This is to certify that the

thesis entitled

MATERIAL OTHER THAN GRAPE (MOG)

IN JUICE AND WINE

presented by

PAO-JUI DAVID HUANG

has been accepted towards fulfillment
of the requirements for

M. S. degree in FOOD SCIENCE

Jerry N. Cash
Major professor

Date June 4, 1979

MATERIAL OTHER THAN GRAPE (MOG)

IN JUICE AND WINE

By

Pao-jui David Huang

A Thesis

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1979

ABSTRACT

MATERIAL OTHER THAN GRAPE (MOG)
IN JUICE AND WINE

By

Pao-jui David Huang

MOG has historically caused operational problems at the processing plant and it seems very likely that it will also create juice and wine quality problems.

Two grape cultivars, Concord grapes and Aurora Blanc were used for grape juice and wine production, respectively. Grape samples taken from the mechanical harvesters were analyzed for total weight of MOG and percent MOG. Hand-picked samples were used as controls and also for the additions of varied amounts of MOG during processing in order to determine the effect of MOG on the overall quality of finished products. Chemical and physical analyses of samples which evaluated the quality included: (1) Total phenolic compounds and various phenolic fractions. (2) pH and total acidity. (3) Brix. (4) Color. (5) Alcohol content. (6) Sensory assessment.

This study indicated that MOG content has a definite affect on the chemical constituents and final quality of juice and wine. The total phenolic, flavonoid and tannin contents of wine and juice increase as MOG content in the harvested grapes increases.

to my father and mother

ACKNOWLEDGEMENTS

I am sincerely grateful to Dr. J.N. Cash for his most valuable guidance and never-ending patience, understanding and encouragement throughout the course of this study and the preparation of this manuscript.

Grateful appreciation is also expressed to the other members of my guidance committee, Drs. P.C. Markakis, G.S. Howell, and M.A. Ubersax, for their helpful suggestions.

Special thanks to Paw Paw Grape Juice Company and Warner Vineyard, Inc., and National Grape Cooperative and Welch's Foods for their generous supply of grapes used in the study.

Acknowledgement is extended to the Michigan Viticultural Advisory Committee for their partial financial support of this work.

Finally, I am most grateful to my parents, sisters, and brother for their encouragement during my graduate program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In recent years mechanical harvesting has become a predominant method for harvesting grapes. The effects of mechanical harvesting on juice and wine quality are complex and result from the interaction of many factors; including operating parameters of the harvester, the specific grape variety, the extent of berry damage and the level of incorporation of MOG (Material Other than Grape). MOG has historically caused operational problems at the processing plant and it seems very likely that it will also create juice and wine quality problems.

Two grape cultivars, Concord and Aurora Blanc were used for grape juice and wine production, respectively. Grape samples taken from the mechanical harvesters were analyzed for total weight of MOG and percent MOG. Hand-picked samples were used as controls and also for the additions of varied amounts of MOG ranging from 1 to 5% during processing in order to determine the effect of MOG on the overall quality of finished products. Chemical and physical analyses of samples which evaluated the quality included:

(1) Total phenolic compounds and various phenolic fractions.

(2) pH and total acidity.

(3) Brix.

(4) Color.

(5) Alcohol content.

(6) Sensory assessment.

The purpose of this study was to find the relationship between MOG content and the wine and juice quality.

LITERATURE REVIEW

Phenols and Related Substances Other than Flavonoids

1. Derivatives of Hydroxylated Benzoic Acid

In the late 1800's, chemical preservatives were added to many foods, including wine. Among these preservatives were salicylic acid, p-hydroxybenzoic acid and the esters of these compounds. Ash (1952) states that 90% of the wine made in 1900 in California had salicylic acid added but most such additions are now prohibited in the major wine-producing and wine-importing countries. Pellet (1906) identified small amounts of salicylic acid which occurred naturally in grapes and untreated wine. Traphagen and Burke (1903) reported the equivalent of at least 0.32 mg of salicylic acid per kg of fresh Concord grapes while Chelle (1925) concluded from his work that small amounts of salicylic acid can occur naturally in wine. Ribereau-Gayon (1963) reported finding very small amounts of salicylic acid in some wines but other researchers (Webb 1962; Stevens et al. 1967) indicated that this acid does not occur in many wines. It appears certain from available data that natural salicylic acid can be no more than 1 mg/liter, either free or readily liberated by alkali in grape must or wine.

Guimberteau and Portal (1961) reported 0.2 to 0.5 mg per liter of hydroxybenzoic acid in saponified samples of all the 18 wines and 4 juices they tested. Ribereau-Gayon (1963) indicated vanillic, syringic, gentisic, protocatechuic, and gallic acid were detected in wine after

saponification. It seems possible that some degradation of flavonoids of the alkaline-fusion and cleavage type was occurring during saponification, so that the syringic acid may have come from malvidin residues, vanillic acid could have come from peonidin and gallic acid from delphinidin. Boettinger (1901) reported protocatechuic acid in the water-soluble substance from grape leaves.

2. Derivatives of Hydroxycinnamic Acid

Simple observation shows that grapevines produce grapes which contain anthocyanins and that the vines are lignified. Therefore from our knowledge of the biosynthesis of such compounds it seems very likely that shikimic acid and cinnamates should occur in grapes and indeed, several researchers (Kliewer 1966; Ribereau-Gayon 1963) have identified these compounds in grape juice and wine. Caffeic acid and p-coumaric acid, or their derivatives, as well as, ferulic and sinapic acid have been reported in grapes and wine by several researchers and there can be little doubt of their general presence (Hennig and Burkhardt, 1957, 1958). Masquelier and Ricci (1964) found chlorogenic acid, while Pifferi (1965) indicated that both chlorogenic and isochlorogenic acid occurred in wine.

3. Other Nonflavonoid Phenols

Since tyrosine is a cinnamic acid precursor, it would be expected to occur in grapes and wine and several investigators have shown this to be the case (Castor 1953; Beridze and Sirbiladze, 1964; Nassar and Kliewer 1966). Free tyrosine occurs in grapes at about 20-60 mg/kg fresh weight, but 75-90% of this disappears during fermentation to wine through conversion to tyrosol by yeasts (Ehrlich 1907). Lingens et al. (1967) indicated that yeast does not require an external source of phenylalanine, tyrosine,

or other benzenoid substances, but is capable of synthesizing tyrosol without the presence of these compounds. Yamamoto et al. (1966) reported the yeast fermentation of a prepared medium containing L-tyrosine produced. In addition to tyrosol, p-hydroxybenzoic acid, p-hydroxyphenyllactic acid, p-hydroxyphenylacetic acid, p-hydroxybenzaldehyde acid or p-hydroxyophenylacetaldehyde, and p-hydroxyphenylpyruvic acid.

Chaudhary et al. (1968) reported gas chromatographic detection of a trace of phenol itself in extracts of wines. They reported m-cresol, vanillin, sinapaldehyde, coniferadehyde, and syringaldehyde in wine, but they thought these compounds may have been extracted from wooden cooperage, or converted from lignin which was extracted from wooden cooperage.

Hennig and Burkhardt (1958) used two dimensional paper chromatography to identify ellagic acid and ellagitannins in red and white wines. They also found ellagic acid in large amounts in juice, but noted that it could have been produced by polyphenoloxidase action on natural tannins during the analysis. Ellagic acid was reported (Hennig et al. 1958) in large amounts in young wine fermented in the presence of grape solids, but it was removed by aging and processing.

Flavonoids

The yellow flavonoids comprise a diverse, almost ubiquitous class of pigments, with chemical structures similar to the anthocyanins. Approximately 400 flavonoids are known and more are being identified. These can be divided into the following families:

1. Catechins (Flavan-3-ols) and Flavones

The natural flavan-3-ol series is d-catechin itself.

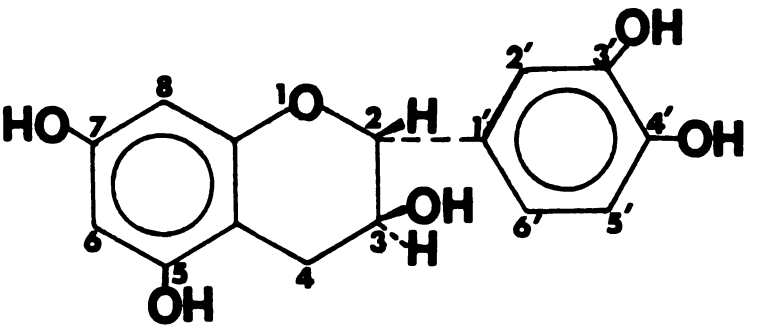
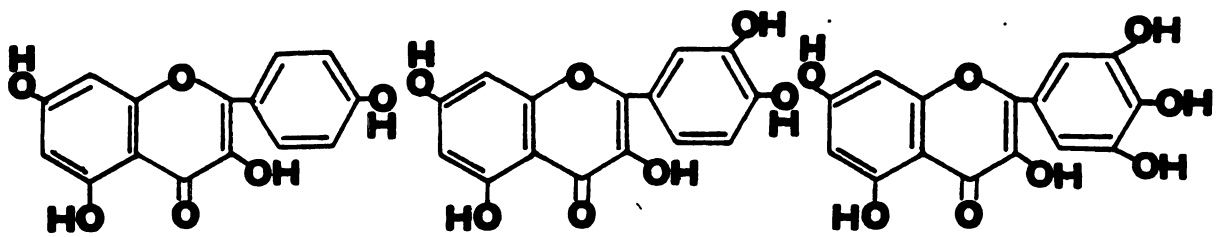


Figure 1. Catechins (Flavan-3-ols)

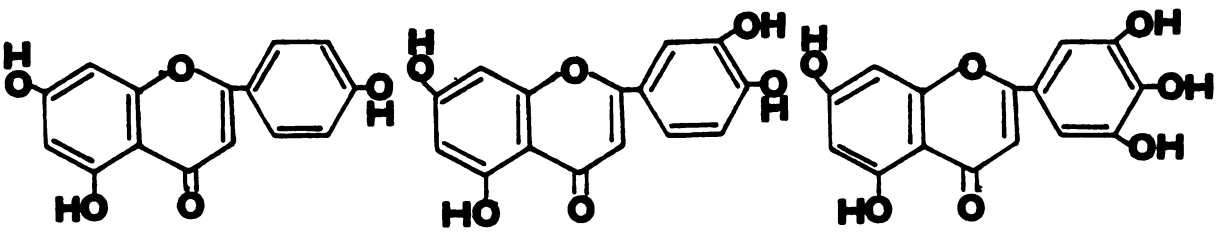
Catechins (flavonols) are the most common and comprise the major group in this family which includes kaempferol, quercetin and myricetin.



Kaempferol Quercetin Myricetin

Figure 2. Three common catechins (flavonols)

Flavone is less common and includes compounds such as apigenin, luteolin, and tricetin.



Apigenin Luteolin Tricetin

Figure 3. Three common flavones

Sixty other aglycones, based on hydroxy derivatives of the flavonoid and flavone structure are known. Five other minor groups are known and these are chalcones, aurones, flavanones, isoflavanones, and biflavonyls.

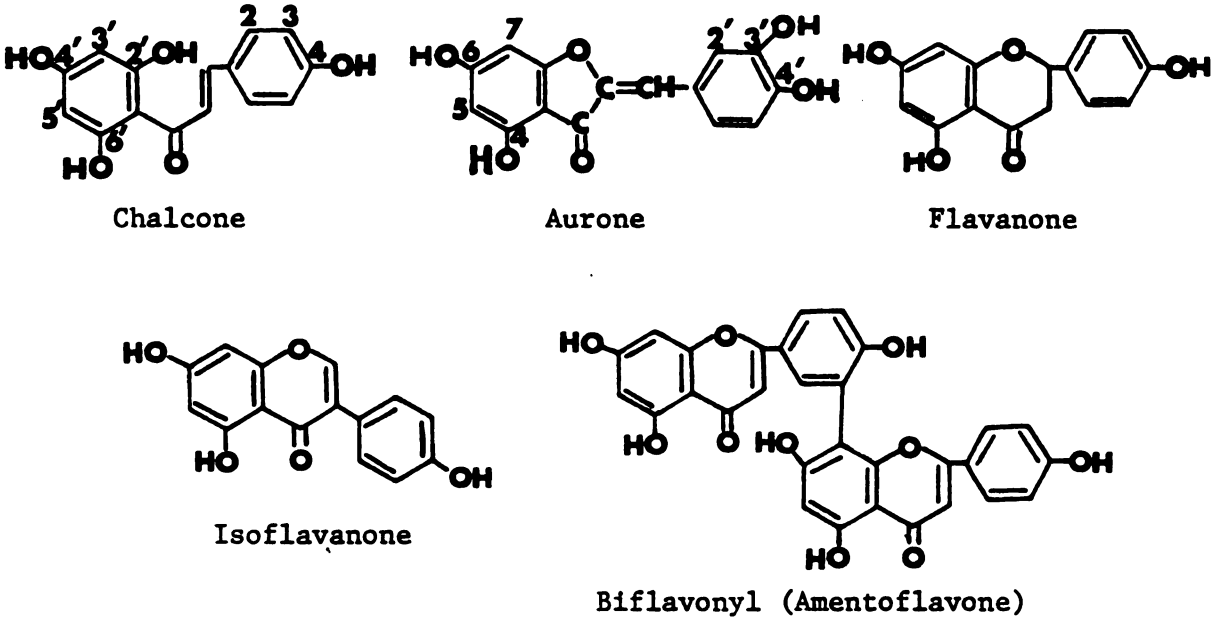


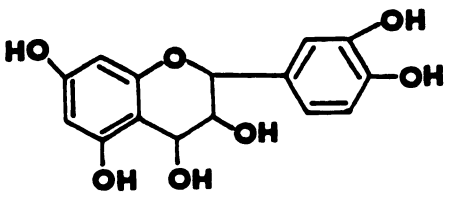
Figure 4. Five minor aglycones

Usually aglycones exist in the glycoside form with glucose, rhamnose, galactose, arabinose, xylose, apiose, or glucuronic acid. The sites of substitution are varied, but the 7; 5; 4'; 7,4'; and 3' positions are common. The flavonoid compounds also may occur with acyl substituents in a manner similar to the anthocyanins.

