storage (table 16) which also implies that brown anthocyanins contributed to hue angle. This confirms reports by Hong (1988).

Percent polymeric color did not contribute to hue angle until after fermentation which was indicated by high positive and negative correlations for juice and fermented juice and young wine, respectively (table 16). In addition, hue angle was highly positively correlated with Hunter b and negatively correlated with L in young wine (table 16).

The combined effects of enzyme treatment and concentration were the only factors which significantly (alpha=0.05) increased hue angle (ca. 2.7%). Hue angle showed a highly significant (alpha=0.001) decrease (enzyme: ca. 13.2%, HTST/enzyme: ca. 4.1%) by the combined effects of enzyme treatment and storage temperature (20°C); less significant (alpha=0.05) were the combined decreasing effects of enzyme and fining in juice (ca. 3.2%) and of enzyme and fermentation (enzyme: ca. 11.6%, HTST/enzyme: ca. 5.2%). (table 12)

HTST application (combined with enzyme treatment) preserved hue angle in fined juices, during fermentation and storage at 20°C. This

inhibitory effect was highly significant (alpha=0.001) in HTST/enzyme-treated young wines which had ca. 8.7% and 4.2% higher hue angle values than enzyme-treated and pulp fermented young wines, respectively; pulp fermented had ca. 4.4% higher values than enzyme-treated young wines (table 12).

After storage the hue angle values of the wines were lower than in the corresponding press juices (mean: 74.5). Unfined HTST/enzyme-treated wine (2°C) had the highest (72.0) and fined enzyme-treated wine (20°C) the lowest (54.4) value. This indicates a significant difference in appearance.

Table 14 summarizes the saturation indices ($(a^2 + b^2)^{1/2}$) for all trials which showed similar changes during processing and storage. Saturation increased during pressing, peaked after fermentation (except for fined enzyme treatment) and decreased during maturation (except for fined enzyme treatment) and storage; it decreased with storage at 20 compared to 2°C. With fining pulp and enzyme treatment showed lower and HTST/enzyme treatment higher saturation indices.

This pattern of change is very similar to those for anthocyanin and Hunter a and also shows some similarity with that for b which was confirmed by high positive correlations during processing and storage (table 16). This confirms the finding by Sapers et al. (1981) that the saturation index is largely influenced by the concentration of anthocyanins. A high negative correlation with % polymeric color, the browning index and Hunter L, respectively, in young wine (table 16) suggests that polymers and brown compounds decreased saturation after

fermentation. During storage haze had a decreasing effect on saturation as indicated by a high negative correlation of these parameters (table 16).

The saturation index showed a highly significant (alpha=0.001) increase by the influence of fermentation (ca. 48.0%). Also significant (alpha=0.01) were the combined effects of HTST application, enzyme treatment and fining (ca. 17.1%) and pressing and fermentation (alpha=0.05; pulp: 92.7%, enzyme: 92.4%, HTST/enzyme: 85.8%).
Enzyme treatment in juice decreased saturation most significantly (alpha=0.001) (ca. 6.5%); other significant (alpha=0.01) influences were storage temperature (20°C) (ca. 9.9% decrease) and the combined effects of fining and fermentation (ca. 6.4% decrease). (table 12)

HTST application had a significant preserving effect on saturation during fermentation and in stored wines. This effect was highly significant (alpha=0.001) in HTST/enzyme-treated young wine which had ca. 24.4% higher values than enzyme-treated and ca. 7.2% higher values than pulp fermented young wines, respectively. After storage HTST/enzyme-treated wine still had ca. 16.3% higher saturation than both other types of wine (alpha=0.05).

After storage pulp fermented and enzyme-treated wines had lower saturation indices than the corresponding juices (pulp: 55.2, mean enzyme: 50.1) while HTST/enzyme-treated wines had higher values (mean of the juices: 39.4). Fined HTST/enzyme-treated wine (2°C) had the highest (53.1) and pulp fermented wine (20°C) the lowest saturation (33.6).

HAZE AND SEDIMENT FORMATION IN BLACKBERRY WINE STORED AT 2 AND 20°C

HAZE

Changes in percent haze during processing and storage for all trials are listed in table 14. During pressing haze increased in pulp fermented and enzyme-treated while it decreased in HTST/enzyme-treated juices. For all trials, haze decreased with fermentation and increased during maturation. During storage haze increased substantially in the pulp fermented and decreased in the fined HTST/enzyme-treated wines (figure 17).

This pattern is similar to that for % polymeric color and opposite to that for anthocyanin for all except enzyme-treated juices and wines; this may imply that increased haze was associated with polymerized anthocyanin. For enzyme-treated juices and wines the pattern for haze is similar to that for anthocyanin and opposite to that for % polymeric color; in this case haze could have been related to shorter oligomeric pigment forms (Somers, 1977, 1986).

Pulp fermentation was the greatest overall and the only significant (alpha=0.05) increasing influence on haze in stored wines (ca. 189%) (table 12). This is probably related to the greatest anthocyanin loss and polymerization found during fermentation on the pulp; no monomeric anthocyanin was detected after storage (20°C) of unfined pulp fermented wines. Native polymers present in the skins were possibly released into the liquid phase during fermentation; these polymers may be more susceptible to haze formation than polymerized anthocyanin. Overall, the lack of pectic enzyme probably prevented the breakdown of the haze causing polymers (medium length).

The significant (alpha=0.05) effect of enzyme treatment combined with fermentation decreased haze more (ca. 82.8%) than combined with fining (ca. 69.2%) in juice. This shows that fining only had a minor effect. HTST application reduced haze by ca. 67.0%. (table 12)

After storage the contents of haze were higher than in the corresponding juices (mean--pulp, unfined enzyme: 8.0%; fined enzyme, HTST/enzyme: 2.7%). Fined pulp fermented wine (20°C) was the most (42.6%) and fined enzyme-treated wine (2°C) the least hazy (3.3%).

SEDIMENT

The development of sediments during six months of storage at 20°C in the dark is shown in figure 18. The amount of sediment remained unchanged after 19 weeks of storage.

Overall, sedimentation was considerably higher than in red raspberry wines produced by the same treatments (Rommel, 1988). None of the blackberry wines was very stable. Fined HTST/enzyme-treated wine was relatively stable in producing only very slight sediments after 9 to 10 and medium amounts after 12 weeks which remained at this level until the end of storage. Second in stability were fined enzyme-treated and unfined HTST/enzyme-treated wines with very slight sediments after 3 and 4, slight sediments after 8 and 5, medium sediments after 10 and 8 and strong sediments after 12 and 15 weeks, respectively. Most unstable were unfined enzyme-treated and pulp fermented wines with very slight sediments after 3 and 2, slight sediments after 4, medium amounts after 6, strong sediments after 9 and 13 and very strong sediments after 11 and 16 weeks, respectively.

This pattern for sediment formation reflects the pattern for haze production except for unfined, enzyme-treated wine. After storage at 20°C unfined enzyme-treated wine had the second lowest level of haze but one of the highest levels of sediments and also the second highest amounts of % polymeric color. Therefore, low levels of haze may have been related to the lengths of the polymers which were too long to stay in solution and hence precipitated soon after their formation.

ANTHOCYANIN COMPOSITION OF BLACKBERRY JUICE, YOUNG AND STORED WINE

The anthocyanin profile for the crushed fruit is shown in figure 19a and for the wine after fermentation on the pulp in 19b. Seven distinctive peaks could be identified in the crushed fruit by reversed phase high pressure liquid chromatography (HPLC): one major peak (2--67.0%), four medium sized peaks (3--7.74%, 4--7.9%, 5--7.76%, and 6--6.65%) and two minor peaks (1--1.04%, 7--0.97%).

The peaks 2 and 3 are cyanidin-3-glucoside (Cy-3-Gl) and cyanidin-3-rutinoside (Cy-3-Ru). Cy-3-Gl is common to a wide range of blackberries studied, usually as the major pigment and Cy-3-Ru as a second pigment in lower concentrations or trace amounts; their identities are therefore well established (Hong, 1988; Jennings, 1980; Sapers, 1986; Spanos, 1987; Torre, 1973, 1977). Cy-3-Gl as the major pigment and trace amounts of Cy-3-Ru are the only anthocyanins which were reported for 'Evergreen' blackberry (Jennings, 1980; Torre, 1973, 1977). The short retention times of the peaks 2 and 3 combined with the presumption that due to genetic effects blackberries are only able to synthesize the aglycone cyanidin and

no diglycosides (Jennings, 1980; Sapers, 1986) further support these assignments. Comparison of the retention times for the peaks 2 and 3 with those for Cy-3-Gl and Cy-3-Ru as identified for 1. Red Raspberry by the same system (Rommel, 1988; Spanos, 1987) and 2. blackberries by a similar system (Sapers, 1986), further establishes the identities of these peaks. The relative amounts are consistent with those reported previously (Hong, 1988; Jennings, 1980; Sapers, 1986; Torre, 1973, 1977).

Sapers et al. (1986) recently reported presence of three additional peaks in 33 thorny and thornless blackberry cultivars and selections. However, the samples investigated did not include 'Evergreen' variety. In blackberry extract from 7 predominantly thornless cultivars Sapers et al. (1986) tentatively identified the following additional peaks, in their order of elution, by reverse phase HPLC: a xylose cyanidin derivative and two dicarboxylic acid acylated derivatives of Cy-3-Gl.

When comparing the relative retention times and area percentages of the peaks 4 to 6 to those for the peaks 2 and 3 it seems possible that peak 4 is a xylose containing anthocyanin and that the peaks 5 and 6 are acid acylated derivatives of Cy-3-Gl or Cy-3-Ru. The retention times and relative amounts are consistent with those found by Sapers et al. (1986) who used a similar separation system. A high positive correlation of the peaks 5 and 1 and their high negative correlations with ^{o}Brix and peak 2 (Cy-3-Gl) in pulp fermented juice (table 8) suggest that peak 5 like peak 1 was increased during fermentation and could be a derivative of Cy-3-Gl. Both peak 6 and % polymeric color which showed a high positive correlation were highly negatively correlated with ^{o}Brix in fermented juice (table

16); this indicates that peak 6 like polymers may be a secondary product which increased during fermentation.