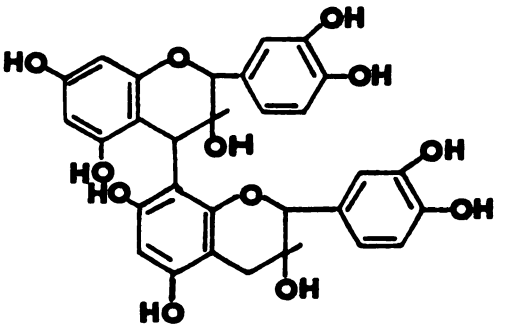
2. Leucoanthocyanins

Although leucoanthocyanins are colorless, under some food handling and processing conditions, they can be converted to colored products. The leucoanthocyanins have some alternate names, such as proanthocyanidins, anthoxanthins, anthocyanogens, flavolans, flavylans and flavylogens.

The basic building block of leucoanthocyanins is flavan-3,4-diol which usually forms a dimer through a 4-8 or 4-6 linkage, but trimers and higher polymers are not too uncommon. When a leucoanthocyanin is heated in the presence of a mineral acid, it will product an anthocyanidin, such as pelarogonidin, cyanidin, petunidin or delphinidin.



Flavan-3,4-diol



Leucoanthocyanin

Figure 5. A dimer leucoanthocyanin

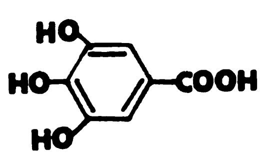
The leucoanthocyanin compounds are of interest in wine and juice because they contribute to the astringency or puckeriness and the flavor of the product. They also contribute to enzymatic browning in wine, because orthodihydroxy phenols can be oxidized to orthoquinones, which then polymerize to brown melanin pigments. Leucoanthocyanin compounds can also cause haze formation when the wine is chilled but this haze will usually reclear when the wine is warmed.

3. Tannins

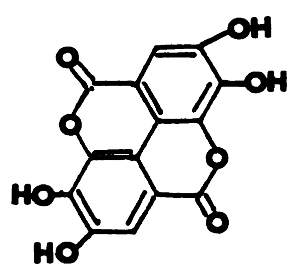
The term tannin, as well as tannic acid, gallotannic acid, and incorrectly digallic acid, is defined in the Merck Index as a complex mixture found in the bark of oak, sumac, and myrobalen. Its appearance

ranges from light yellow to brown in color. Tannic acid, as purchased from a chemical supply house, has the formula $C^{76}H^{52}O^{46}$, a molecular weight of 1,701 and probably consists of nine molecules of gallic acid and one of glucose.

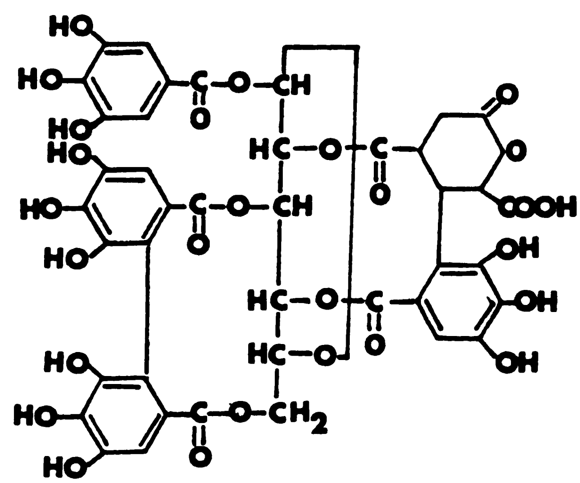
The term "tannin" as used in foods includes two types of compounds. The first type is the condensed tannin which may be 4,8 or 2,8 C-C dimers or 3,3-ether-linked dimers of catechin and related compounds. Other linkages, such as 2-carbon, 7-oxygen or 4-carbon, 7-oxygen compounds have been reported. The second type are the "hydrolyzable tannins" including the gallotannins and the ellagitannins which are both polymers of gallic acid and ellagic acid. A typical ellagitannin containing gallic acid, ellagic acid, and one molecule of glucose is shown in the following structures.



Gallic acid



Ellagic acid



Chebulagic acid

Figure 6. A typical ellagitannin structure

Although both types of tannins may exist in food, the tannins of grapes and wine have been known for many years to be condensed tannins (Thudichum and Dupre, 1872; Dekker, 1913). When Pointe and Gualdi (1931) used formaldehyde and hide powder on wine they obtained equal precipitates with each solvent so they concluded that the true tannins of wine were all of the flavanol-derived condensed type. Since oak gallotannic acid may be added to wine under certain processing conditions, it might be found in wine, but tannins of the pentadigalloyl glucose type or any other of the hydrolyzable tannin types do not occur naturally in wine or grape juice. Both types of tannins contribute to enzymatic browning reactions, but their mechanisms of action are not well understood.

4. Anthocyanins

Anthocyanins are glycosidic forms of anthocyanidins (aglycones), whose basic skeletal structure is derived from the flavylium nucleus (2-phenyl-benzopyrylium).

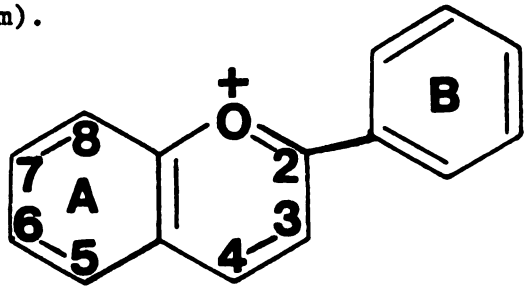


Figure 7. 2-Phenyl-benzopyrylium (flavylium)

Only five sugars have been found as portions of anthocyanin molecules. They are in order of relative abundance glucose, rhamnose, galactose, xylose, and arabinose. Anthocyanins may also be "acylated" which adds a third component to the molecule when one or more molecules of p-coumaric, ferulic, caffeic, or acetic acids may be esterified to the sugar molecule.

Twenty anthocyanidins are known but only six, pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin are important in foods.

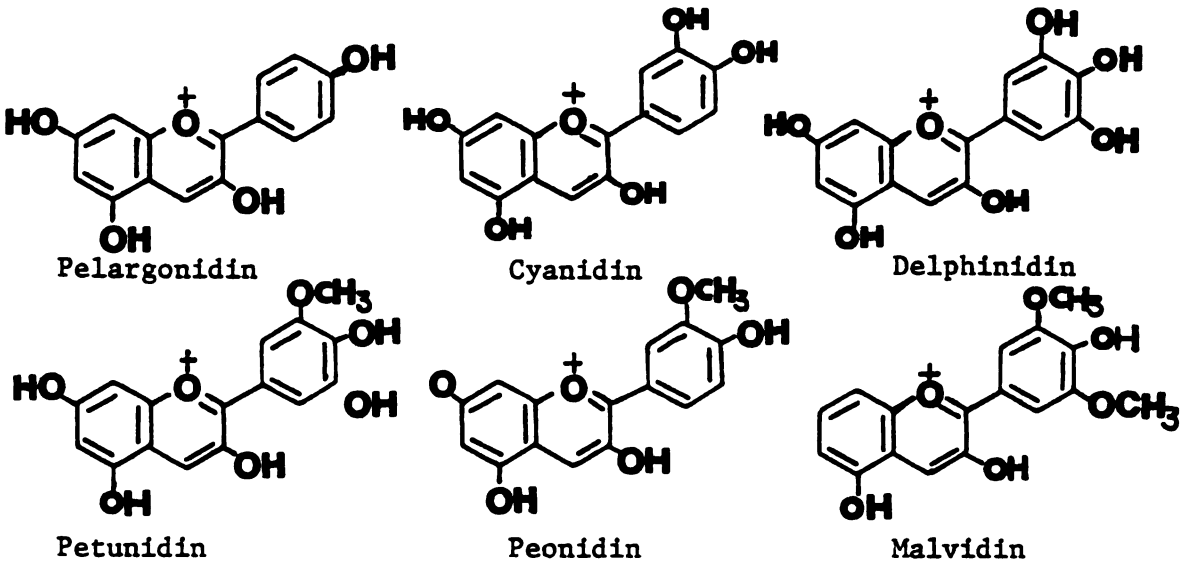


Figure 8. Six common anthocyanidins

Rice (1965), in paper chromatographic studies of Concord grape pigments, found cyanidin, peonidin, delphinidin, petunidin, and malvidin monoglucosides, diglucosides, and in most cases one or more acylated forms for each glucoside. Shewfelt (1966) found 14 paper chromatographic spots of pigments in concord grape juice, and the aglycone proportions were 45.7% delphinidin, 37.1% cyanidin, 8.8% petunidin, and 8.4% malvidin.

The anthocyanins of wine would be expected to be the same as those of grapes, and if the wines are young enough this may be true. The acyl groups are hydrolyzed very readily and will rapidly disappear from linkage in anthocyanins and appear as free acids in the wine. The glycosides have been shown to hydrolyze to free anthocyanidins, and anthocyanogens which have been reported to give cyanidin in wine (Ribereau-Gayon *et al.*

1958). Very little anthocyanidin can usually be recovered from wine, and the major change appears to be the incorporation of anthocyanidins into polymeric red, tannin-like molecules.

Amount and Distribution of Phenols in the Grape

Usually hand picked grapes are harvested by cutting entire clusters from the vines and the harvested weight of fruit includes the cluster stems which are removed during preparation of the grapes for wine and juice. Mechanical harvesting, which has become a predominant harvest method, usually involves some type of vine agitation to shake the berries from the rachises and a vibrating chain belt to separate the stems, petioles, leaves and canes from the harvested fruit (Black and Cargill et al. 1977), but this method leaves many stems, petioles and leaves in the product and these materials have a relatively high phenol content. In 7 varieties, Amerine and Bailey (1959) found 2,400 to 21,900 mg phenol, calculated as tannic acid, per kg of fresh weight of main stem, and 9,100 to 43,700 mg/kg in lateral stems. Durmshidze (1955) reported "tannin" to be 40,000 mg/kg of the dry weight of grape stems, and 20,000 mg/kg of the leaves. Diemair et al. (1951) reported 1320 - 3600 mg tannin per kg in fresh leaves and 1040-2200 mg/kg in their stems.

Singleton and Esau (1969) indicated that a ton of grapes contains an average of 3.7% stems, with the remaining 96.3% representing the typical berry weight. If all the tannin of the stem was extracted and dissolved in the juice or wine produced from the ton of grapes it could contribute from 90 to 1700 mg of phenol/liter, calculated as gallic or tannic acid with an estimated average of 1000 mg/liter. In this situation the stems could be a very significant source of phenols if they were not completely removed before processing operations begin. Another reason the stems

should be removed is because they contribute to off flavor and adsorb anthocyanins which reduces the color (Pacottet, 1904; Laborde, 1910). Singleton (1966, 1967) concluded that the total phenol of the berry was distributed, on the average, about 1% in the solid pressed pulp, 5% in the juice, 50% in the skins for red varieties and 25% for white, and the remaining 46-69% in the seeds. But he added for any specific sampling of grapes, actual analysis would be necessary owing to considerable variation by variety, vineyard, year and processing technique. The following table presents values considered typical for the amount of total phenol calculated as gallic or tannic acid in the major fraction of the grape berry (Singleton et al. 1969).