The most dramatic change in the anthocyanin profile was the loss of Cy-3-Gl (peak 2) during fermentation (ca. 39.1%, combined with enzyme ca. 75.2%) (fig 19b; table 15). This was supported by highly colored sedimentation during maturation (after racking), strong increases in % polymeric color during processing and a high negative correlation of Cy-3-Gl with ^{o}Brix and peak 1 (polymers?) in pulp fermented juice (table 16). The latter indicates that Cy-3-Gl was very actively involved in polymerization. This finding also confirms the unstable nature of the monoglycoside Cy-3-Gl whose complete disappearance was reported for red raspberry during fermentation (Rommel, 1988).

Due to its shortest retention time which indicates high polarity and greater molecular weight, peak 1 could be a dimer, a degradation product of high polarity or possibly polymerized anthocyanin. This is supported by the fact that this peak was present in very low quantities in the crushed fruit and press juices and increased largely during fermentation. However, even after storage peak 1 had a peak area of only ca. 6% despite the almost complete transformation of anthocyanins into polymers (table 15). This could indicate that peak 1 is not a polymer peak; however, it is more likely to be due to the strong precipitation of polymers observed during maturation and storage.

Peak 7 was detected in some but not all crushed fruit samples and increased largely throughout processing and particularly during storage.

Because of its very late retention time this peak is likely to be a cyanidin derivative or cyanidin.

TREATMENT EFFECTS ON THE ANTHOCYANIN PROFILE

Table 15 lists the changes in anthocyanin composition (HPLC area percentages) during processing and storage for the different treatments. It has to be noted that with such pronounced loss of Cy-3-Gl there is an apparent increase of other peak areas whose absolute changes are difficult to predict. The HPLC areas do not always add up to 100%; in these cases the integrator excluded minor peaks or shoulders which developed particularly during storage and remain unidentified. Table 12 summarizes the significant treatment effects at three stages of processing and after storage. Figure 20 shows the treatment effects on the HPLC chromatograms after fermentation.

HTST pasteurization generally slowed down degradation reactions and was therefore the only positive influence on the anthocyanin profile of the wines. This great impact of HTST application indicates that the fruit contained large quantities of native enzymes, possibly glycosidases or PPO enzyme. This seems to be a particular problem for the blackberry variety 'Evergreen' which was found to produce excessive amounts of haze and sediment in commercial winemaking as well as in this study. For this variety HTST application is a necessity in order to obtain an acceptable product.

This preserving effect of HTST treatment was shown to be highly significant (alpha=0.001) on other peaks in the profile than 5 (probably

on Cy-3-Gl) and peak 6; the fact that peak 5 was not influenced by pasteurization combined with the assumption that peak 6 decreased relative to other peaks, further support the hypothesis that the peaks 5 and 6 are acylated derivatives of other cyanins. Acylated anthocyanins (acylated with acid or containing at least two acyl groups) were reported to be very stable during heating, storage and exposure to light (Hong, 88; Sapers, 81). However, Sapers et al. (1986) report that anthocyanins acylated with simple dicarboxylic acids are extremely unstable and display complex acid hydrolysis behavior. This leads to the conclusion that the peaks 5 and 6 are acylated with other acids.

In addition, HTST application had an inhibitory effect on peak 1 formation in juice and a significant (alpha=0.01) preserving effect on this peak during storage (3 X more than the other two treatments); also significant (alpha=0.01) were the preserving effects on peak 2 (Cy-3-Gl) during fermentation, peak 4 and on other peaks than 3 (Cy-3-Ru) which indicates a greater stability of Cy-3-Ru compared to other anthocyanins.

Fining did not influence the profile except for a significant (alpha=0.01) decreasing effect on peak 4 combined with enzyme treatment in juice (ca. 22.7%). This instability of peak 4 compared to the above mentioned greater stability of the acylated anthocyanins (peaks 5 and 6) supports the hypothesis that peak 4 is a xylose cyanidin derivative which are known to break down more easily (reference?). This result also supports the tentative identification of this peak as a xylose-containing anthocyanin by Sapers et al. (1986) whose synthesis had been believed impossible in blackberries by previous investigators (Jennings, 1980;

Markakis, 1982).

Fermentation (including pulp) had the greatest negative influence on the anthocyanin profile. It was responsible for the highly significant (alpha=0.001) loss of Cy-3-Gl (peak 2) (pulp 39.1%, combined with enzyme 75.2%). Pulp fermentation had a significant (alpha=0.01) decreasing influence on peak 3 (Cy-3-Ru) combined with storage temperature (61%). The highly significant (alpha=0.001) increasing influence of fermentation on the peaks 5 (108%), 6 (pulp 61%, combined with enzyme 2.6 X) and 3 (pulp 131%, combined with enzyme 3 X) are believed to be proportional to the greater instability of other peaks and supports the stability of these peaks as discussed earlier; the latter was also supported by high negative and positive correlations of peak 3 with ^{o}Brix in juice and fermented juice, respectively (table 16). The significant (alpha=0.01) increasing influence on peak 4 (pulp 73%) is also proportional because this assumed xylose derivative degraded rapidly after fermentation. The highly significant (alpha=0.001) increasing influence on peak 1 (pulp 2.5 X, combined with enzyme 5 X) is likely to be absolute because this peak only existed in one of the fruit samples.

Enzyme treatment enhanced the negative effects of fermentation in most cases. Its highly significant (alpha=0.001) increasing effect on peak 1 (in juice 102%, combined with fermentation 5 X) is believed to be absolute while the effects on the peaks 3 (Cy-3-Ru) (3 X) and 6 (2.6 X) are probably proportional due to their previously discussed greater stabilities. Enzyme treatment had a significant (alpha=0.01) decreasing

effect on the peaks 3 (Cy-3-Ru) (combined with storage time 47.5%), 4 (combined with fining in juice, 22.7%) and 2 (Cy-3-Gl) (alpha=0.05; combined with fermentation 75.2% and storage 34.2%).

Storage temperature (20°C) had a destructive effect on three peaks of the anthocyanin profile: this effect was highly significant (alpha=0.001) for peak 5 (44.2%) and significant (alpha=0.01) for the peaks 3 (Cy-3-Ru) (combined with fermentation 61%) and 2 (Cy-3-Gl) (alpha=0.05; 17.9%).

Storage time (2°C) was less influential than temperature. It only had a significant (alpha=0.01) decreasing effect on peak 3 (Cy-3-Ru) (combined with enzyme 47.5%). The significant (alpha=0.01) increasing influence on peak 7 (71.3%) supports the possibility that this peak represents cyanidin.

Storage at both temperatures was a significant (alpha=0.01) increasing influence on peak 6 (34.9%). This could be an indication for further acylation or a proportional decrease of other peaks in the profile which confirms the great stability of this compound. The other assumed acylated cyanidin derivative (peak 5) and Cy-3-Ru were not affected in their stabilities until storage.

MULTIPLE CORRELATION STUDIES

A high correlation was assumed for multiple correlation coefficients greater than 0.85 (alpha=0.05). The individual correlations for blackberry juice and wine at four stages of processing are listed in table 16.

A high positive correlation at three stages of processing was found between: 1. Hunter L and b, 2. Hunter a and the saturation index, 3. hue angle and anthocyanin, 4. hue angle and color density, 5. hue angle and browning, 6. anthocyanin and color density, and 7. color density and browning. 8. Anthocyanin and browning showed a high positive correlation at two stages of processing.

A high negative correlation at three stages of processing was found between 9. Hunter L and browning, and at two stages between 10. °Brix and % polymeric color, 11. Hunter L and anthocyanin, and 12. Hunter L and color density.

High positive and negative correlations at three stages of processing existed between 13. color density and % polymeric color, and at two stages between 14. °Brix and peak 3 (Cy-3-Ru), 15. Hunter L and a, 16. Hunter L and hue angle, 17. Hunter L and the saturation index, 18. Hunter L and peak 3 (Cy-3-Ru), 19. hue angle and peak 3 (Cy-3-Ru) and, 20. % polymeric color and browning.

In addition to the above correlations at several stages the wines after storage did not show any significant correlations except for the combined occurence of high levels of polymers and browning; this was indicated by a high positive correlation of these two parameters. The high correlations found until storage are therefore of less importance for the final product.

CONCLUSIONS

1. The major causes for anthocyanin loss were storage time and the combined effect of fermentation and enzyme treatment. Compared to the juices anthocyanin loss was in the range of 85.3 to 100% for unfined HTST/enzyme ($2^{o}C$) and unfined pulp ($20^{o}C$) treatment, respectively. HTST pasteurization had a highly significant influence on anthocyanin retention throughout processing with 111% retention after storage. Unlike red raspberry, blackberries do not contain diglucosides which may explain the greater color instability of blackberry wines. After storage blackberry wine exhibited 52.6-82.3% more anthocyanin loss than raspberry wine.

2. The great reactivity of Cy-3-Gl as found for red raspberry was confirmed. In contrast to raspberry, blackberry has Cy-3-Gl as its major pigment and therefore contains greater total amounts of this anthocyanin. This may explain why fermentation did not eliminate Cy-3-Gl completely (combined with enzyme treatment 75.2% loss). Transformation of Cy-3-Gl into Cy-3-Ru through loss of rhamnose possibly aggravated this strong decrease; native enzymes were probably another contributing factor. Cy-3-Gl was very actively involved in polymerization as shown by a strong increase in polymeric color and correlation analyses.

3. Four additional peaks were detected by HPLC which have not been reported previously for 'Evergreen' blackberry. Peaks 5 and 6 are

believed to be acylated derivatives of Cy-3-Gl or Cy-3-Ru; the great stability of 5 and 6 throughout processing and storage supports this assumption. Peak 4 could be a xylose-cyanidin derivative; peak 7 might be cyanidin. The retention times and relative amounts of the peaks 4 to 6 are in agreement with previous reports.

4. Pulp fermentation was the greatest overall and the only significant increasing influence on haze, particularly during storage (1.9 and 4.3 times increase compared to other treatments and pulp fermented juice, respectively). The major reducing influences on haze were fermentation combined with enzyme treatment (82.8%) and HTST application (67%). After storage blackberry wine was 2 to 3.5 times hazier than raspberry wine.

5. Sediment formation was a problem, with none of the blackberry wines being stable over six months. Fined HTST/enzyme-treated wines were relatively stable for the first 9 to 10 weeks. Sedimentation reflected haziness except for unfined enzyme treatment with strong sediment but little haze formation. After storage blackberry wine exhibited approximately twice the amount of sediment compared to raspberry wine.

6. HTST pasteurization had a great impact in retarding degradation processes which indicates presence of large quantities of native enzymes in 'Evergreen' blackberry. It was the only positive

influence on total anthocyanin and the anthocyanin profile, particularly on Cy-3-Gl and peak 4. Pasteurization slowed down polymerization, reduced haze and preserved color density and the Hunter measurements. However, it had an increasing effect on browning.