Table 1. Total Phenol Distribution in Vitis vinifera Wine Grapes[a]

Portion	Red varieties	White varieties
Skins	1859	904
Pressed pulp	41	35
Juice	206	176
Seeds	3525	2778
Total	5631	3893

[a]In gallic or tannic acid equivalents, mg/kg berries.

The Role of Phenolic Compounds in Juice and Wine Organoleptic Tests

1. Flavor Effect

Although it is often assumed that phenols only contribute bitterness and astringency to grape juice and wine, this is not always the case and the presence of phenolic compounds in these products may be very desirable because they can give body and fullness of flavor. The materials which

are most likely to be responsible for astringency are the small polymeric, yet soluble tannins and the leucoanthocyanins, while bitterness is a property of many substances in addition to some phenols (Bate-Smith et al. 1953). It is generally agreed that some level of astringency and bitterness is desirable in red wine, but it is not very clear what that level should be or how it can be defined. The astringency of tannin is proportional to its protein-binding capacity, which may change with storage. Joslyn and Goldstein (1964a) indicated that a tannin content of 200 mg/liter is necessary for detection in wine or juice and increases above 200 mg/liter must be greater than 40 mg/liter increments to be detectable.

It is generally recognized that the loss of astringency during fruit ripening is related to increased polymerization with decreased solubility of the tannin (Joslyn and Goldstein, 1964a) and this loss of astringency leads to tastelessness, rather than bitterness. Depolymerization, such as conversion of a polymeric anthocyanogen to anthocyanidins, may also lead to loss of astringency. These observations are consistent with astringency being dependent upon protein-binding capacity, because the simple flavonoids bind protein poorly, conversely, large condensed tannins bind protein easily, which causes precipitation and the larger the tannin molecule the greater the precipitation. It appears that astringency can be produced by polymerization of small phenolic molecules to larger ones or by breaking up very large molecules to smaller ones, although either of these mechanisms may also cause a loss of astringency, under certain conditions. Maier et al.(1964) mentioned that polymerization by oxidative or other mechanisms might produce increased astringency as molecular weight increased, and then decreased astringency as the optimal astringency size was exceeded.

2. Color

Appearance is an important feature of wine, and a wine's color, along with clarity, is a good indicator of its past history, condition, and probable present and future quality. The colors of most wines are almost wholly the result of their content of various phenolic substances. The substances responsible for the yellow colors of white wine were thought for many years to be flavonols, particularly quercetin, based largely on the fact that quercetin was the only known wine-soluble yellow substance identified in grapes (Genevois, 1951). The color of red wine may vary visibly in intensity from very pale rose to dark so as to appear nearly black. This hue depends upon factors such as pH, co-pigmentation, metallic lakes which affect the anthocyanins themselves, and upon the amount of brown color coexisting in the wine. The major effect of pH on anthocyanin color and structural form has been clarified by Jurd (1963) and Geissman (1963). In the pH range of 2.0 to 6.0 the flavylium salts evidently undergo the following transformations:

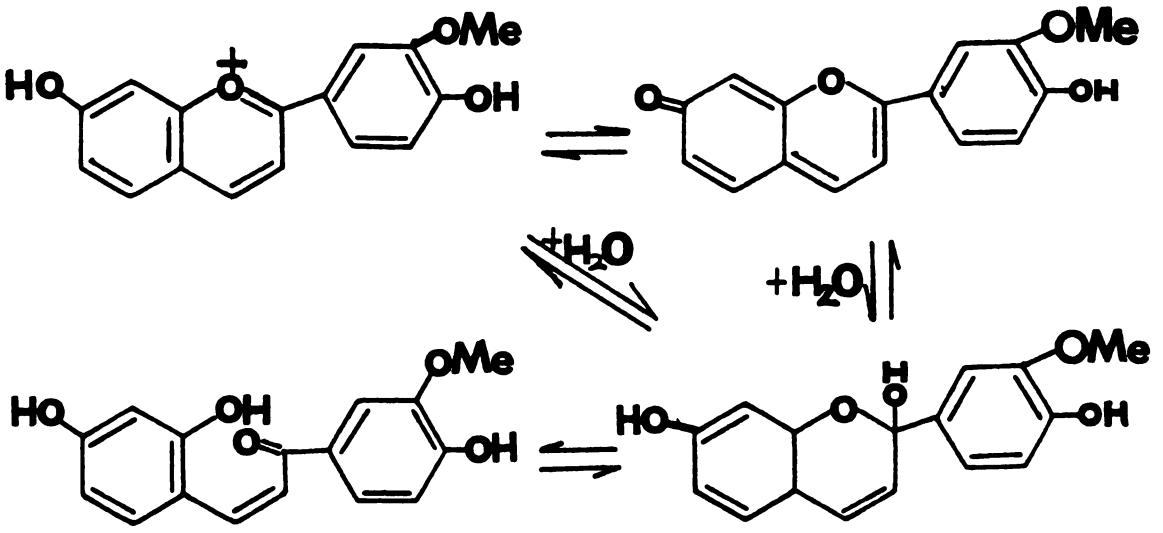


Figure 9. The effect of pH on anthocyanin color and structural transformations

Many other factors may also influence visable color. Sulfur dioxide tends to prevent browning, and bleaches not only some of the brown color but anthocyanins as well. There are a number of reports that the presence of tannin intensifies the red color of wine or juice and shifts it slightly toward blue (Winkler and Amerine, 1938). Gallic Acid has been reported to have a similar, but weaker effect. In the presence of metal ions such as aluminum and iron, complexes between other phenols, the metal, and anthocyanins modify the color exhibited, often in the blue direction.

Effects of Machine Harvesting on Juice and Wine Quality

The effects of mechanical harvesting on juice and wine quality are complex and result from the interaction of many factors, including operating parameters of the harvester, relative vine vigor, trellising practices, the specific grape variety, the extent of berry damage and the incorporation of material other than grapes (MOG), such as canes, bark, petioles, and leaves. There have been few papers published dealing with the effect of MOG content on juice and wine quality.

Petrucci and Siegfried (1975) reported the cultivars Ruby Cabernet and Emerald Riesling were mechanically harvested and analyzed for MOG content. Emerald Riesling, which harvested comparatively easily, had a low MOG content and a small variation in MOG content between samples. Ruby Cabernet had a higher MOG content and a greater variation in MOG content between samples but in both cultivars, about half of the total MOG was small leaf fragments which would adhere to the grapes and not be easy to remove before processing.

Johnson and Grgich (1975) compared the quality of White Riesling and Cabernet Sauvignon wines from grapes harvested; (A) by conventional

handpicking and handling methods and; (B) by Redwood's Up-Right Vectur mechanical harvester which gave a MOG content of less than 1%. For Cabernet Sauvignon the wine from the machine-harvested grapes had identical characteristics to the wine from the handpicked grapes. For White Riesling, the machine-harvested grapes arrived at the winery free from oxidation, had fewer leaves than the handpicked, and yielded a wine with superior fragrance and slightly higher acidity. The must from the handpicked grapes was slightly higher in sugar content.

Noble and Ough et al. (1975) investigated the effects of added grape leaves, berry damage, and mechanical harvest on wine quality. The results indicate that there were overall adverse effects on wine quality from mechanical harvesting of grapes or from adding shredded grape leaves. In all cases, the white wines made from damaged or machine-harvested grapes, or from grapes to which leaves had been added, were significantly darker by visual assessment as well as by absorbance at 420 nm. The phenolic content of wines increased with the addition of leaves or with berry damage.

MATERIALS AND METHODS

Since MOG content seems to be correlated with ease of harvest and the quality of both juice and wine, two cultivars which differ markedly in harvestability and final product characteristics were used for quality evaluations. Samples taken from mechanical harvest were analyzed for total weight of MOG and per cent MOG. Handpicked samples were used as controls and also for the additions of varied amounts of MOG during processing in order to determine the effect of MOG on the overall quality of finished products. Chemical and physical analyses of samples included:

(1) Total phenolic compounds and different phenolic fractions

(2) pH and total acidity

(3) Brix

(4) Color

(5) Alcohol

(6) Sensory assessment

Sample Preparation:

During the 1978 harvest, concord grapes for grape juice and red wine production and Aurora Blanc grapes for white wine production were supplied by Welch's Inc. and Warner Vineyards, respectively.

Aurora Blanc grapes were harvested by two methods, (a) by conventional handpicking and (b) by mechanical harvester. The mechanically harvested grapes were stored in a cold room (4^{o} C) for approximately

12 hours. Green and senescent leaves were obtained from the vines which were hand harvested. Weighed amounts of leaves, which were shredded by hand were added to weighed amounts of hand harvested grapes to give 1% (W/W) of added leaf tissue (HH + 1% leaf). Intact, hand harvested (HH) grapes served as the control.

For mechanically harvested grapes, the MOG content was determined on five, 2000 gram samples. Shredded leaf tissue was added to mechanically harvested grapes to give 1% (W/W) added leaf (MH + 1% leaf) while mechanically harvested (MH) samples served as the control.

White Wine Making Procedure:

All mechanical and hand harvested treatments were held overnight at 21-24^{o}C, then crushed and de-stemmed and 100 PPM sulfur dioxide was added to sterilize the crushed grapes (must). Juice was pressed and filtered through cheese cloth into fermentation containers where sugar and acid content were checked and the sugar was adjusted to 22^{o} Brix. Saccharomyces cerevisiae--Montrachet yeasts were added to the must, approximately 36 hours after the addition of sulfur dioxide and the fermentors were placed in a 10 to 15.5^{o}C cubicle for fermentation. When fermentation was complete (approximately 20 days) the sugar level was 6.0^{o} Brix and the acidity was around 0.65%. The wine was racked and topped off, then stored for 3 months at 10^{o}C before bottling. Samples were taken during different stages of fermentation and analyzed for acidity, sugar, alcohol and phenolic content.

In early October, 1978, Concord grape samples were obtained by conventional handpicking and from covered grape gondolas which had been harvested by a mechanical harvester. Green and senescent leaves and petioles, stems and rachis were obtained from the vines which were hand

harvested. Weighed amounts of each of these were added to weighed amounts of handpicked grapes to give the following treatments: (1) hand harvested (HH) grapes (2) hand harvested grapes plus 1% leaves and 1% petioles (3) hand harvested grapes plus 5% leaves and 5% petioles (4) hand harvested grapes plus 1% leaves (5) hand harvested grapes plus 5% leaves (6) hand harvested grapes plus 1% petioles (7) hand harvested grapes plus 5% petioles.

For mechanically harvested Concord grapes, five-2000 gram samples were checked for their MOG contents.

Concord Grape Juice Making Procedure:

Samples of the various treatments were crushed and destemmed before heating to 68-70°C where they were held for 2 minutes to inactivate enzymes and facilitate the extraction of pigment. The samples were then cooled to 45-50°C and commercial pectinase (Irgazyme from Ciba-Geigy Corp.) was added to hydrolyze pectins. These batches were held for 18 hours at 21°C, then pressed. The juice was collected in plastic containers which were covered and refrigerated for detartration. After 3-4 weeks, the detartration process was complete and the juice was pasteurized, bottled and stored. Samples of the juice were analyzed for the phenolic fractions.

Concord Red Wine Making Procedure:

Samples of grapes from each of the various treatments were crushed and destemmed. The must was sterilized by adding 125 ppm sulfur dioxide and then refrigerated for approximately 24 hours. Sugar and acid content was adjusted to 22° Brix. After 24-36 hours the sulfur dioxide had dissipated sufficiently and a montrachet strain of Saccharomyces cerevisiae

was added to the must in a plastic primary fermentor which was then covered with cheesecloth. The must was stirred twice a day for four days to aerate the mixture and break the cap of floating pomace. The free run wine was drawn off and the pulp was pressed in a pilot plant juice press. The wine was placed in secondary fermentors with fermentation locks and held for three weeks at 25-30^{o}C before the first racking. After racking the wine was stored for approximately three months before it was filtered and bottled. At different times during the fermentation and holding periods, samples were removed and checked for pH, titratable acidity, sugar content, alcohol content and analyzed for phenolic content.

Total Phenolic Compounds and Different Phenolic Fractions:

Total Phenolic Compounds:

Total phenolic compounds were determined according to the method of Singleton and Rossi (1965) with the following modifications:

(1) For white wines, 0.5 ml sample plus 65 mls of distilled water were placed in a 100-ml volumetric flask. Red wines and juice were diluted 1:3 with distilled water then 0.5 ml. diluted sample plus 65 mls of distilled water were placed in a 100-ml volumetric flask.

(2) 5 mls of Folin-Ciocalteu reagent were added and mixed thoroughly.

(3) 15 mls of 20% sodium carbonate solution were added and the solution was made to 100 ml volume.

(4) Solutions were left standing for 2 hours at room temperature to fully develop the color.

(5) Absorbance was read at 765 nm.

(6) Total phenolic content was calculated (as gallic acid) from a standard curve (Appendix I).

The Folin-Ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. This reagent oxidizes phenolates and the heteropoly acid becomes partially reduced from the +6 to a mixture of +6 and +5 valence states, resulting in the production of complex molybdenum-tungsten blue. Complete reduction to the lower valence state destroys the color. The phenols are oxidized rapidly only in solution sufficiently alkaline to give appreciable concentrations of the phenolate ions. Most phenols are about 50% ionized at pH 9-10 but as the ionized portion reacts with the Folin reagent the equilibrium will shift and more phenolate will be produced. Time would be required for this reaction to approach completion.

Flavonoid and Nonflavonoid Phenol Determination:

Total phenolic compounds in wine can be generally separated into flavonoid and non-flavonoid phenols. Therefore, if the total and non-flavonoid phenolics can be accurately determined, the flavonoid content can be calculated very easily. The quantitative estimation of flavonoid and non-flavonoid phenols (Kramling and Singleton, 1969) in wine is based upon the determination of the phenol content before and after precipitation and removal of the flavonoids through reaction with formaldehyde under selected condition as follows:

(1) 10 mls of wine were pipetted into a 30 ml test tube, then 10 ml of 1:4 conc. HCl added.

(2) 5 ml of a standard formaldehyde solution contain 8 mg/ml were added, sparged with nitrogen and stoppered.

(3) Samples were incubated 24 hours at room temperature then filtered through a membrane filter of 0.45 u pore size. Under these conditions the filtrate contained only non-flavonoid phenols because phenols lacking a meta-dihydroxy grouping (non-flavonoid) did not precipitate. The non-flavonoid phenol content could then be tested with the Folin-Ciocalteu reagent and measured colorimetrically.

Tannin and Nontannin Determination:

Precipatation of tannins with cinchonine sulfate was carried out according to the method of Peri and Pompei (1971) and adapted to wine as follows:

(1) A 50 ml sample was placed in a 100 ml centrifuge tube and neutralized to pH 7.0 with sodium hydroxide solution.

(2) Precipitation was carried out by the addition of 25 ml of pH 7.9 phosphate buffer and 12.5 ml of the cinchonine sulfate solution (1.5 g cinchonine base, 2 ml 1:3 sulfonic acid, water to 100 ml.).

(3) After 20 minutes at room temperature the cinchonine tannate precipitate was separated by centrifuging and the clear supernatant was transferred to a 200 ml volumetric flask.

(4) The precipitate remaining in the centrifuge tube was washed twice with 10 ml of a 10% solution of sodium sulfate in water.

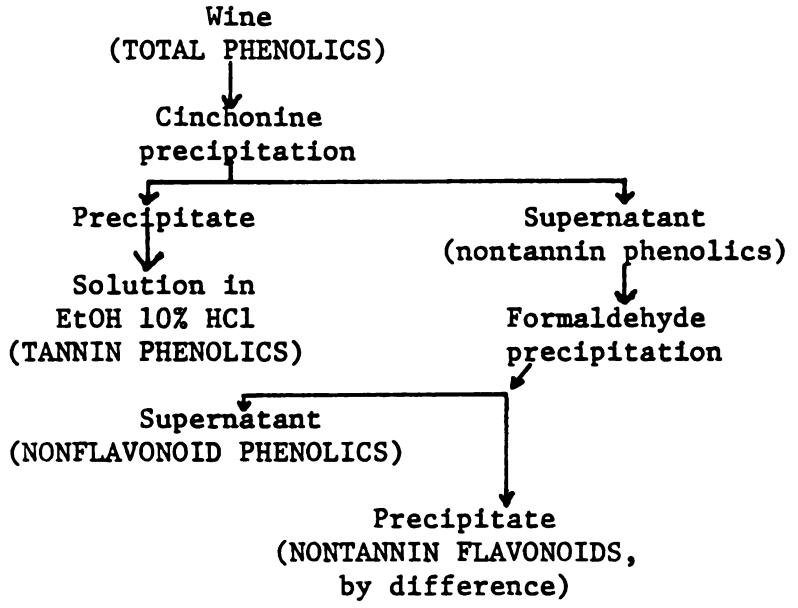
(5) The supernatant together with the washing, was acidified to pH 3.5 with HCl, brought to volume with distilled water, and used for the determination of nontannin phenolics with the Folin-Ciocalteu reagent.

(6) The cinchonine tannate precipitate was dissolved in ethanol-

10% HCl, brought to 50 ml with the same solvent, and used for the determination of tannin phenolics with the Folin-Ciocalteu reagent.

Each analysis was replicated twice and mean data were reported.

The results obtained were double checked by carrying out the precipitation with cinchonine and formaldehyde on two different portions of the same wine. The fractionation mechanism may be schematized as follows:



pH and Total Acidity:

The juice and wine pH and total acidity were measured with a pH meter as follows:

(1) 10 ml of sample and 75 ml distilled water were transferred to a 250 ml beaker.

(2) The pH of the samples was taken then they were titrated with 0.1N sodium hydroxide to the phenolphthalein endpoint of 8.1 with the pH meter.

(3) Per cent acidity was calculated as tartaric acid.

Color:

The color of Concord juice and wine was measured using a Hunter Color Difference Meter, Model D25 with an inverted head. After standardizing with a pink tile, L=67.6, a=+21.4, b=+11.9, a 50 ml sample was filled into an optical glass cylinder cup 3.5 inch x 0.75 inch and a standard white plaque was placed under the bottom of the cup. Two readings were taken on each sample and averaged to give individual means for L, a, b values.

Alcohol Content:

The alcohol content of white and red wine was determined by distillation using the following procedure:

(1) 200 ml of the sample were placed in a 500 ml Kjeldahl flask with 50 ml distilled water. The flask was connected to a condenser and the wine was distilled to give 190 ml distillate collected in a 200 ml volumetric flask.

(2) The 200 ml volumetric flask was held in a water bath at 15.5^{o}C and made to volume with distilled water, mixed and held until the temperature had equilibrated.

(3) The sample was transferred to a cylinder and alcohol content was determined with an alcohol hydrometer.

(4) Every sample was replicated and mean data were reported.

Sensory Assessment:

Panelists evaluated four white wines and nine red wines, and nine Concord juice samples. These evaluations were carried out by 12

panelists who tasted the product each day for five different days. All evaluations were made in individual booths under white light. Samples were scored 10 points for each of the factors which included flavor acceptability, aroma acceptability, aroma intensity, bitterness and astringency (Appendix II, Taste Panel Ballots). All taste panel data were analyzed for variance and Duncan's Multiple Range Test (1957) was then used to pinpoint significant differences among variables revealed by the analysis of variances.

RESULTS AND DISCUSSION

Table 1 shows the average weights of total MOG found in both cultivars of mechanically harvested grapes used in this study.

TABLE 1. Material other than grapes (MOG) in mechanically harvested grapes.

Variety	No. of Samples	Avg. Sample Wt. (g)	Total MOG (mean) Wt. (g)	%
Aurora Blanc	5	2,000	94	4.7
Concord	5	2,000	52	2.6

Mechanically harvested Concord grapes contained slightly more than half the MOG that mechanically harvested Aurora Blanc grapes did. This probably reflects the inherent variability in ease of harvesting which exists between the cultivars.

White Wine Analysis:

Table 2 presents the must analyses for all samples. With the longer holding times, total acidity declined and pH increased in all samples. These results are similar to those of Ough (1969), Ough *et al.* (1971) and Noble and Ough (1975) who found changes occurring, due to longer skin contact times. The pH of the final wine was related to the initial pH of the must while the decrease in total acidity was due to tartrate precipitation and subsequent removal of the precipitate during the fining

procedure. Adding leaf tissue to the hand harvested and mechanically harvested grapes caused the pH to increase slightly. The MH, MH + 1% leaf, and HH + 1% leaf samples did not differ significantly from the control sample (HH) in pH and total acidity.

TABLE 2. Aurora Blanc must and wine analyses of all treatments at various stages of fermentation.

	MUST				WINE	
	pH		Total Acidity			
Treatment	4 hr.	24 hr.	4 hr.	24 hr.	pH	Total Acidity
Hand Harvested	3.2	3.3	0.83	0.75	3.40	0.64
Hand Harvested + 1% Leaf	3.2	3.49	0.83	0.76	3.55	0.66
Mechanically Harvested	3.45	3.55	0.81	0.76	3.58	0.61
Mechanically Harvested + 1% Leaf	3.45	3.65	0.81	0.79	3.68	0.58

The initial Brix of the hand harvested (HH) grapes differed somewhat from that of the mechanically harvested (MH) fruit, reflecting slight differences in the maturity of the samples, but after adjusting the Brix and fermenting the samples, Table 3 shows that final Brix and alcohol content had very little difference in any of the treatments, which means that adding small amounts of leaf tissue and MOG may not affect the fermentation processes. Even in the MH + 1% leaf, where the total phenolic content was almost double that in the control sample (HH), (Table 11), the yeast fermentation did not appear to be significantly inhibited.

Tables 4 through 7 present results of the analysis of the phenolics in white wine made from HH, HH + 1% leaf, MH and MH + 1% leaf at various

time intervals during production. In all four treatments, the total phenolic, flavonoid, and tannin contents decreased during wine processing. This is probably due to the fact that parts of the flavonoids and tannins precipitate with other substances, particularly potassium bitartrate, and is largely removed during fining and/or filtration. Also, during the fermentation process, some of the small soluble tannin or flavonoid compounds may polymerize to large insoluble polymers or they may form insoluble complexes with proteins which then precipitate and decrease the tannin, flavonoid and total phenolic contents.

TABLE 3. The soluble solid and alcohol content of white wine in final stage of fermentation.

Treatment	Initial Brix	Adjusted Brix	Final Wine Soluble Solid (Brix)	Alcohol Content (%)
Hand Harvested	13.0	22	6.0	13.4
Hand Harvested + 1% Leaf	13.8	22	6.0	13.0
Mechanically Harvested	15.0	22	6.2	13.1
Mechanically Harvested + 1% Leaf	15.0	22.2	6.2	14.0

TABLE 4. Analysis of phenolic fraction of white wine from hand harvested grapes at various stages of production.

(mg gallic acid/100 ml)

Date	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
9/12/78 Original Juice	38.00	21.56	16.44	31.2	6.80	+0.4
10/31/78	31.50	21.56	9.94	25.8	5.70	+0.6
11/31/78	29.98	21.70	8.28	25.1	4.88	+0.3
2/7/79	26.00	21.75	4.25	22.0	4.00	+0.3

[1]The percent error was calculated as: $\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$

TABLE 5. Analysis of phenolic fraction of white wine from hand harvested grapes with 1% leaf added. (mg gallic acid/100 ml)

Date	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
9/12/78	39.5	22.98	16.52	32.17	7.33	+0.3
10/31/78	32.5	23.75	8.75	28.60	3.90	+0.5
11/31/78	28.0	23.20	4.80	24.50	3.50	+0.4
2/7/79	26.0	23.00	3.00	23.40	2.60	+0.2

[1]The percent error was calculated as:

$$\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$$

TABLE 6. Analysis of phenolic fraction of white wine from mechanically harvested grapes at various stages of production.

(mg gallic acid/100 ml)

Date	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
9/12/78	83.50	37.50	46.00	47.20	36.30	+0.6
10/31/78	55.50	26.25	29.25	45.00	10.50	-0.3
11/31/78	51.70	28.25	23.45	45.15	6.55	-.04
2/7/79	49.75	30.63	19.12	45.30	4.45	+0.3

[1]The percent error was calculated as:

$$\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$$

TABLE 7. Analysis of phenolic fraction of white wine from mechanically harvested grapes with 1% leaf added.

(mg gallic acid/100 ml)

Date	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
9/12/78	84.0	36.0	48.0	46.0	3.8	-0.4
10/31/78	55.5	29.3	26.2	47.1	8.4	+0.4
11/31/78	51.0	32.5	18.5	45.5	5.5	+0.4
2/7/79	49.0	32.0	17.0	44.5	4.5	+0.4

[1]The percent error was calculated as:

$$\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$$

Tables 8 through 11 compare the phenolic fractions of the white wine for each of the treatments at the same stage of production. The addition of 1% leaf to hand harvested and mechanically harvested grapes at the time of harvest, had little influence on the total phenolic, flavonoid, and tannin contents at that stage. The MH and MH + 1% leaf samples, however, had much higher total phenolic, flavonoid and tannin contents than the HH and HH + 1% leaf samples, due to the leaching of these materials from the MOG during the harvesting procedure. One month, two months, and four months fermentation data (Table 9, 10, & 11) show that in the MH and MH + 1% leaf samples, the total phenolic content is still much higher than HH and HH + 1% leaf, but the difference in tannin content continously decreased. After four months fermentation the tannin contents of all treatments are very nearly the same. This indicates that in MH and MH + 1% leaf samples the tannins precipitate much more than in HH and HH + 1% leaf. It may be that MH and MH + 1% leaf samples contained some MOG substance which caused tannins to precipitate more easily.