7. Fining decreased browning and increased saturation. It was of some significance in reducing haze in combination with fermentation. Fining reduced total anthocyanin, particularly peak 4.

8. Percent polymeric color showed high negative correlations with total anthocyanin (Cy-3-Gl), color density, browning, Hunter L, hue angle, and positive correlations with Hunter a and saturation during fermentation and maturation. Fined enzyme-treated wine had by far the highest % polymeric color after storage at 20°C. After storage blackberry wine contained 2.4 to 2.8 more % polymeric color than raspberry wine.

9. Pulp fermented blackberry wines were excessively astringent and bitter as determined by preliminary sensory evaluation. For an acceptable product HTST pasteurization was neccessary which combined with enzyme treatment and fining gave the best overall quality. HTST application minimized haze and sediment formation which increased both color stability and appearance; it also reduced astringency and bitterness in retarding polymerization. Fining influenced color appearance positively in reducing browning, and

increasing saturation (redness).

Table 9-Blackberry weights, juice volumes and yields of the three treatments

TREATMENT		BERRY WEIGHT (kg)	FRJ (l)	YIELD FRJ (%)	JUICE (l)	YIELD PJ (%)	FRJ+PJ (l)	TOTAL YIELD (%)
PULP FERMENTION	replicate 1	37.1	7.2	19.4	16.3	43.9	23.5	63.9
	replicate 2	36.9	9.5	25.7	16.7	45.3	26.1	70.7
	means	37.0	8.4	22.6	16.5	44.6	24.8	67.0
ENZYME	replicate 1	38.0	9.8	25.8	17.0	44.7	26.9	70.8
	replicate 2	38.9	14.0	36.0	12.5	32.1	26.5	68.3
	means	38.5	11.9	30.9	14.8	38.4	26.7	69.6
HTST/ ENZYME	replicate 1	32.9	11.4	34.7	11.7	35.6	23.1	70.2
	replicate 2	36.5	15.9	43.6	11.4	31.2	27.3	74.8
	means	34.7	13.7	39.2	11.6	33.4	25.2	72.6

FRJ free run juice (before pressing); PJ-press juice.

Table 10-Compositional analyses of blackberry juice and wine at several stages of processing and storage[a]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	COMPOSITION							
PULP CONTROL	pH	3.63	3.62	3.61[b]	3.62	3.63	3.73	3.68
	TA[d]	1.05	1.42	1.42[b]	1.37	1.04	1.28	1.25
	°BRIX[e]	12.39	1.95	.40[b]	.30			
PULP FINED	pH			3.60[b]	3.64	3.64	3.73	3.64
	TA			1.42[b]	1.37	1.03	1.22	1.25
	°BRIX			.50[b]	.35			
ENZYME CONTROL	pH	3.81	3.55	3.68	3.71	3.72	3.77	3.79
	TA	1.07	1.29	1.17	1.15	.99	1.04	1.00
	°BRIX	11.49	13.25	.10	.00			
ENZYME FINED	pH		3.62	3.68	3.70	3.75	3.78	3.81
	TA		1.24	1.11	1.09	.91	.98	.94
	°BRIX		12.17	.10	.25			
HTST/ENZYME CONTROL	pH	3.84	3.68	3.77	3.71	3.71	3.73	3.69
	TA	1.08	1.41	1.29	1.26	1.45	1.16	1.28
	°BRIX	12.77	13.29	.50	.45			
HTST/ENZYME FINED	pH		3.68	3.77	3.72	3.71	3.75	3.70
	TA		1.34	1.22	1.19	1.03	1.09	1.14
	°BRIX		13.04	.10	.20			

[a]All values reported are means of duplicate analyses and replicates of processing trials; [b]Separate lots, no fining; [c]Crushed berries; [d]Titratable acidity (g citric/100 mL), [e]Weight % soluble solids (measured with Abbe refractormeter and hydrometer), methods by Amerine et al. (1980). PRESS JUICE including pulp fermented juice, 50 ppm SO_2 added; FERMENTATION after amelioration with sugar during fermentation; YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2 C, dark) for 2 months, 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark.

Table 11a-Blackberry, F values

	SOURCE	DF	pH	TA	°BRIX	ACN	C.D.	%P.C.	BI	HUNTER L	HUNTER a	HUNTER b	HUE	SI	%HAZE(TM)
	PROCESS(P)	2	1.15	5.48 *	229.60 ***	74.61 ***	142.54 ***	34.43 ***	81.75 ***	27.84 ***	27.17 ***	50.71 ***	28.24 ***	29.52 ***	1.60
Juice	JUICE (J)	2	2.02	62.62 ***	38.21 ***	9.14 **	61.39 ***	0.09	14.54 **	31.21 ***	98.58 ***	77.78 ***	8.27 **	99.26 ***	0.98
	P X J	4	0.55	2.07	61.69 ***	10.93 *	21.50 ***	3.03 *	12.16 **	5.26 *	4.50 *	10.16 **	6.12 *	5.00 *	3.37
	MSE(ERROR)	9	0.02	0.00	0.46	18.18	0.59	8.12	0.09	3.89	6.95	0.69	0.24	7.42	5.11
	PROCESS(P)	1	9.20 *	21.08 **	1.95	80.61 ***	257.42 ***	1.55	192.13 ***	117.59 ***	1.54	115.68 ***	115.15 ***	0.16	0.40
Juice/	FERMENT(F)	1	17.32 **	39.45 ***	2919 ***	531.76 ***	1159 ***	60.71 ***	656.37 ***	458.73 ***	36.44 ***	1244 ***	368.51 ***	69.58 ***	5.22
ferm.	FINED (f)	1	1.12	6.69	2.01	10.89 *	48.94 ***	0.14	39.80 ***	13.62 **	0.63	37.58 ***	21.36 **	1.20	4.27
Juice,	P X F	1	6.09 *	0.00	0.30	25.00 **	39.08 ***	0.19	15.25 **	39.21 ***	141.98 ***	55.10 ***	53.06 ***	139.65 ***	10.12 *
enz./	P X f	1	0.60	0.07	0.13	0.36	1.87	0.16	0.81	1.29	3.32	0.00	5.90 *	3.21	2.98
HTST-	F X f	1	1.12	0.03	2.01	2.20	21.32 **	0.37	13.94 **	0.09	22.07 **	42.61 ***	0.30	23.16 **	4.12
enz.	P X F X f	1	1.80	0.00	2.01	0.00	2.44	0.37	2.01	0.19	1.12	8.54 *	0.06	1.14	7.30 *
	MSE	8	0.00	0.00	0.22	11.14	0.35	34.00	0.05	5.19	3.62	0.16	0.41	3.50	1.67
	PROCESS(P)	2	5.07	14.82 **	0.52	140.83 ***	31.55 ***	0.95	35.52 ***	40.52 ***	32.07 ***	5.42 *	27.31 ***	29.19 ***	2.04
Young	FINED (f)	1	0.09	1.20	0.01	57287 ***	7.50 *	0.26	10.12	7.44 *	5.48	0.31	5.95	5.15	0.45
wine	P X f	2	0.16	0.38	0.62	5.36 *	0.17	0.21	0.42	0.37	1.78	1.79	0.62	1.83	0.68
	MSE	6	0.00	0.00	0.10	0.34	0.27	49.73	0.04	9.60	5.01	0.32	1.21	4.62	1.42
	PROCESS(P)	2	11.42 ***	9.14 **		77.07 ***	89.97 ***	31.29 ***	24.07 ***	27.49 ***	9.41 **	5.11 *	91.04 ***	6.09 *	4.10 *
	FINED (f)	1	0.14	4.15		3.55	26.97 ***	8.63 *	8.57 *	9.39 **	0.24	0.51	8.95 **	0.07	1.07
Stored	STORED (S)	2	3.67	1.82		33.26 ***	2.02	148.20 ***	7.59 **	1.66	9.77 **	1.50	39.49 ***	7.03 **	1.63
wine,	P X f	2	0.70	0.45		1.34	0.68	9.67 **	0.34	0.26	0.94	0.43	2.89	0.79	0.01
six	P X S	4	1.30	1.01		0.77	2.12	9.36 ***	2.28	2.09	1.10	2.54	12.81 ***	1.19	1.49
months	f X S	2	0.07	1.32		0.35	0.95	11.26 ***	0.55	0.23	0.41	0.39	0.03	0.39	0.09
	P X f X S	4	0.12	0.11		0.50	0.25	5.06 **	0.15	0.13	0.11	0.08	0.62	0.11	0.06
	MSE	15	0.00	0.01		1.01	0.36	15.43	0.29	31.50	29.91	7.18	1.73	36.02	249.23

Table 11b-Blackberry, F values (continued)

	SOURCE	DF	%PEAK 1	%PEAK 2	%PEAK 3	%PEAK 4	%PEAK 5	%PEAK 6	%PEAK 7
	PROCESS(P)	2	62.70 ***	101.33 ***	71.79 ***	35.70 ***	55.37 ***	69.26 ***	
Juice	JUICE (J)	2	56.22 ***	26.41 ***	7.17 *	22.52 ***	16.50 ***	2.40	
	P X J	4	17.67 ***	23.34 ***	19.20 ***	12.35 **	13.44 ***	18.11 ***	
	MSE(ERROR)	9	0.06	7.42	1.28	0.38	0.67	0.34	
	PROCESS(P)	1	1.47	37.69 ***	41.69 ***	1.85	7.18 *	3.89	
Juice/	FERMENT(F)	1	11.25 **	1387 ***	521.92 ***	2.19	180.31 ***	701.53 ***	
ferm.	FINED (f)	1	0.55	1.39	0.52	1.87	0.27	0.00	
juice.	P X F	1	0.09	8.21 *	29.01 ***	1.08	0.04	1.10	
enz./	P X f	1	0.14	0.50	0.09	1.51	0.36	0.12	
HTST-	F X f	1	0.30	2.24	0.68	0.01	1.00	0.35	
enz.	P X F X f	1	0.12	0.16	0.60	0.36	1.17	0.02	
	MSE	8	4.27	6.99	2.56	3.53	1.20	1.19	
	PROCESS(P)	2	0.50	11.23 **	21.92 **	4.33	1.41	10.21 *	
Young	FINED (f)	1	0.42	1.33	0.49	0.38	0.62	0.00	
wine	P X f	2	0.20	0.45	0.13	0.93	0.68	0.15	
	MSE	6	5.63	13.10	4.42	4.45	1.57	2.27	
	PROCESS(P)	2	8.02 **	9.64 **	16.50 ***	1.69	2.47	3.91 *	3.13
	FINED (f)	1	2.38	0.01	2.97	0.53	0.15	0.02	0.13
Stored	STORED (S)	2	3.46	4.14 *	53.66 ***	1.69	20.10 ***	11.16 **	10.68
wine,	P X f	2	1.21	1.32	1.66	0.85	0.05	0.96	0.54
six	P X S	4	0.23	2.75	6.42 **	1.75	1.26	0.72	1.53
months	f X S	2	1.76	0.16	0.46	0.13	1.24	0.22	0.64
	P X f X S	4	0.35	1.09	1.38	1.13	0.98	1.95	0.69
	MSE	15	0.63	22.12	7.76	3.62	10.79	15.94	36.44