Results for the analysis of variance for taste panel data are presented in Table 12. It should be noted that the values for replications are not significant in any of these tables, indicating that the 12 panelists who tasted the samples and rated each of the various factors every day for five different days were very consistent in their ratings. The values for the factors of flavor acceptability, aroma acceptability, and aroma intensity are significant at the 1% level, but astringency and bitterness are not significant. The lack of significance for the last two variables is probably correlated with the decreases in the tannin content, since this compound is most likely to be responsible for astringency and bitterness. Joslyn and Goldstein (1964a) has indicated

TABLE 8. Analysis of phenolic fraction of white wine with different treatments at the time of grape harvest (9/12/78).

(mg gallic acid/100 ml)

Treatment	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
Hand Harvest	38.00	21.56	16.44	31.20	6.80	+0.4
Mechanical Harvest	83.50	37.50	46.00	47.20	36.30	+0.6
Hand Harvest + 1% Leaf	39.50	22.98	16.52	32.16	7.34	+0.3
Mech. Harvest + 1% Leaf	84.00	36.00	48.00	46.00	38.00	-0.4

[1]The percent error was calculated as:

$$\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$$

TABLE 9. Analysis of phenolic fraction of white wine with different treatments after one month of fermentation (10/31/78).

(mg gallic acid/100 ml)

Treatment	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
Hand Harvest	31.5	21.56	9.94	25.80	5.7	+0.6
Mechanical Harvest	55.5	26.25	29.25	45.00	10.5	-0.3
Hand Harvest + 1% Leaf	32.5	23.75	8.75	28.60	3.9	+0.5
Mechanical Harvest + 1% Leaf	55.5	29.25	26.25	47.10	8.4	+0.4

[1]The percent error was calculated as:

$$\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$$

TABLE 10. Analysis of phenolic fraction of white wine in different treatments after 2 months of fermentation (11/31/78).

(mg gallic acid/100 ml)

Treatment	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
Hand Harvest	29.98	21.70	8.28	25.10	4.88	+0.3
Mechanical Harvest	51.70	28.25	23.45	45.15	6.55	-0.4
Hand Harvest + 1% Leaf	28.00	23.20	4.80	24.50	3.50	+0.4
Mechanical Harvest + 1% Leaf	51.00	32.50	18.50	45.50	5.50	+0.4

[1]The percent error was calculated as:

$$\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$$

TABLE 11. Analysis of phenolic fraction of white wine in different treatments after 4 months of fermentation (2/7/79).

(mg gallic acid/100 ml)

Treatment	Total Phenolic Compounds	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
Hand Harvest	26.00	21.750	4.250	22.0	4.00	+0.3
Mechanical Harvest	49.75	30.625	19.125	45.3	4.45	+0.3
Hand Harvest + 1% Leaf	26.00	23.000	3.000	23.4	2.60	+0.2
Mechanical Harvest + 1% Leaf	49.00	32.00	17.000	44.5	4.50	+0.4

[1]The percent error was calculated as:

$$\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$$

TABLE 12. Analysis of variance mean square for sensory attributes of white wine.

Source	df	Aroma Acceptability M.S.	Flavor Acceptability M.S.	Aroma Intensity M.S.	Astringency M.S.	Bitterness M.S.
Total	19	0.649	0.46	0.548	0.218	0.325
Variable	3	3.6^{**}	1.72^{**}	2.74^{**}	$0.098^{n.s.}$	$0.486^{n.s.}$
Replication	4	$0.03^{n.s.}$	$0.231^{n.s.}$	$0.228^{n.s.}$	$0.46^{n.s.}$	$0.557^{n.s.}$
Error	12	0.224	0.224	0.107	0.169	0.208

**Significance at 1% level

* Significance at 5% level

$^{n.s.}$Non-significant F ratio

that 200 mg tannin/liter is a minimum detectable level and it can readily be seen in Table 11 that the tannin content of any of the treatments after fermentation is only about one fifth this amount. Flavor and aroma in wine are also influenced by the phenolics of the MOG although there are probably other MOG factors which also contribute to the significant difference that the panelists noted in these components. Tables 13 and 14 show the taste panel means, standard deviation, and a comparison of the factors which were rated by the panelists. On the 1 to 10 scale used in this study, 10 indicates the most acceptable, 1 indicates the least acceptable. In aroma acceptability, the panelists found HH and HH +1% leaf to be definitely better than MH and MH better than MH + 1% leaf but all the wines scored above 5 and were considered to have acceptable aromas. In flavor acceptability, HH, HH + 1% leaf and MH had the same level of acceptability and were definitely better than MH + 1% leaf which scored in the unacceptable range. In aroma intensity, the panelists found that HH had the strongest aroma, MH and MH + 1% leaf had about equal aroma levels, and the aroma of the HH + 1% leaf seemed to be the least intense of all the treatments. The panelists seemed to indicate that wine made from the HH fruit had the best all around quality but the addition of 1% leaf to the HH had only a small overall effect in quality. Wine made from the MH grapes had a slightly lower quality than either of the HH samples and the addition of 1% leaf to the MH fruit produced a wine that was significantly lower in quality than that made from HH grapes.

Table 13. Mean and standard deviation of the quality characteristics for sensory evaluation (white wine).

	Aroma Acceptability	Flavor Acceptability	Aroma Intensity
Hand Harvest	$6.57^{a} \pm 0.19$	$5.98^{a} \pm 0.33$	$5.79^{a} \pm 0.28$
Mech. Harvest	$5.98^{b} \pm 0.24$	$5.63^{a} \pm 0.52$	$4.80^{b} \pm 0.41$
Hand + 1% Leaf	$6.82^{a} \pm 0.29$	$5.80^{a} \pm 0.53$	$3.98^{c} \pm 0.35$
Mech. + 1% Leaf	$5.26^{c} \pm 0.35$	$4.67^{b} \pm 0.26$	$4.72^{b} \pm 0.25$

$^{a-c}$Average superscripted by the same letter are not significantly different at the 5% level of probability (Duncan, 1957).

Table 14. Mean and standard deviation of the quality characteristics for sensory evaluation (white wine).

	Astringency	Bitterness
Hand Harvest	$6.15^{a} \pm 0.40$	$7.15^{a} \pm 0.28$
Mech. Harvest	$6.13^{a} \pm 0.57$	$7.42^{a} \pm 0.44$
Hand Harvest + 1% Leaf	$5.90^{a} \pm 0.24$	$7.12^{a} \pm 0.73$
Mech. Harvest + 1% Leaf	$5.90^{a} \pm 0.48$	$6.67^{a} \pm 0.38$

$^{a-c}$Average superscripted by the same letter are not significantly different at the 5% level of probability (Duncan, 1957).

Concord Juice and Red Wine Analysis:

Table 15 shows the pH and total acidity (T.A.) data for all the Concord juice and wine samples at various stages of processing. The data shows that total acid declined and pH increased in all samples before the first racking. These results are similar to those of Ough et al. (1971) and Nobel and Ough (1975) who observed similar acid and pH changes in samples which had longer skin contact times and this is possibly due to tartrate precipitation. After aging one month, the pH decreased slightly and total acid increased slightly as compared with the sample taken before the first racking. Table 16 shows that the Brix and alcohol contents of the finished wine from the nine treatments did not differ appreciably, which would indicate that the various amounts of MOG did not affect the fermentation process.

Tables 17, 18, and 19 present Hunter color measurements expressed as L, +a, +b, hue (a/b) and saturation $(a^2+b^2)^{1/2}$ of Concord grape juice, wine after one month of fermentation, and after two months fermentation, respectively. Objective color measurements have several advantages over visual descriptions in specifying juice and wine color. The "L" value, which measures lightness or darkness of a product, actually describes the relationship between reflected and absorbed light. The Hunter "a" value denotes redness in the "+" mode or greenness in the "-" mode, while the Hunter "b" value measures yellowness in the "+" mode or blueness in the "-" mode. Hue is expressed by the angle whose cotagent is a/b. Saturation is measured by the expression $(a^2 + b^2)^{1/2}$, which describes reflection at a given wavelength and shows how much a color differs from gray. The L value, hue, and saturation express the interdependence of these three color parameters, and because of this, none of them used alone can give an accurate

description of wine but the combination of the three defines the product very well (Robinson, et al. 1969). Comparing Tables 17 through 19, one can find no relationship between the color parameters and the amount of MOG added in any of the samples, or between those parameters and the amount of MOG added in any of the samples, or between those parameters, and processing time in each treatment, which simply means that MOG had little affect on the wine color in this study. Table 20 shows the total phenolic content, flavonoid and non-flavonoid content, tannin and nontannin content of all the nine different treatments in Concord juice. The sample containing 5% leaf and 5% petiole, had the highest total phenolic, flavonoid, and tannin content, followed in order by the 5% petiole, 5% leaf, MH (which both contain 2.6% MOG), 1% petiole, HH (control sample), 1% leaf plus 1% petiole, and finally the 1% leaf samples. These results generally fit the experimental hypothesis which holds that a sample containing higher MOG content will have higher total phenolic, flavonoid, and tannin content. The two exceptions, 1% leaf and 1% leaf + 1% petiole contained total phenolic, flavonoid and tannin levels below the control sample but this may be due to some substance in the leaf tissue which precipitates phenolic compounds in the wine. However, if a large amount of leaf tissue is added, the phenolics in the leaf tissue may exceed the small amount precipitated from wine so that the total effect is an increase in phenolics. Tables 21 and 22 show the phenolic fractions in Concord wine after three and five months of fermentation. It can be readily seen that for each treatment, total phenolic, flavonoid and tannin content all decreased, just as it did in the white wine and probably for the same reasons. It should also be noted that the amounts of the different phenolic fractions in the various treatments follows

TABLE 15. Concord juice and wine pH and total acidity.

Treatment	At Harvest		Juice		Must		Before 1st Racking		After 1 Month Aging (Fermentation 4 months)	
	pH	T.A.[1]	pH	T.A.	pH	T.A.	pH	T.A.	pH	T.A.
HH	3.20	0.96	3.29	0.94	3.25	0.93	3.45	0.90	3.42	0.99
HH + 1% Leaf + 1% Petiole	3.20	0.96	3.50	0.84	3.52	0.83	3.55	0.81	3.48	0.95
HH + 5% Leaf + 5% Petiole	3.20	0.96	3.53	0.83	3.56	0.81	3.57	0.80	3.54	0.96
HH + 1% Leaf	3.20	0.96	3.35	0.96	3.45	0.96	3.47	0.94	3.43	0.997
HH + 5% Leaf	3.20	0.96	3.40	0.84	3.55	0.80	3.55	0.74	3.43	0.91
HH + 1% Petiole	3.20	0.96	3.38	0.88	3.45	0.84	3.47	0.82	3.30	0.96
HH + 5% Petiole	3.20	0.96	3.39	0.88	3.55	0.84	3.55	0.84	3.42	0.91
MH (1)	3.30	0.92	3.39	0.87	3.45	0.85	3.48	0.83	3.25	0.95
MH (2)	3.30	0.92	3.39	0.87	3.45	0.85	3.48	0.83	3.25	0.95

[1] Total Acidity (g Tartaric Acid/100 ml)

TABLE 16. Brix and alcohol contents of red wines

Treatment	Initial Juice Brix	Adjusted Brix	After Finish Fermentation Brix	Alcohol Content (%)
HH	12.8	22.0	6.6	11.8
HH + 1% Leaf + 1% Petiole	13.2	22.0	6.4	12.3
HH + 5% Leaf + 5% Petiole	14.2	22.0	6.7	11.4
HH + 1% Leaf	13.0	22.0	6.4	12.5
HH + 5% Leaf	13.8	21.8	7.0	11.0
HH + 1% Petiole	13.2	21.8	7.2	10.6
HH + 5% Petiole	14.0	21.8	7.0	11.0
MH (1)	13.4	22.0	6.8	11.4
MH (2)	13.4	22.0	6.8	11.4

TABLE 17. Color measurement of Concord juice in different treatment.