* significant difference between the sources at alpha=0.05
** significant difference between the sources at alpha=0.01
*** significant difference between the sources at alpha=0.001

Table 12a-Blackberry, significant influences over time[a]

PARAMETER / SOURCE	°BRIX	pH	TA	ACN	C.D.	%PC	BI	H L	H a	H b	HUE	SI	%HAZE
PULP (JUICE)	(↓)[g]					↑[e]		↑	↑	↑	-	↑	
PULP (JUI/WI[b])													
PULP (WINE)			↑	↓*f					↑	↑			
PULP (STORAGE)				↓					↑	↓			↑
HEAT (JUICE)				↑	↑		↑	-*f	-	-		- ↑*f	
HEAT (JUI/WI[b])		↑	↑	-	↑(1)	(↑)	(↑)	-	-(1) ↑*E*F	-	↑ -(1)	- ↑*F	↓
HEAT (WINE)				-	-		↑	-	↑	-	↑	↑*F	
HEAT (STORAGE)				-	-	-					(↑)		
ENZ. (JUICE)	↑		↓	↑	↑		↑	↑*f	↑*f	↑(*f)	↑		
ENZ. (JUI/WI[b])				↓	↓*F			↑	↑(*H*F) ↓*F	↑ ↓*f*F	↓*F or*f	↑ ↓*F	↓*f
ENZ. (WINE)				↓*f	↓		↓	↑			↓	↓*F	
ENZ. (STORAGE)		↑	↓	↓	↓	↑*f*20°C	↓	↑		↑	(↓)		
PRESS (JUICE)	↑*E		↑									(↑)	
FINE (JUICE)					↓*E		↓	↑*E	↑*E	↑(*E)		↑*H	
FINE (JUI/WI[b])				↓	↓(1)		↓	↑	↑(1) ↓*F	↑ ↓*F*E	↓*E	↑(1) ↓*F(4)	↓*E
FINE (WINE)				↓	↓			↑					
FINE (STORAGE)					↓	↑*E*20°C	↓	↑					
FERM (JUICE)	(↓)					↑(2)		↑(2)	↑	↑(2)			
FERM (JUI/WI[b])	↓	↑	↑	↓	↓*E	↑	↓	↑	↑(*E*H) ↓*E	↑ ↓*f(*E)	↓(*E)	↑(*H) ↓*E	
FERM (WINE)									↑(2)	↑(2)			
2°C (STORAGE)						↑							
20°C (STORAGE)						↑(*E*f)	↑		↑		↓(3,5)	↓	

[a]At alpha ≥ 0.05; [b]Except pulp treatment; [c]Stabilizing effect on other peaks; [d]Preservation; [e]Increase; [g]Decrease; [o]Brix soluble solids; TA titratable acidity; H L,a,b Hunter L, a and b; SI saturation index; ACN total anthocyanin; C.D. color density; %P.C. % polymeric color; BI browning index; %P1-P8 HPLC area % of peaks 1-8 (Cy-3-Sop, Cy-3-GlRu, Cy-3-Gl, Pel-3-Sop, Cy-3-Ru, Pel-3-GlRu, Pel-3-Gl?, Pel-3-Ru?); (1) juice; (2), (3) pulp, HTST/enzyme treatment; (4) wine; (5) enzyme treatment; T, t storage temperature, time; f fining; E enzyme; H HTST; F fermentation; (r), (a) relative, absolute change of area % compared to other peaks.

Table 12b-Blackberry, significant influences over time[a] (continued)

PARAMETER / SOURCE	%P1	%P2	%P3	%P4	%P5	%P6	%P7
PULP (JUICE)	↑(a+r)	↓(a)	↑(r)	↑(r)	↑(r)	↑(r)	
PULP (JUI/WI[b])							
PULP (WINE)		↓(a)				↓(a)	
PULP (STORAGE)							
HEAT (JUICE)	-		↓*f(r)	↑(a)		↓*P(r)	
HEAT (JUI/WI[b])		-			↓(r)		
HEAT (WINE)		-	↓(r)				
HEAT (STORAGE)	↑*E(a)		↓(r)				
ENZ. (JUICE)	↑						
ENZ. (JUI/WI[b])		↓*F(a)					
ENZ. (WINE)		↓(a)					
ENZ. (STORAGE)	↑*H(a)	↓(a)					
PRESS (JUICE)		↓(2,a)				↓*H(r)	
FINE (JUICE)			↓*H(r)	↓(a)			
FINE (JUI/WI[b])							
FINE (WINE)							
FINE (STORAGE)							
FERM (JUICE)	↑(2a,r)	↓(2,a)	↑(2,r)	↑(2,r)	↑(2,r)	↑(2,r)	
FERM (JUI/WI[b])	↑(r)	↓(a)			↑(r)	↑(r)	
FERM (WINE)							
2°C (STORAGE)			↓*E(a,r)			↑(a)	
20°C (STORAGE)		↓(a)	↓(a)		↓(r)	↑(a)	↑(a)

See previous page for legend.

Table 13-Spectral analyses of blackberry juice and wine at several stages of processing and storage[a]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	COMPOSITION							
PULP CONTROL	ACN[f]	26.2	18.3	8.2[b]	8.4	6.4	5.0	0.0
	C.D.[f]	7.8	7.8	6.1[b]	5.2	5.6	5.6	6.6
	% P.C.[f]	25.7	33.1	45.7[b]	45.2	42.4	60.9	67.2
	BI	4.0	3.3	2.9[b]	2.6	2.6	3.1	4.3
PULP FINED	ACN			8.4[b]	6.8	5.9	4.0	2.8
	C.D.			6.1[b]	4.2	4.6	4.6	5.7
	% P.C.			44.7[b]	44.6	42.3	60.1	71.8
	BI			3.0[b]	2.1	2.3	2.5	4.0
ENZYME CONTROL	ACN	29.9	39.6	7.0	6.9	5.1	3.9	2.9
	C.D.	7.7	12.4	4.2	3.3	3.6	4.4	4.1
	% P.C.	22.0	18.6	43.4	46.1	42.8	57.6	69.9
	BI	3.7	4.5	2.1	1.7	1.9	2.4	2.6
ENZYME FINED	ACN		32.5	6.1	5.0	5.2	3.5	2.1
	C.D.		9.8	3.7	2.5	3.0	3.4	2.2
	% P.C.		19.9	43.8	40.3	48.2	56.8	100.0
	BI		3.6	1.9	1.3	1.6	1.8	1.4
HTST/ENZYME CONTROL	ACN	34.0	63.8	11.7	14.6	12.6	9.4	7.1
	C.D.	9.8	19.8	8.0	6.1	7.8	7.9	8.7
	% P.C.	20.0	16.8	40.3	38.2	41.8	53.1	59.5
	BI	4.6	6.8	3.7	2.8	3.7	4.0	4.8
HTST/ENZYME FINED	ACN		54.9	9.7	10.5	9.8	8.1	6.0
	C.D.		15.5	6.5	5.4	6.4	6.2	6.6
	% P.C.		16.9	43.6	38.3	38.3	49.4	59.9
	BI		5.4	3.1	2.6	3.1	3.1	3.7

[a]All values are reported as means of duplicate analyses and replicates of processing trials; [b]Separate lots, no fining; [c]Crushed berries; [f]Methods by Wrolstad (1974): ACN total monomeric anthocyanin (mg/100 mL), C.D. color density ($A_{420nm} + A_{520nm}$), % P.C. % polymeric color = P.C./C.D.. BI browning index (A_{420nm}); PRESS JUICE including pulp fermented juice, 50 ppm SO_2 added; FERMENTATION after amelioration with sugar during fermentation; YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2°C, dark) for 2 months, 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark.

Table 14-Hunter analyses of blackberry juice and wine at several stages of processing and storage[a]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	COMPOSITION							
PULP	HUNTER L	12.7	26.0	33.6[b]	35.9	33.4	26.8	24.2
CONTROL	HUNTER a	27.5	52.7	52.3[b]	51.5	50.9	40.7	34.3
	HUNTER b	8.3	16.5	19.6[b]	19.4	18.8	14.6	14.2
	HUE	73.3	72.6	69.5[b]	69.3	69.7	70.5	67.6
	SI	28.7	55.2	55.8[b]	55.1	54.2	43.2	37.1
	%HAZE	3.2	7.9	4.0[b]	3.5	13.7	26.4	41.5
PULP	HUNTER L			33.0[b]	41.4	40.2	34.8	24.3
FINED	HUNTER a			52.5[b]	49.9	48.0	45.4	31.1
	HUNTER b			19.3[b]	20.2	19.7	16.8	12.7
	HUE			69.8[b]	68.0	67.6	69.7	68.7
	SI			56.0[b]	53.6	51.9	48.4	33.6
	%HAZE			4.1[b]	2.2	8.5	14.2	42.6
ENZYME	HUNTER L	14.2	17.2	42.7	47.9	42.7	38.3	44.6
CONTROL	HUNTER a	31.6	46.1	45.1	45.9	45.3	43.5	36.9
	HUNTER b	9.5	12.0	19.9	19.5	19.8	18.0	22.8
	HUE	73.2	75.4	66.2	67.0	66.4	67.6	58.3
	SI	33.0	47.7	49.3	49.9	49.4	47.1	43.4
	%HAZE	4.9	8.0	2.2	1.4	12.2	12.0	8.6
ENZYME	HUNTER L		21.9	47.3	54.2	50.7	47.3	52.2
FINED	HUNTER a		50.6	44.7	39.5	40.5	40.5	30.1
	HUNTER b		15.1	19.7	18.8	19.5	18.6	21.6
	HUE		73.4	66.2	64.5	64.3	65.3	54.4
	SI		52.5	48.9	43.8	45.0	44.6	37.1
	%HAZE		2.5	2.2	2.0	4.6	3.3	9.9
HTST/ENZYME	HUNTER L	10.6	12.8	26.2	30.2	24.2	23.9	24.0
CONTROL	HUNTER a	25.3	35.2	52.8	55.7	50.8	47.4	45.9
	HUNTER b	7.3	8.9	16.8	18.2	15.9	15.4	17.8
	HUE	74.0	75.9	72.5	71.1	72.6	72.0	68.8
	SI	26.3	36.3	56.5	58.7	53.2	49.9	49.2
	%HAZE	6.6	2.6	4.1	3.6	7.6	12.0	13.0
HTST/ENZYME	HUNTER L		15.9	30.1	33.0	30.8	30.3	30.6
FINED	HUNTER a		41.1	54.5	54.7	53.3	50.0	47.2
	HUNTER b		10.9	18.0	18.8	18.4	17.6	18.6
	HUE		75.2	71.4	71.1	71.0	70.7	68.5
	SI		42.5	56.4	57.9	56.4	53.1	50.8
	%HAZE		2.9	3.6	3.0	8.3	4.7	5.6

[a]All values are means of duplicate analyses and replicates of processing trials; [b]Separate lots, no fining; [c]Crushed berries. PRESS JUICE including pulp fermented juice, 50 ppm SO_2 added; FERMENTATION after amelioration with sugar during fermentation; YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2°C, dark) for 2 months, 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark; HUNTER L lightness, a redness, b yellowness; HUE = $\tan^{-1}$ X Hunter a/Hunter b; SI saturation index.

Table 15-Anthocyanin composition (%) of blackberry juice and wine at several stages of processing and storage[a,h]

STAGE		BERRIES[c]	PRESS JUICE	FERMENTION	YOUNG WINE	BOTTLING	STOR.,2°C	STOR.,20°C
TREATMENT	ANTHOCYANIN							
PULP	PEAK (1)	.00	2.53	3.55[b]	3.62	.78	.55	.00
CONTROL	CY-3-GL (P2)	72.6	44.2	22.2[b]	21.5	14.6	28.6	11.8
	CY-3-RU (P3)	6.89	15.9	14.1[b]	26.2	20.5	25.1	7.60
	PEAK (4)	6.39	11.1	.00[b]	8.72	8.30	5.49	6.05
	PEAK (5)	6.28	12.8	27.8[b]	14.4	10.4	11.9	4.44
	PEAK (6)	6.70	10.8	20.7[b]	15.8	20.1	19.3	26.0
	PEAK (7)	1.04	1.97	2.26[b]	3.25	11.6	3.98	20.0
PULP	PEAK (1)			2.90[b]	3.63	1.22	.28	.00
FINED	CY-3-GL (P2)			19.1[b]	21.3	25.3	25.3	20.0
	CY-3-RU (P3)			21.2[b]	26.3	31.0	24.3	13.8
	PEAK (4)			.00[b]	9.23	6.82	4.70	3.55
	PEAK (5)			24.8[b]	14.4	10.5	14.3	4.39
	PEAK (6)			21.2[b]	16.4	14.7	21.7	20.2
	PEAK (7)			4.07[b]	3.34	12.4	3.94	22.8
ENZYME	PEAK (1)	.00	1.02	5.89	3.88	1.65	.98	1.29
CONTROL	CY-3-GL (P2)	72.9	70.1	21.6	18.5	18.6	10.1	17.6
	CY-3-RU (P3)	6.56	5.74	21.5	28.3	24.5	13.1	12.7
	PEAK (4)	7.33	7.84	.00	6.14	5.20	3.15	4.09
	PEAK (5)	6.26	7.55	23.0	13.7	14.5	18.4	1.24
	PEAK (6)	6.49	5.84	19.6	21.1	20.3	27.5	19.7
	PEAK (7)	.00	.92	3.77	4.82	9.35	14.7	30.6
ENZYME	PEAK (1)		1.25	5.67	5.95	1.14	.00	.00
FINED	CY-3-GL (P2)		70.9	19.2	16.4	19.9	11.4	12.8
	CY-3-RU (P3)		6.51	22.6	29.2	25.8	13.1	12.3
	PEAK (4)		6.06	.00	3.03	4.09	4.24	5.11
	PEAK (5)		7.02	26.3	15.4	10.7	17.7	8.38
	PEAK (6)		5.88	24.6	20.6	20.6	23.8	31.5
	PEAK (7)		.84	1.66	3.86	10.6	18.8	17.4
HTST/ENZYME	PEAK (1)	.10	.11	2.88	3.05	1.73	2.11	2.56
CONTROL	CY-3-GL (P2)	73.7	74.8	33.6	31.9	25.5	23.7	26.8
	CY-3-RU (P3)	7.44	5.74	17.0	18.4	18.3	13.1	12.6
	PEAK (4)	5.67	7.55	6.15	6.67	2.53	5.89	6.51
	PEAK (5)	6.18	5.71	15.5	13.2	15.8	13.1	13.8
	PEAK (6)	6.96	5.08	21.5	19.3	18.2	27.4	30.2
	PEAK (7)	.00	.54	3.41	3.09	6.17	8.75	7.31
HTST/ENZYME	PEAK (1)		.29	2.35	3.65	2.56	1.45	.28
FINED	CY-3-GL (P2)		74.8	29.0	26.9	27.1	23.9	18.5
	CY-3-RU (P3)		4.80	19.6	20.1	18.9	16.2	9.09
	PEAK (4)		6.96	7.64	7.01	5.99	2.71	7.62
	PEAK (5)		5.71	13.9	13.1	11.5	16.6	8.73
	PEAK (6)		5.64	22.8	19.1	17.3	25.4	27.2
	PEAK (7)		.57	4.68	5.88	8.80	9.32	16.1

[a]All values reported are means of duplicate analyses and replicates of processing trials; [b]separate lots, no fining; [c]Crushed berries; [h]Integrator excluded minor peaks and peak shoulders where the summation of the peak areas does not add up to 100%. PRESS JUICE including pulp fermented juice, 50 ppm SO_2 added; FERMENTATION after amelioration with sugar during fermentation; YOUNG WINE after fermentation to dryness; BOTTLING after cold storage (2°C, dark) for 2 months, 25 ppm SO_2, 180 ppm K-sorbate and 3% sugar added, filtered; STOR. storage for 6 months in the dark; P1-P7 peaks 1-7: (1) polymers?, (P2) cyanidin-3-glucoside, (P3) cyanidin-3-rutinoside, (4) xylose cyanidin derivative, (5) and (6) dicaffeoylic acid acylated derivatives of CY-3-RU, (7) ?.

Table 16-Blackberry, multiple correlation analyses on compositional, spectral and Hunter parameters[a]

	CORRELATION PARAMETERS	JUICE	JUI/FERM JUI ENZ, HTST/ENZ	YOUNG WINE	STORED WINE SIX MONTHS
	PH -T.A.	***	***	***	-0.844
	BRIX -HU L	***	-0.889	***	***
	BRIX -HU b	***	-0.876	***	***
	BRIX -HUE	***	0.947	***	***
	BRIX -ACN	***	0.954	***	***
	BRIX -C.D.	***	0.966	***	***
**	BRIX -%P.C.	-0.881	-0.909	***	***
	BRIX -BI	***	0.956	***	***
	BRIX -%PEAK1	-0.876	***	***	***
	BRIX -%PEAK2	0.961	***	***	***
**	BRIX -%PEAK3	-0.876	0.950	***	***
	BRIX -%PEAK4	-0.880	***	***	***
	BRIX -%PEAK5	-0.936	***	***	***
	BRIX -%PEAK6	-0.940	***	***	***
**	HU L -HU a	0.927	***	-0.974	***
***	HU L -HU b	0.989	0.996	***	0.857
**	HU L -HUE	***	0.974	-0.974	***
**	HU L -SI	0.936	***	-0.970	***
**	HU L -ACN	***	-0.895	-0.860	***
**	HU L -C.D.	***	-0.899	-0.987	***
	HU L -%P.C.	***	***	-0.940	***
***	HU L -BI	***	-0.907	-0.985	-0.902
	HU L -%PEAK1	0.906	***	***	***
	HU L -%PEAK2	-0.856	***	***	***
**	HU L -%PEAK3	***	-0.921	0.862	***
	HU L -%PEAK5	0.871	***	***	***
	HU a -HU b	0.960	***	***	***
	HU a -HUE	***	***	0.978	***
***	HU a -SI	0.999	***	0.999	0.983
	HU a -C.D.	***	***	0.945	***
	HU a -%P.C.	***	***	0.912	***
	HU a -BI	***	***	0.939	***
	HU b -HUE	***	***	0.939	***
	HU b -SI	0.966	***	***	***
	HU b -ACN	***	-0.871	***	***
	HU b -C.D.	***	-0.885	***	***

	CORRELATION PARAMETERS	JUICE	JUI/FERM JUI ENZ, HTST/ENZ	YOUNG WINE	STORED WINE SIX MONTHS
	HU b -BI	***	-0.896	***	***
	HU b -%PEAK1	0.908	***	***	***
	HU b -%PEAK3	***	-0.909	***	***
	HUE -SI	***	***	0.972	***
***	HUE -ACN	0.854	0.953	0.898	***
***	HUE -C.D.	0.872	0.966	0.965	***
	HUE -%P.C.	***	-0.857	***	***
***	HUE -BI	0.861	0.969	0.944	***
**	HUE -%PEAK3	***	0.950	-0.895	***
	SI -%HAZE	***	***	***	-0.871
	SI -C.D.	***	***	0.937	***
	SI -%P.C.	***	***	0.915	***
	SI -BI	***	***	0.933	***
	SI -%PEAK1	0.849	***	***	***
***	ACN -C.D.	0.933	0.959	0.878	***
	ACN -%P.C.	***	-0.896	***	***
**	ACN -BI	0.908	0.943	***	***
	ACN -%PEAK3	***	0.911	***	***
***	C.D. -%P.C.	***	-0.903	0.926	0.887
***	C.D. -BI	0.957	0.995	***	0.893
	C.D. -%PEAK3	***	0.942	***	***
**	%P.C.-BI	***	-0.881	***	0.959
	%P.C.-%PEAK3	***	-0.846	***	***
	%P.C.-%PEAK6	0.932	***	***	***
	BI -%PEAK3	***	0.942	***	***
	%PEAK1-%PEAK2	-0.899	***	***	***
	%PEAK1-%PEAK5	0.896	***	***	***
	%PEAK2-%PEAK3	-0.977	***	***	***
	%PEAK2-%PEAK4	-0.916	***	***	***
	%PEAK2-%PEAK5	-0.992	***	***	***
	%PEAK2-%PEAK6	-0.923	***	***	***
	%PEAK3-%PEAK4	0.865	***	***	***
	%PEAK3-%PEAK5	0.962	***	***	***
	%PEAK3-%PEAK6	0.927	***	***	***
	%PEAK4-%PEAK5	0.918	***	***	***
	%PEAK5-%PEAK6	0.895	***	***	***