Treatment	L	a	b	Hue (a/b)	Saturation $(a^2 + b^2)^{1/2}$
HH	28.4	47.4	13.5	3.51	49.28
HH + 1% Leaf + 1% Petiole	38.5	44.3	9.6	4.61	45.33
HH + 5% Leaf + 5% Petiole	39.6	41.8	10.3	4.06	43.05
HH + 1% Leaf	29.8	48.0	13.1	3.66	49.76
HH + 5% Leaf	37.1	43.6	10.8	4.04	44.92
HH + 1% Petiole	42.5	42.6	8.5	5.01	43.44
HH + 5% Petiole	43.6	40.3	7.5	5.37	40.99
MH (1)	36.8	48.8	11.0	4.44	50.02
MH (2)	35.0	49.5	11.7	4.23	50.86

TABLE 18. Color measurement of red wine with different treatment after 1 month fermentation.

Treatment	L	a	b	(a/b)	Saturation $(a^2 + b^2)^{1/2}$
HH	24.0	37.5	9.5	3.95	38.68
HH + 1% Leaf + 1% Petiole	29.1	34.9	9.4	3.71	36.14
HH + 5% Leaf + 5% Petiole	24.3	39.2	9.6	4.08	40.36
HH + 1% Leaf	30.4	36.6	9.9	3.70	37.92
HH + 5% Leaf	28.9	38.1	10.5	3.63	39.52
HH + 1% Petiole	25.0	41.6	9.1	4.57	42.58
HH + 5% Petiole	35.2	31.6	9.7	3.26	33.06
MH (1)	23.1	42.3	8.6	4.92	43.16
MH (2)	22.7	44.1	10.2	4.32	45.26

TABLE 19. Color measurement of red wine with different treatment after 2 months fermentation.

Treatment	L	a	b	(a/b)	Saturation $(a^2 + b^2)^{1/2}$
HH	25.5	37.7	10.3	3.66	40.46
HH + 1% Leaf + 1% Petiole	30.2	35.8	10.6	3.38	37.33
HH + 5% Leaf + 5% Petiole	25.2	39.6	10.5	3.77	40.96
HH + 1% Leaf	31.7	39.3	12.0	3.27	41.09
HH + 5% Leaf	30.5	40.3	11.2	3.60	41.83
HH + 1% Petiole	30.3	45.0	11.5	3.91	46.45
HH + 5% Petiole	35.7	33.6	9.7	3.46	34.97
MH (1)	25.7	46.8	12.1	3.87	48.34
MH (2)	26.6	45.8	11.0	4.16	47.10

TABLE 20. Analysis of phenolic fraction of Concord juice in different treatments. (mg gallic acid/100 ml)

	Total Phenol	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
HH	194.00	81.375	112.625	110.75	83.25	+0.74
1% Leaf + 1% Petiole	176.70	78.200	98.500	100.70	76.00	-0.60
5% Leaf + 5% Petiole	230.25	100.880	129.370	117.00	113.25	+0.54
1% Leaf	168.90	69.125	101.775	94.80	74.10	+0.40
5% Leaf	220.60	100.100	120.500	124.10	96.5	-0.50
1% Petiole	204.00	82.350	121.650	117.25	86.65	+0.64
5% Petiole	223.50	98.750	124.750	122.10	101.40	+0.50
MH (1) (2.6% MOG)	213.00	90.000	123.000	120.00	93.00	+0.40
MH (2) (2.6% MOG)	218.09	93.590	124.500	122.09	96.00	+0.40

[1]The percent error was calculated as: $\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$

TABLE 21. Analysis of phenolic fractions of red wine after 3 months fermentation. (mg gallic acid/100 ml)

	Total Phenol	Formaldehyde Precipitation		Cinchonine Precipitation		Error %[1]
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
HH	163.5	73.12	90.38	90.0	73.5	+0.90
1% Leaf + 1% Petiole	141.0	65.62	75.38	83.2	57.8	+0.74
5% Leaf + 5% Petiole	186.0	90.38	95.62	102.0	84.0	-0.40
1% Leaf	138.0	63.75	74.25	81.0	57.0	+0.60
5% Leaf	183.0	73.12	109.88	103.0	80.0	+0.37
1% Petiole	172.5	66.75	105.75	98.4	74.1	+0.40
5% Petiole	186.0	74.25	111.75	104.0	82.0	+0.30
MH (1) (2.6% MOG)	177.0	64.50	112.50	94.8	82.2	+0.50
MH (2) (2.6% MOG)	180.0	72.00	108.50	99.0	81.0	+0.50

[1]The percent error was calculated as: $\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$

TABLE 22. Analysis of phenolic fractions of Concord wine after 5 months fermentation.

(mg gallic acid/100 ml)

	Total Phenol	Formaldehyde Precipitation		Cinchonine Precipitation		Error%
		Supernatant (non-flavonoids)	Precipitate (flavonoids)	Supernatant (non-tannin)	Precipitate (tannin)	
HH	135.80	57.00	78.80	79.20	56.60	+0.40
1% Leaf + 1% Petiole	123.69	54.74	68.95	70.49	53.20	+0.40
5% Leaf + 5% Petiole	161.00	70.44	90.56	83.99	77.01	-0.60
1% Leaf	118.23	46.99	71.24	66.56	51.87	+0.74
5% Leaf	150.00	68.06	81.94	84.38	65.62	+0.70
1% Petiole	138.72	56.00	82.72	79.80	58.92	+1.30
5% Petiole	151.98	64.65	87.33	83.03	68.95	+0.60
MH (1) (2.6% MOG)	144.84	61.20	83.64	81.60	63.24	+0.70
MH (2) (2.6% MOG)	148.30	63.54	84.76	83.02	65.28	+0.70

[1]The percent error was calculated as: $\frac{(\text{non-tannin phenolics} + \text{tannin phenolics}) - \text{total phenolics}}{\text{total phenolics}} \times 100$

the same pattern as seen in the Concord juice, with the higher MOG samples containing higher total phenolic, flavonoid, and tannin contents.

Results of the analysis of variance of the sensory assessment data for the Concord wine is shown in Table 23. The values for flavor acceptability, aroma acceptability, bitterness and astringency are all significant at the 1% level, but aroma intensity is not significant. These results differ somewhat from those found for the white wine, which did not show significant difference in bitterness or astringency, probably because of the tannin loss during fermentation. The red wine also lost tannin during fermentation, however, the initial tannin content in the Concord grapes was considerably higher than in the Aurora Blanc fruit and a comparison of the tannin contents of the red and white wine after fermentation will reveal that the red wine had considerably more tannin than the white wine. The F ratio for replications within the categories of flavor acceptability, aroma acceptability, bitterness, and astringency are significant at the 5% level indicating that the panelists were somewhat less consistent in the ratings of the red wine than they were in their ratings for the white wine. Tables 24 and 25 show the taste panel means, standard deviations, and a comparison of means for the factors which were rated by the panelist. In aroma acceptability, HH, MH, HH + 1% Petiole, and HH + 1% Leaf + 1% Petiole had almost the same level of acceptability and were definitely better than HH + 5% Petiole, HH + 5% Leaf and HH + 5% Leaf + 5% Petiole. For flavor acceptability, the panelists indicated that HH had the best flavor acceptability of all the different treatments. In order of acceptability, MH, HH + 1% Petiole, and HH + 1% Leaf + 1% Petiole were ranked next and were definitely better than HH + 5% Petiole, and HH + 5% Leaf. The least acceptable

sample was the HH + 5% Leaf + 5% Petiole. For astringency and bitterness, panelists thought HH, HH + 1% Leaf, and HH + 1% Petiole had the least astringency and least bitterness. When this fact is related to the tannin content after 5 months fermentation, it can be seen that those same samples also had the lowest tannin levels of the nine different treatments. In aroma intensity, the panelists found no differences between the nine treatments.

Juice Sensory Analysis:

Sensory evaluation of Concord grape juice consisted of rating the factors of flavor strength, flavor acceptability and astringency. The means for each of these factors, as rated by 12 panelists for each treatment, are shown in Figures 10 - 12. It can be readily seen that the ratings for each of the juice factors follows the basic pattern which was seen in both the red and white wines, with the HH sample scoring highest and the 5% Leaf plus 5% Petiole sample scoring lowest. In between these extremes, it can be noted for each quality factor that, generally, the panel ratings decreased as MOG increased.

TABLE 23. Analysis of variance mean squares for sensory attributes of red wine.

Source	df.	Aroma Intensity M.S.	Flavor Acceptability M.S.	Astringency M.S.	Aroma Acceptability M.S.	Bitterness M.S.
Total	44	0.78	0.849	0.55	0.87	0.40
Variable	8	$1.13^{N.S.}$	2.59^{**}	1.59^{**}	2.56^{**}	0.78^{**}
Replication	4	$0.78^{N.S.}$	1.28^{*}	1.04^{*}	1.88^{*}	1.50^{*}
Error	32	0.69	0.36	0.23	0.23	0.17

** = Significance at 1% level

* = Significance at 5% level

N.S. = Non-significant F ratio

TABLE 24. Means and standard deviations of certain quality characteristics for sensory evaluation of red wine.

Treatment	Aroma Acceptability	Flavor Acceptability	Aroma Intensity
Hand Harvest (Intact)	7.35 ± 0.53^{a}	6.40 ± 0.29^{a}	6.18 ± 1.25
1% Leaf + 1% Petiole	6.56 ± 0.39^{bcd}	4.82 ± 0.61^{bcd}	4.93 ± 0.57
5% Leaf + 5% Petiole	5.71 ± 0.29^{ef}	4.08 ± 0.26^{d}	5.95 ± 0.38
1% Leaf	6.08 ± 0.92^{de}	4.31 ± 0.62^{cd}	5.25 ± 1.09
5% Leaf	5.26 ± 0.87^{f}	4.32 ± 0.29^{cd}	5.28 ± 0.29
1% Petiole	6.90 ± 0.41^{abc}	4.89 ± 1.02^{bcd}	5.07 ± 0.39
Mech. Harvest (1)	7.12 ± 0.33^{abc}	5.04 ± 0.60^{bc}	4.90 ± 0.52
Mech. Harvest (2)	7.22 ± 0.20^{ab}	5.49 ± 0.52^{b}	4.87 ± 1.08

a - f = Averages followed by the same letter are not significantly different at the 5% level of probability (Duncan, 1957).

TABLE 25. Means and standard deviation for sensory evaluation of astringency and bitterness in red wine.

Treatment	Astringency	Bitterness
Hand Harvest (Intact)	6.20 ± 0.82^{a}	6.84 ± 0.24^{a}
1% Leaf + 1% Petiole	5.37 ± 0.20^{bc}	6.04 ± 0.28^{b}
5% Leaf + 5% Petiole	5.05 ± 0.51^{bcd}	5.83 ± 0.44^{b}
1% Leaf	5.68 ± 0.37^{d}	6.63 ± 0.24^{a}
5% Leaf	4.46 ± 0.58^{d}	5.60 ± 0.69^{b}
1% Petiole	4.59 ± 0.72^{d}	5.86 ± 0.59^{b}
5% Petiole	4.94 ± 0.43^{cd}	5.97 ± 0.40^{b}
Mech. Harvest (1)	4.72 ± 0.22^{cd}	6.04 ± 0.50^{b}
Mech. Harvest (2)	4.81 ± 0.45^{cd}	5.95 ± 0.83^{b}

a - d = Average superscripted by the same letter are not significantly different at the 5% level of probability (Duncan, 1957).