[a]High correlation was assumed for correlation coefficients > 0.85 (alpha=0.05); ***, ** high correlation at three and two stages of processing; BRIX soluble solids content; HU L,a,b Hunter L (lightness), a (redness), b (yellowness); SI saturation index; ACN total anthocyanin; C.D. color density; %P.C. % polymeric color; BI browning; %PEAK1-6 HPLC area % of peaks 1-6

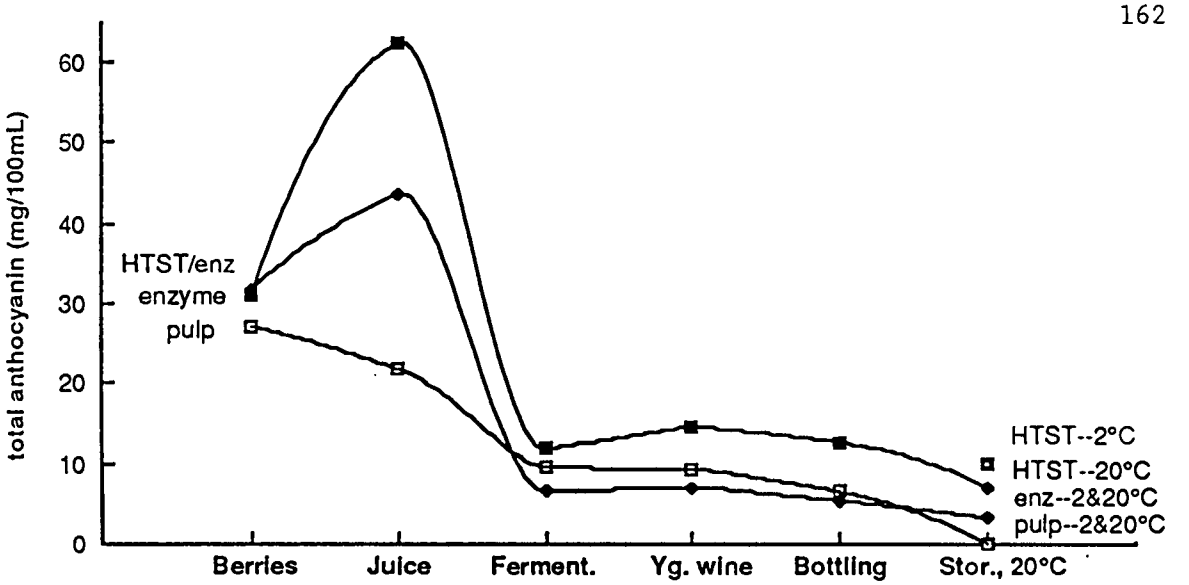


Fig. 10-Changes in total monomeric anthocyanin pigment during processing and storage of blackberry fruit, juice and wine (replicate 1).

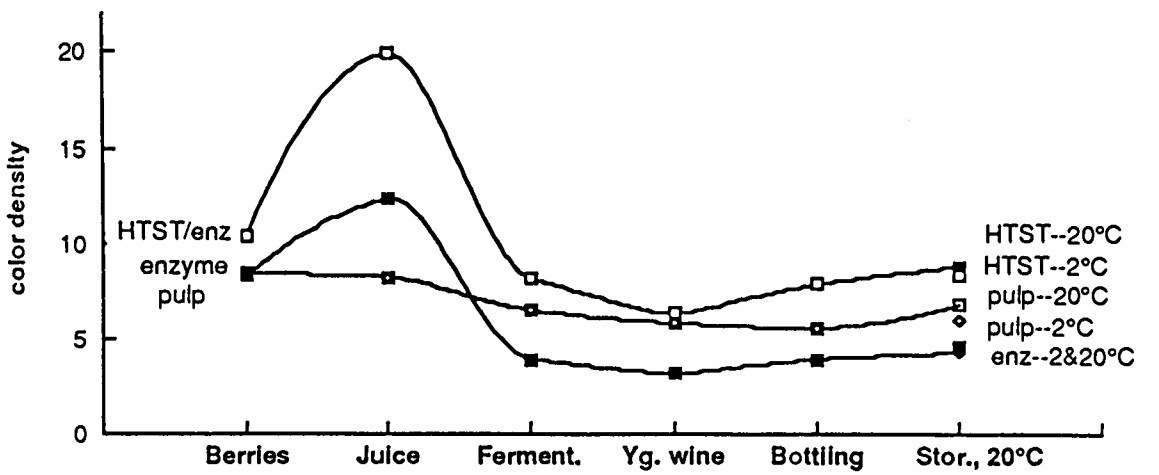


Fig. 11-Changes in color density during processing and storage of blackberry fruit, juice and wine (replicate 1).

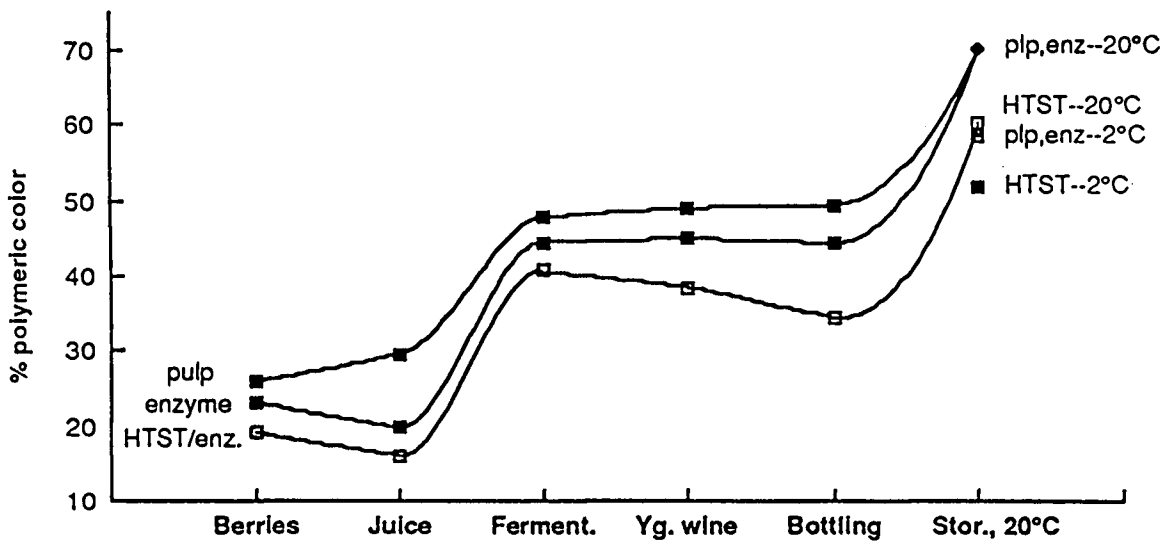


Fig. 12-Changes in % polymeric color during processing and storage of blackberry fruit, juice and wine (replicate 2).

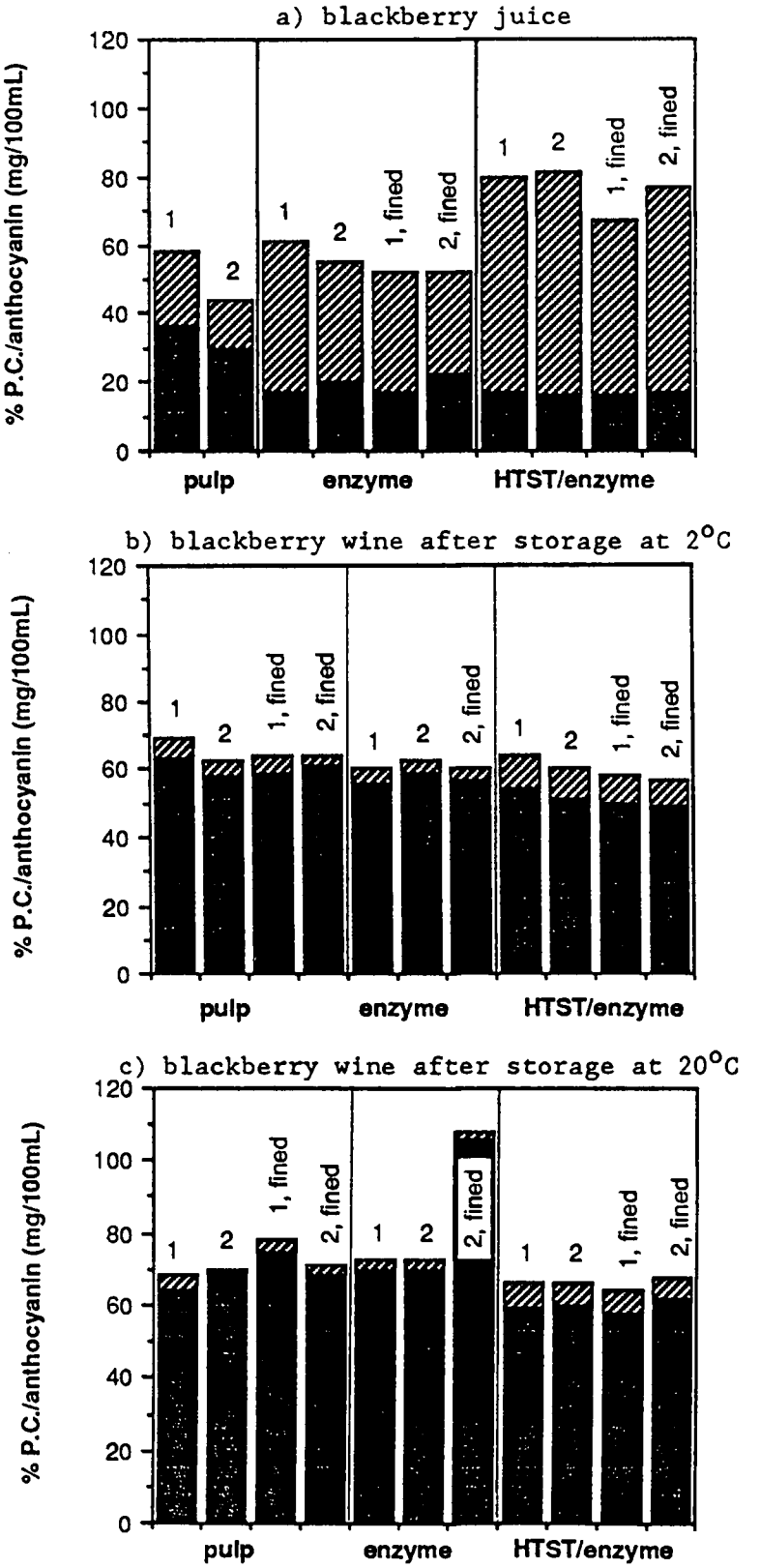


Fig. 13-Anthocyanin to % polymeric color ratios of blackberry juice and wine after storage (in processing trial replicates, with and without fining).