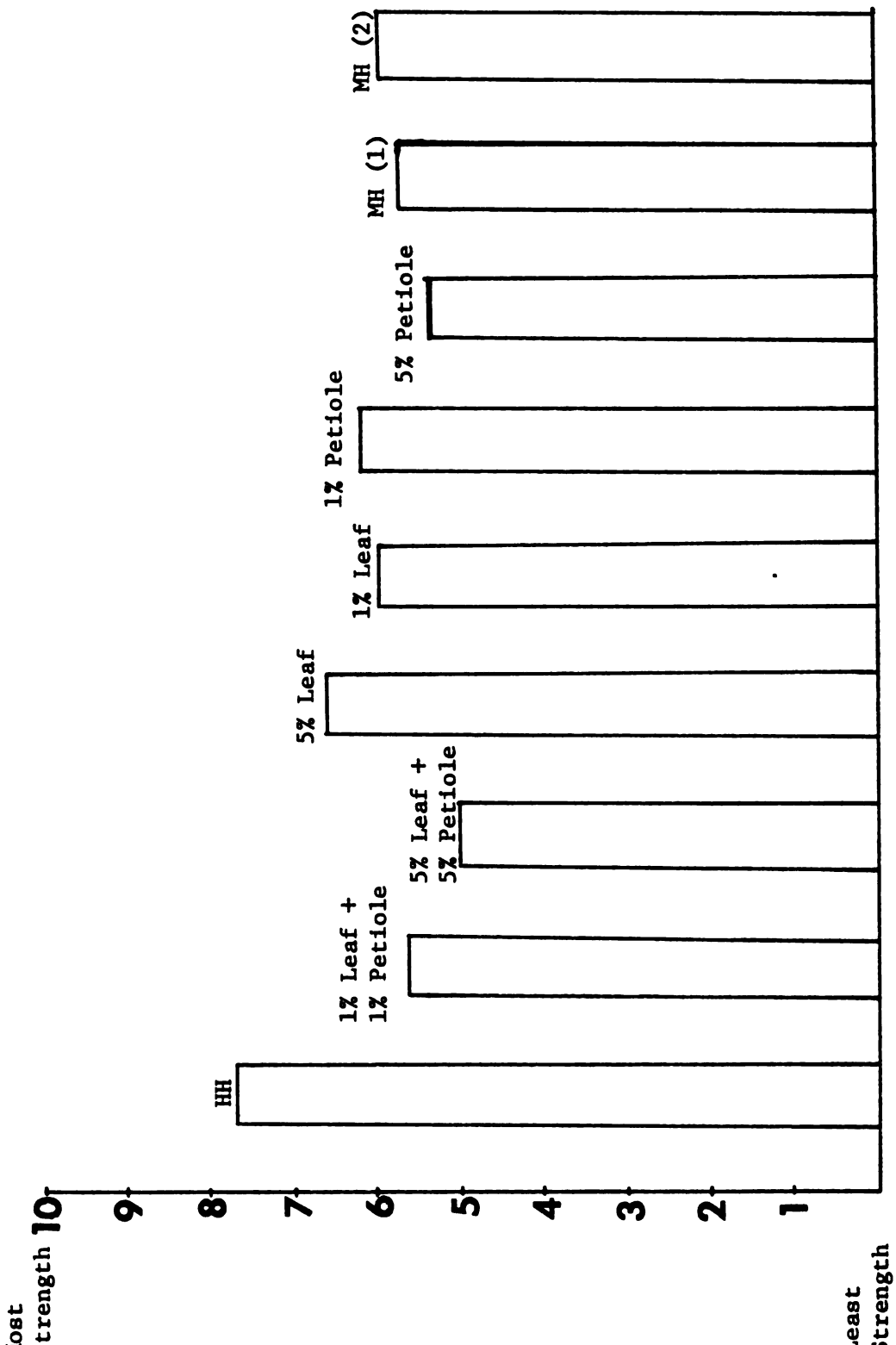


Figure 10. Mean of flavor strength for sensory evaluation of Concord juice.

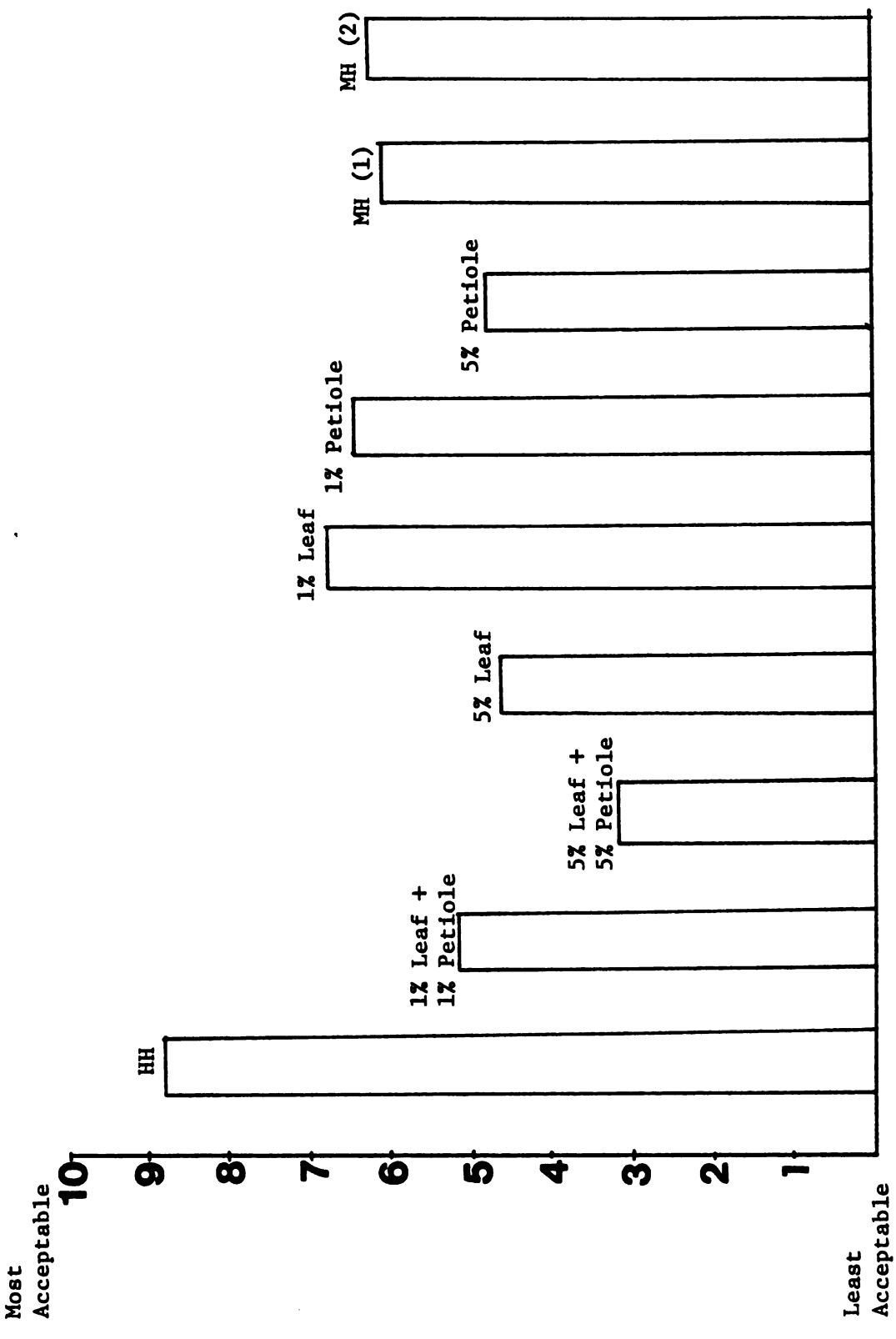


Figure 11. Mean of flavor acceptability for sensory evaluation of Concord juice.

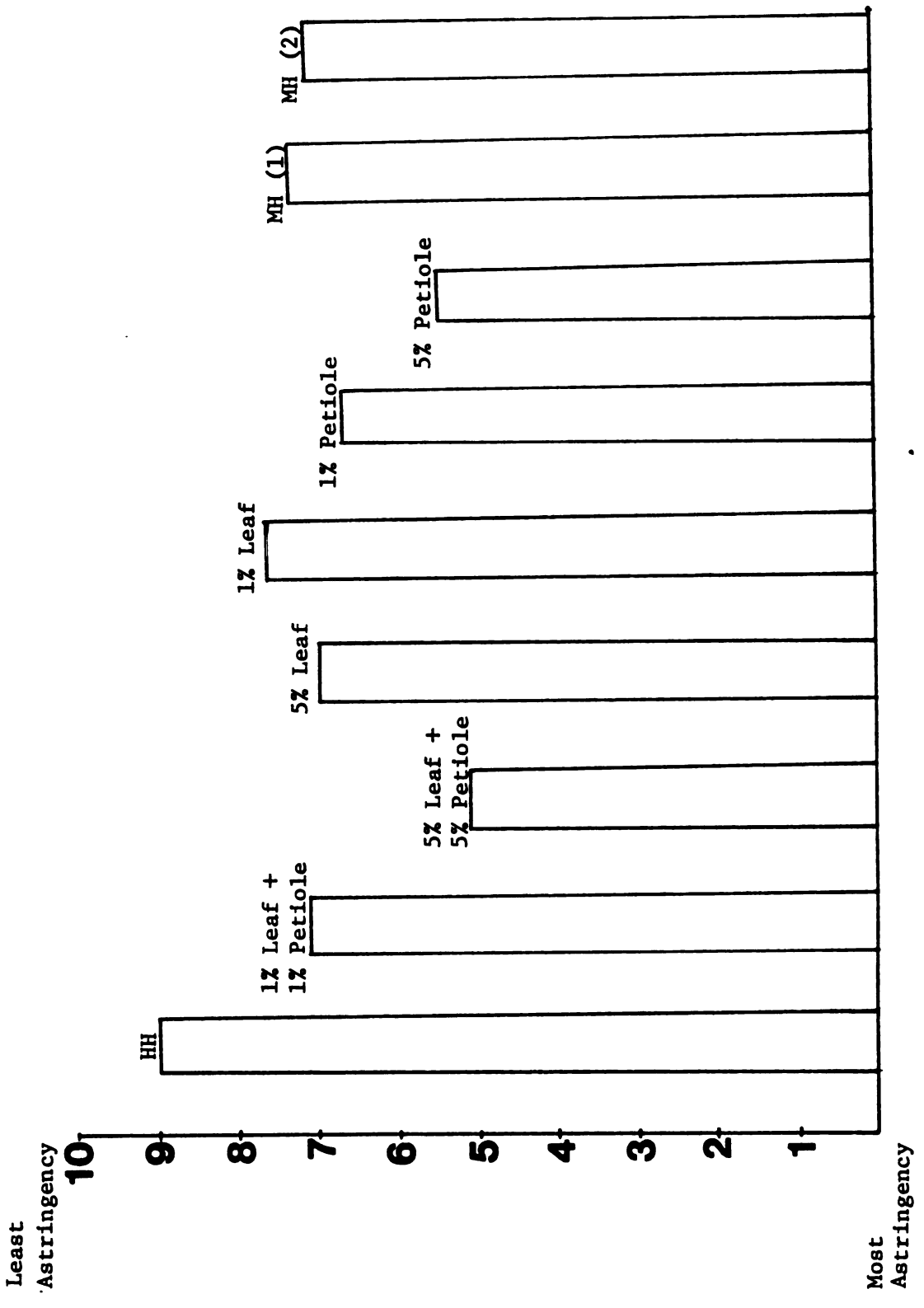


Figure 12. Mean of astringency for sensory evaluation of Concord juice.

SUMMARY AND CONCLUSIONS

This study indicates that MOG content has a definite affect on the chemical constituents and final quality of juice and wine. The total phenolic, flavonoid and tannin contents of wine and juice as MOG content in the harvested grapes increases.

The quality of white wine made from mechanically harvested Aurora Blanc grapes was not as good as that made from hand harvested grapes and it was felt that this was due to the 4.7% MOG content of the mechanically harvested samples, although some of the differences could possibly have been due to maturity differences between the samples.

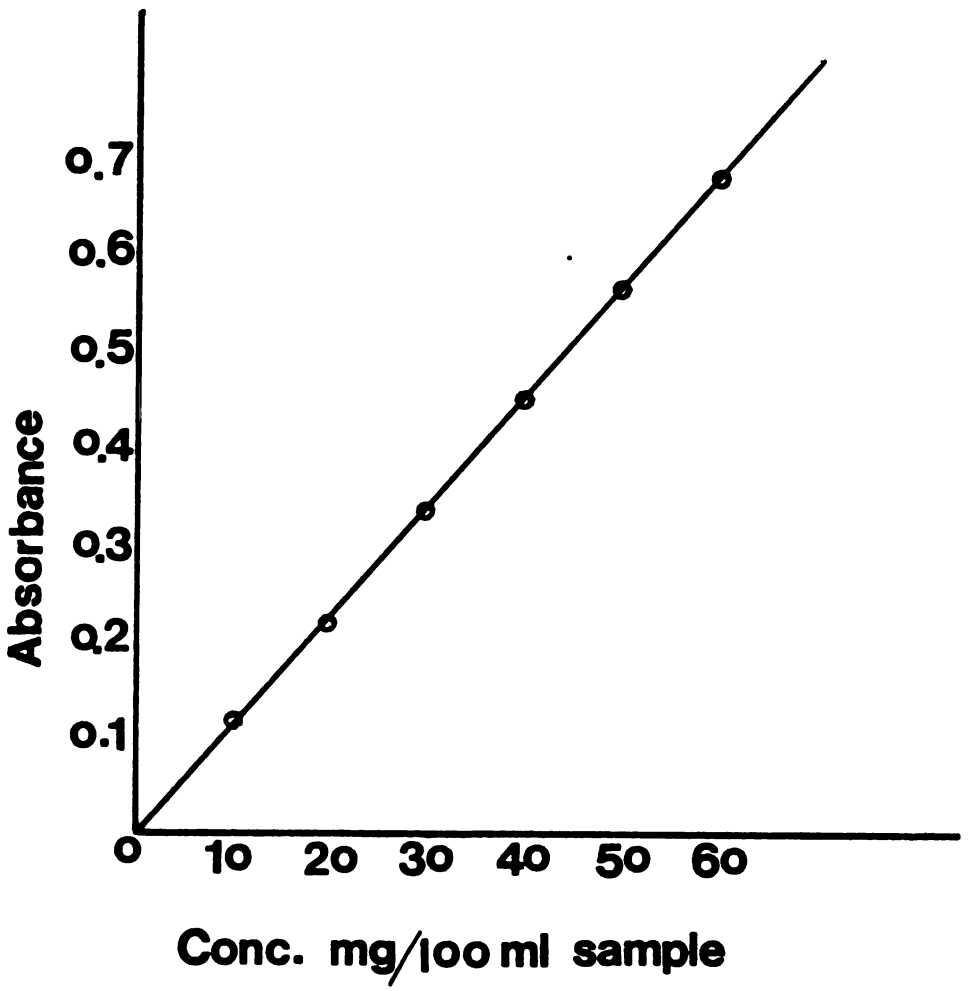
The MOG content of mechanically harvested Concord grapes contained only 2.6% MOG and many of the sensory qualities (i.e. flavor and aroma) were heartier and more pronounced in the final products from this fruit than those white Aurora Blanc grapes. However, it was found that MOG also adversely affected the quality of juice and wine made from the Concord grapes. The samples with added leaf tissue were less acceptable than those which only contained petiole tissue. This was probably due to the greater surface area presented by the leaf tissue, which allowed for more leaching of the leaf constituents into the wine, so leaf tissue seemed to have a greater effect than petiole tissue.