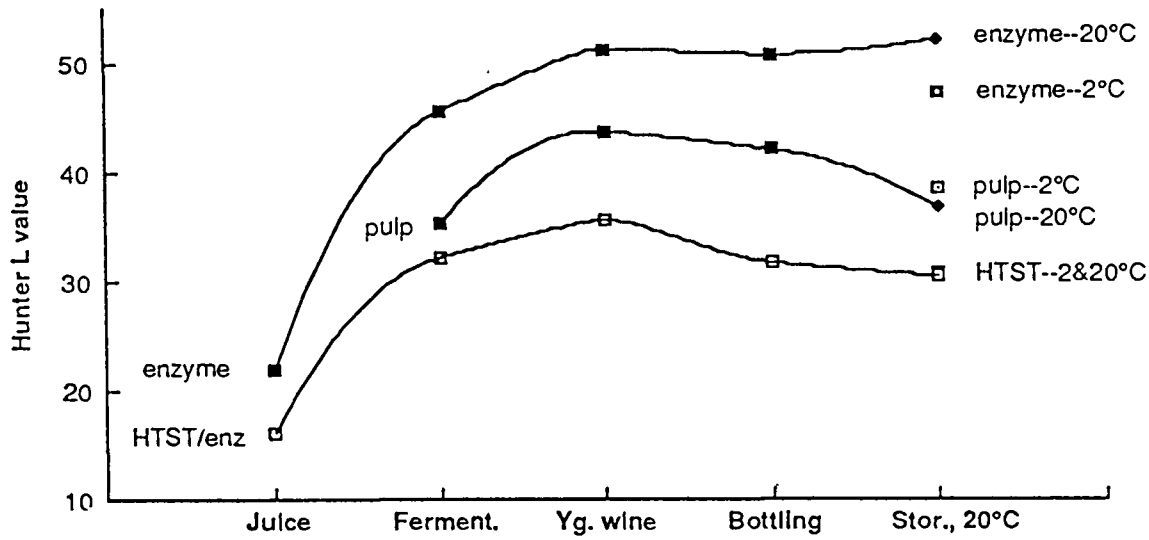


Fig. 14-Changes in Hunter "L" values during processing and storage of fined blackberry juice and wine (replicate 2).

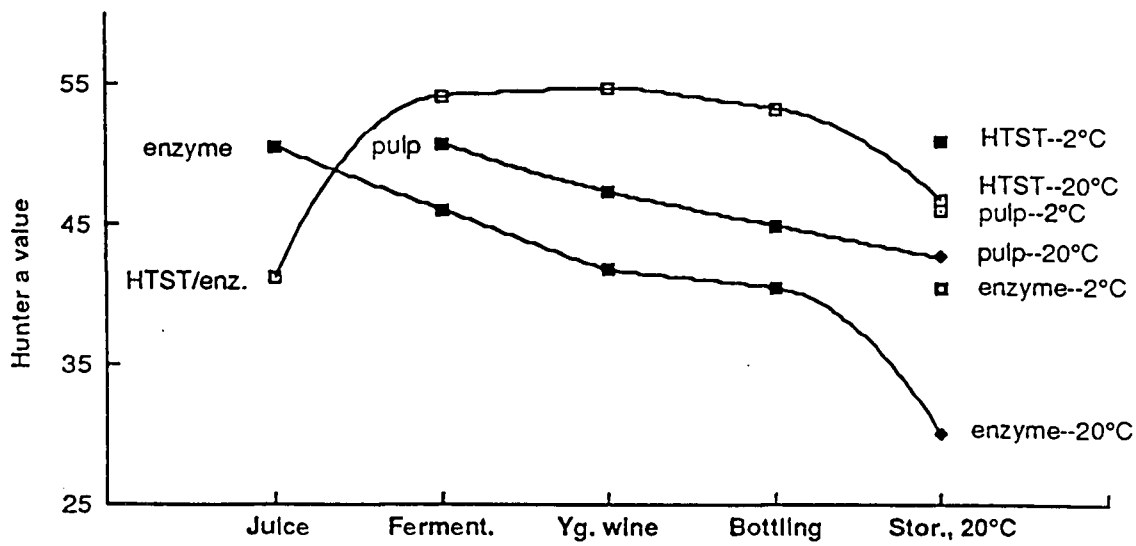


Fig. 15-Changes in Hunter "a" values during processing and storage of fined blackberry juice and wine (replicate 2).

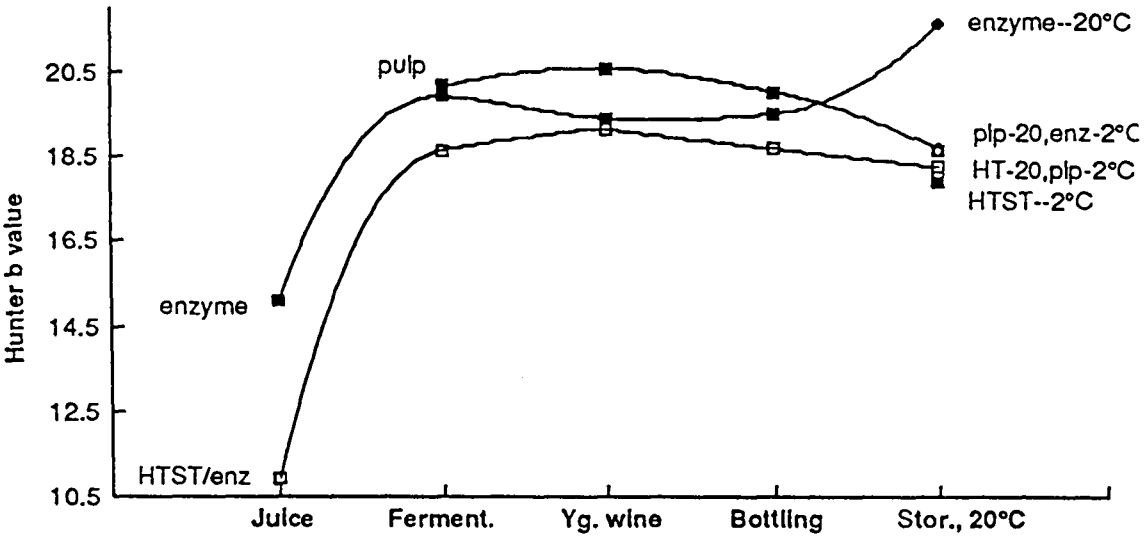


Fig. 16-Changes in Hunter "b" values during processing and storage of fined blackberry juice and wine (replicate 2).

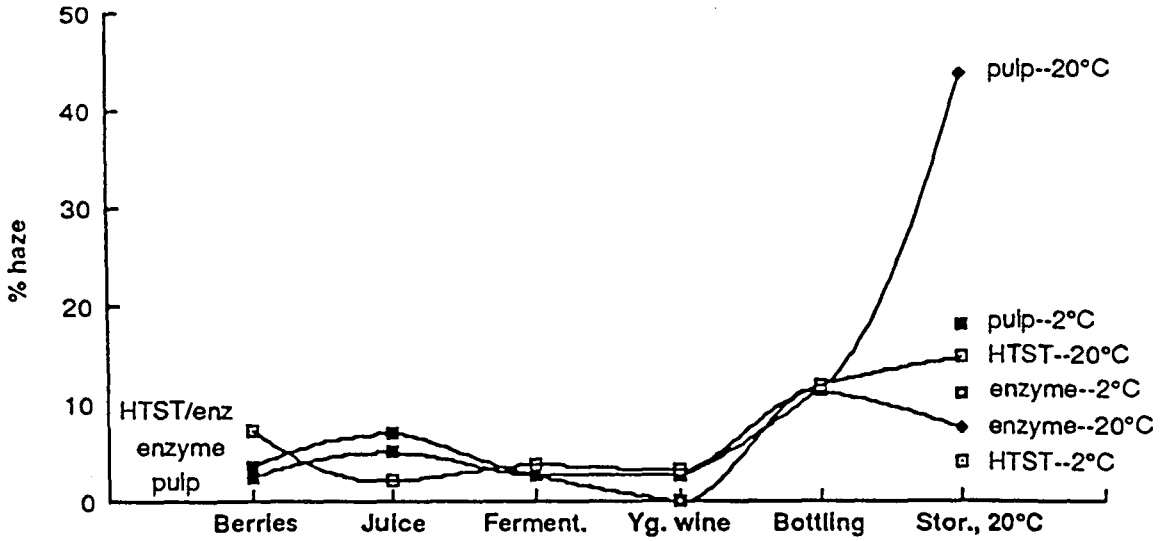


Fig. 17-Changes in % haze during processing and storage of blackberry fruit, juice and wine (replicate 2).

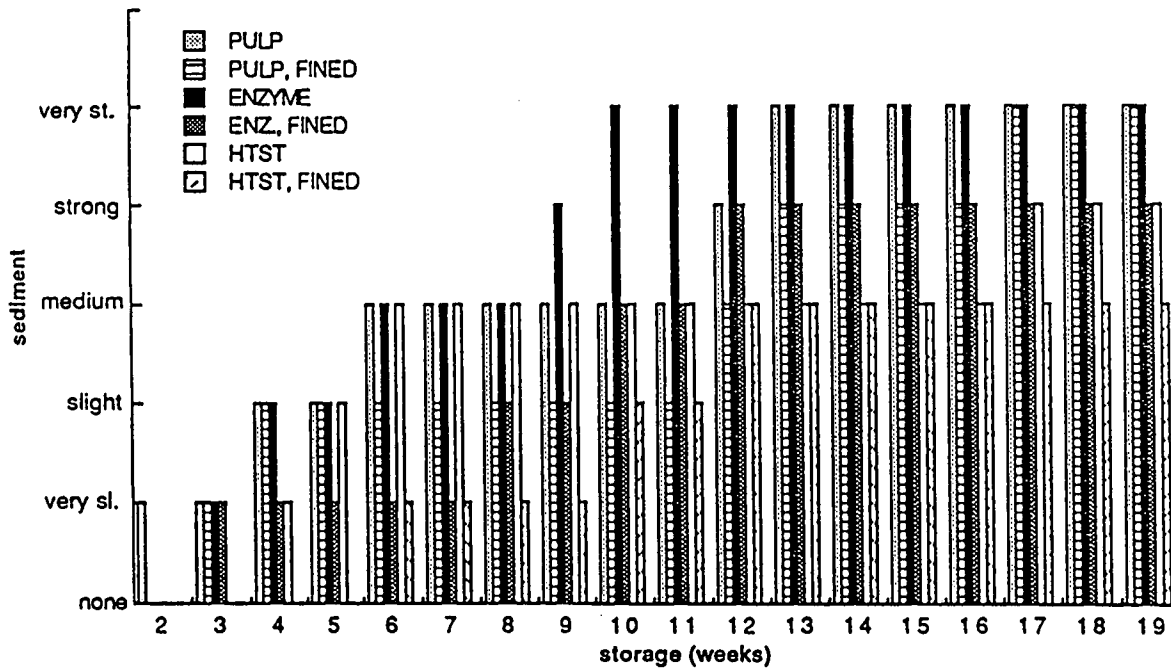


Fig. 18-Sediment formation of blackberry wine during storage at 20°C (replicate 2).

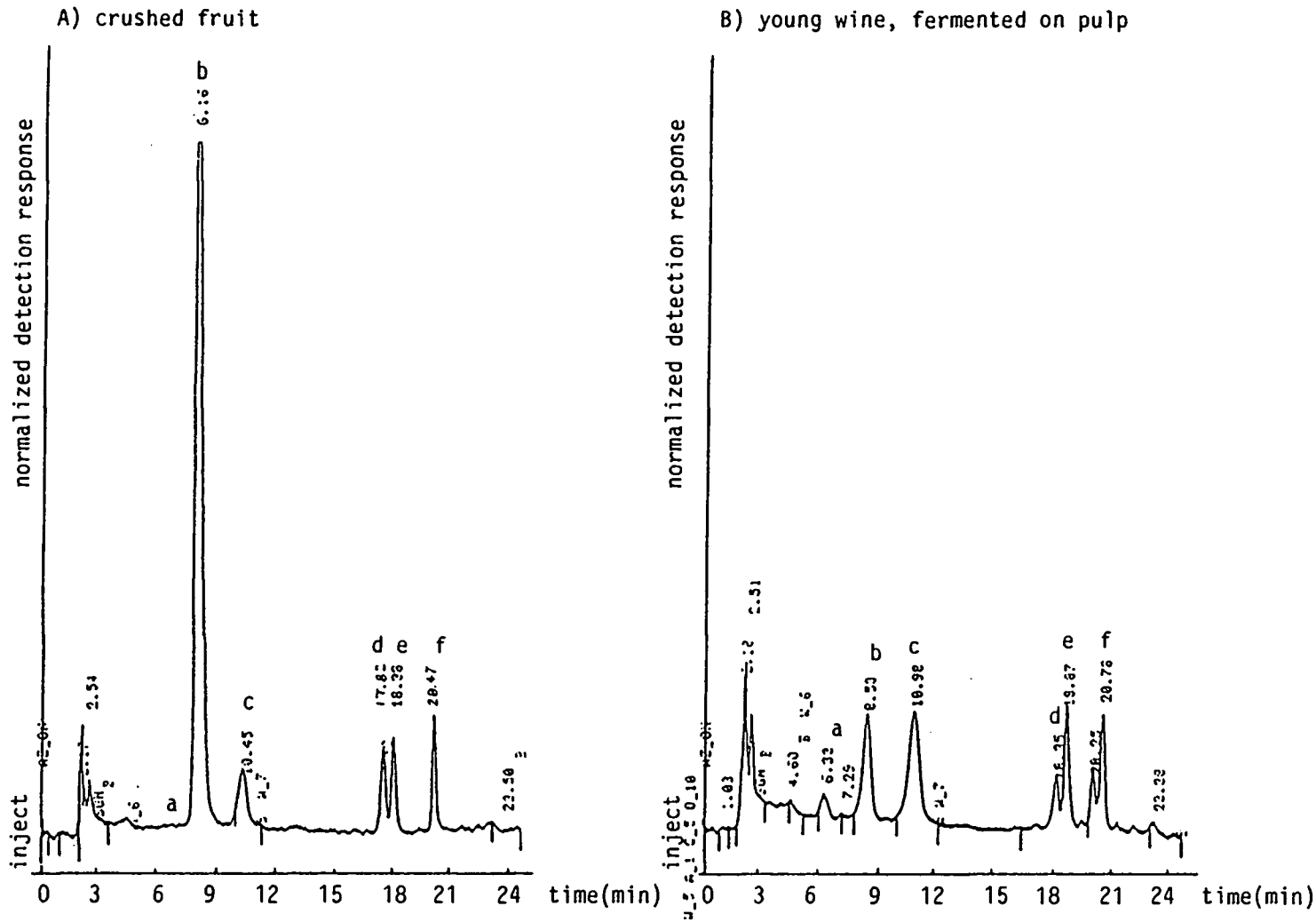
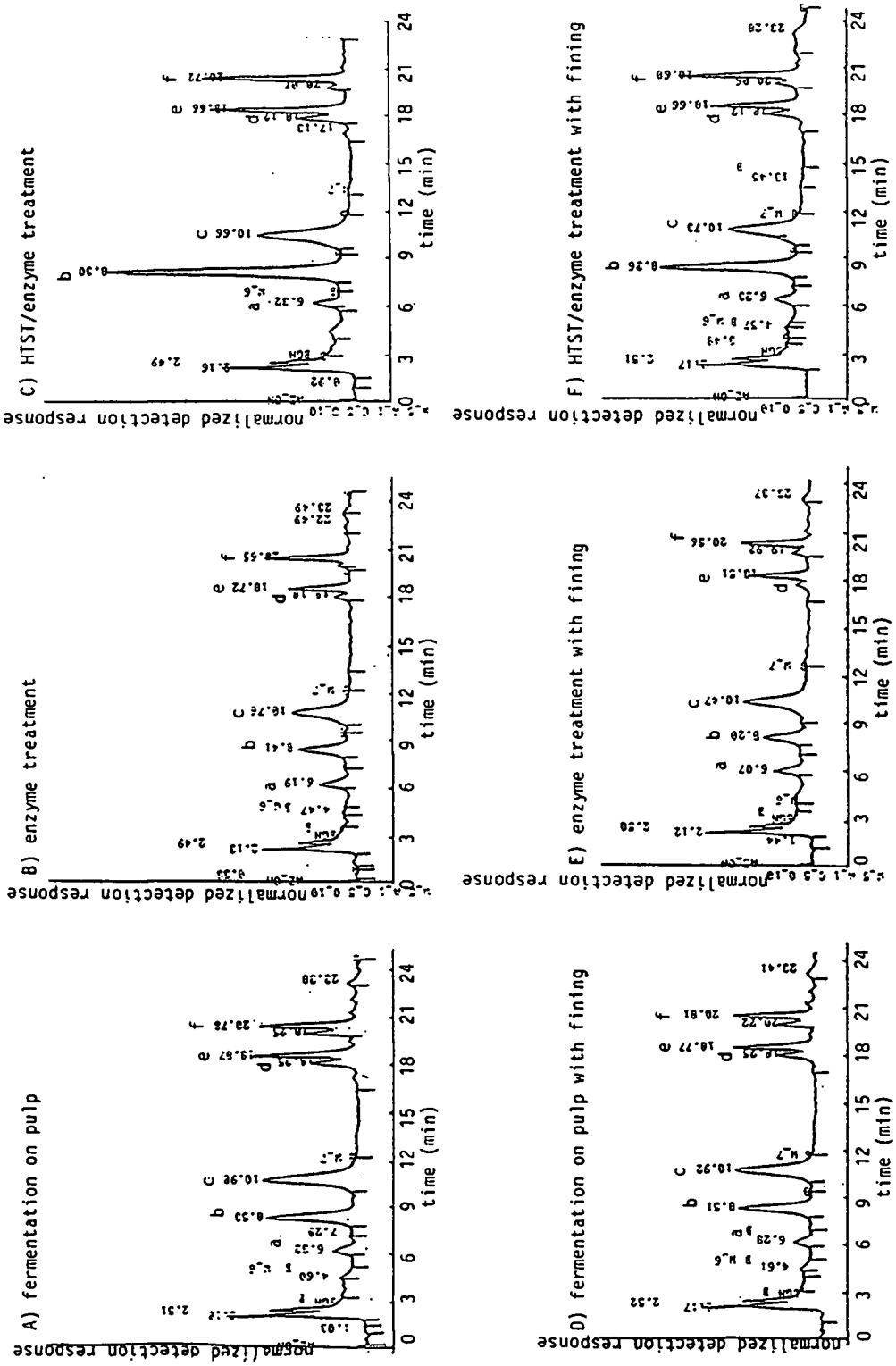


Fig. 19-HPLC chromatograms of the anthocyanins in blackberry fruit (A) and young wine (B) (replicate 1). Relative peak areas calculated with detection at 520nm in parentheses for (A) and (B), respectively: peak identification, a. polymers? (0, 3%); b. cyanidin-3-glucoside (72, 19%); c. cyanidin-3-rutinoside (7, 25%); d. xylose cyanidin derivative? (6, 9%); e. and f. acid acylated derivatives? (6, 15%) and (6, 17%), respectively.

Fig. 20-Treatment influences on the HPLC chromatograms of the anthocyanins; blackberry young wines (replicates 1). Peak identification: a. polymers?; b. cyanidin-3-glucoside; c. cyanidin-3-rutinoside; d. xylose-cyanidin derivative?; e. and f. acid acylated derivatives? (detection at 520nm).



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