APPENDICES

APPENDIX I

Gallic Acid Standard Curve

A=765nm



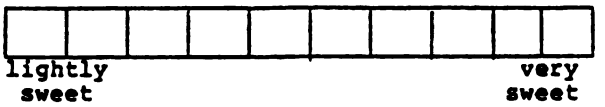
APPENDIX II

GRAPE JUICE TASTE PANEL

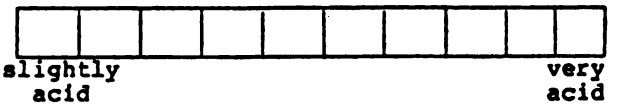
Name _____ Sample No. _____ Date _____

Each characteristic increases in intensity from left to right. Rate each of the characteristics by tasting the grape juice and then placing an "X" in the appropriate box.

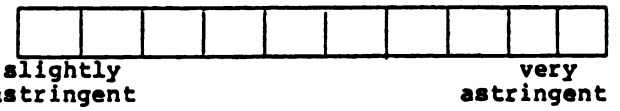
SWEETNESS



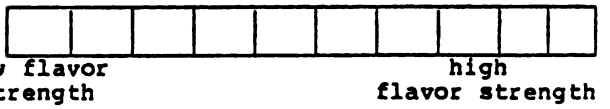
ACIDITY



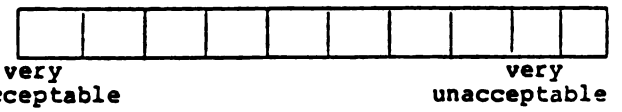
ASTRINGENCY (see below)



FLAVOR STRENGTH



FLAVOR ACCEPTABILITY



NOTE: If you are unsure about the astringency factor you may wish to first taste the very astringent sample which is provided.

APPENDIX III

WINE TASTE PANEL

Name_____ Sample No. _____ Date _____

Each characteristic increases in intensity from left to right. Rate each characteristic by first smelling then tasting the sample and placing an "X" in the appropriate box.

SMELL

Aroma Intensity

imperceptible very strong

Aroma Acceptability

pleasant unpleasant

TASTE

Acidity

slightly acid very acid

Astringency

slightly astringent very astringent

Flavor Strength

low flavor strength high flavor strength

Flavor Acceptability

very acceptable very unacceptable

Aftertaste

no aftertaste strong aftertaste

Bitterness

slightly bitter very bitter

*Astringency (if you are unsure about the astringency factor you may wish to first taste the very astringent sample which is provided).

LIST OF REFERENCES

CITED REFERENCES

Amerine, M.A., and Bailey, C.B. 1959. Carbohydrate content of various parts of the grape cluster. Am. J. Enol. Viticult. 10:196-198.

Ash, C. 1952. Reminiscences of pre-prohibition days. Proc. Am. Soc. Enol. 3:39-44.

Bate-Smith, E.C., and Swain, T. 1953. Identification of leucoanthocyanins as tannin in foods. Chem. & Ind. 1953, 377-378.

Beridze, G.I., and Sirbiladze, M.G. 1964. Nitrogenous substances in vines and wines. Chem. Abstr. 59:10735 (1965).

Black, J.M., Brown, G.K., Marshall, D.E., and Cargill, B.F. 1977. A mechanical system for grape trash removal. Annual Meeting of the American Society of Agricultural Engineers. June 26, 1977.

Boettinger, C. 1901. Studien über Weingildung. 4. Die im Wasser löslichen Bestandteile der Weintraubenblatter. Chem. Ztg. 52:6-8, 17-18, 24-25.

Castor, J.G.B. 1953. The free amino acids of musts and wines. I. Microbiological estimation of 14 amino acids in California musts. Food Res. 18:139-145.

Chaudhary, S.S., Webb, A.D., and Kepner, R.E. 1968. GLC investigation of the volatile compounds in extracts from Sauvignon Blanc wines from normal and botrytised grapes. Am. J. Enol. Viticult. 19:6-12.

Chelle, J.L. 1925. L'acide salicylique et l'acide benzoique dans les vins normaux. Ann. Fals. Fraudes. 18:134-148.

Dekker, J. 1913. "Die Gerbstoffe. Botanische-Chemische Monographie der Tannide," p. 636. Gebrüder Borntraeger, Berlin.

Diemair, W., Janecke, H., and Krieger, G. 1951. Über eine Methode der Gerbstoffbestimmung in der Rebe und im Wein. I.Z. Anal. Chem. 133:346-352.

Durmishidze, S.V. 1955. "Dubil'nye Veshchestva i Antotsiany Vinogradnoi Lozi i Vina," p. 323. Izda. Akad. Nauk. SSSR, Moscow.

Durmishidze, S.V. 1958. Vitamin P v vinograde. Vinodelie i Vinogradarstvo. SSSR 18(2):15.

Ehrlich, F. 1907. Über die Bedingungen der Fuselölbildung und über den Zussammenhang mit dem Eiweissabbau der Hefe. Ber. Deut. Chem. Ges. 40B, 1027-1047.

Geissman, T.A. 1963. Leucoanthocyanins. Plant Phenolics Group N. Am. Proc. 3rd Symp., 1-18.

Genevois, L. 1951. Matières colorantes et viellissement des vins. Rev. Ferment. Inds. Aliment 6, 111-115.

Goldstein, J.L. and Swain, T. 1963. Changes in tannins in ripening fruits. Phytochemistry 2(4):371-383.

Guimberteau, G., and Portal, E. 1961. Contribution á la recherche de l'acide benzoique et des acides-phenols dans les vins. Ann. Fals. Expert Chim. 54:330-337.

Hennig, K., and Burkhardt, R. 1957. Über die Gerbstoffe und Polyphenole der Weine. Naturwissenschaften. 44:328-329.

Hennig, K., and Burkhardt, R. 1958. Über Nachweis Phenolartiger Verbindungen und Hydroaromatischer Oxycarbonsauren in Trauben. Weinberg Keller 5, 542-552. Am. J. Enol. Vitic. 11:64-79.

Johnson, W., and Grgich, M. 1975. Another look at machine harvesting/ wine quality. Wines & Vines. p.40.

Joslyn, M.A. and Goldstein, J.L. 1964. Astringency of fruits and fruit products in relation to phenolic content. Advan. Food Res. 13:179-217.

Joslyn, M.A. and Goldstein, J.L. 1964. Conversion of leucoanthocyanins into the corresponding anthocyanidins. Science 143:954-955.

Jurd, L. 1969. Review of polyphenol condensation reactions and their occurance in the aging of wines. Am. J. Enol. and Vitic. 20:191-195.

Kliewer, W.M. 1966. Sugars and organic acids of Vitis Vinifera. Plant Physiol. 41:923-931.

Kramling, T.E., and Singleton, V.L. 1969. An estimate of the non-flavonoid phenols in wines. Am. J. Enol. and Vitic. 20:86-92.

Laborde, J. 1908-1910. Etude sur les matières tannoides du vin: matière colorante et oenotanin. Rev. Viticult. 30:169-242.

Lingens, F., Goebel, W., and Uesseler, H. 1967. Regulation der Biosynethese der aromatischen Aminosaüren in Saccaromyces cerevisiae. 2. Repression, Induktion und Aktivierung. Eur. J. Biochem. 1, 363-374.

Maier, V.P., Metzler, D.M., and Huber, A.F. 1964. Effects of heat processing on the properties of dates. Date Growers Inst. Rept. 41, 8-9.

Masquelier, J., and Ricci, R. 1964. Chromatographie des dérivés cinnamiques du vin. Qualitas Plant. Mater. Vegetabiles 11:244-248.

Nassar, A.R., and Kliewer, W.M. 1966. Free amino acids in various parts of Vitis vinifera at different stages of development. Proc. Am. Soc. Hort. Sci. 89:281-294.

Noble, A.C., Ough, C.S., and Kasimatis A.N. 1975. Effect of leaf content and mechanical harvest on wine quality. Annual Meeting of the American Society of Enologists, San Francisco, Cal., June 27, 1975.

Ough, C.S. 1969. Substances extracted during skin contact with white musts. I. General wine composition and quality changes with contact time. Am. J. Enol. Vitic. 20:93-100.

Ough, C.S., Berg, H.W., and Coffelt, R.J. 1971. The effect on wine quality of simulated mechanical harvest and gondola transport of grapes. Am. J. Enol. Vitic. 22:65-70.

Pacottet, P. 1904. Rôle des pépins, des pellicules, des rafles en vinification. Rev. Viticult. 22(568)458-488; (571)581-584.

Pellet, H. 1906. L'acide salicylique; propriétés, recherche et dosage; de la présence normale de l'acide salicylique dans le règne végétal; la question des vins portugais (1903).

Peri, C., and Pompei, C. 1971. An assay of different phenolic fractions in wine. Am. J. of Enol. Vitic. 22:55-58.

Petrucci, V.E., Siegfried R. 1975. The extraneous matter in mechanically harvested wine grapes. Annual meeting of the American Society of Enologists, June 27, 1975.

Pifferi, P.G. 1965. Thin-layer chromatography on silica gel G of some phenol carboxylic acids. Vitis 5(1):24-26.

Ponte, A., and Gualdi, G. 1931. Tannin in wines and its estimation. Boll. Uffic. Staz. Sper. Ind. Pelli Mat. Concianti, 391-407; Chem. Abst. 26:3326 (1932).

Ribéreau-Gayon, J., and Ribéreau-Gayon, P. 1958. The anthocyans and leucoanthocyans of grapes and wines. Am. J. Enol. 9:1-9.

Ribereau-Gayon, P. 1963. Les acides-phénols de Vitis vinifera. Compt. Rend. 256:4108-4111.

Rice, A.C. 1965. Identification of grape varieties. J. Assoc. Offic. Agr. Chemists. 47:671-676.

Robinson, B.W., Bertino, J.J., and Whitecombe, E.J. 1966. Objective measurement and specification of color in red wines. Am. J. Enol. Viticult. 17:118-125.

Shewfelt, A.L. 1966. The nature of the anthocyanin pigments in South Carolina Concord grapes. S. Carolina Agr. Expt. Sta. Tech. Bull. 1025, 1-14.

Singleton, V.L. 1967. Fining-phenolic relationships. Wines and Vines. 3:23-26.

Singleton, V.L. 1966. The total phenolic content of grape berries during the maturation of several varieties. Am. J. Enol. Viticult. 17:126-134.

Singleton, V.L., and Esau, P. 1969. "Phenolic Substances in Grapes and Wine, and Their Significance". Academic Press, New York and London.

Singleton, V.L., and Rossi, J.A., Jr. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16:144-158.

Stevens, K.L., Bomben, J.L., and McFadden, W.H. 1967. Volatiles from grapes. Vitis Viniferra (Linn.) Cultivar Grenache. J. Agr. Food Chem. 15:378-380.

Thudichum, J.L.W., and Dupre, A. 1872. "A Treatise on the Origin, Nature, and Varieties of Wine: Being a Complete Manual of Viticulture and Oenology," p. 760. MacMillan, New York.

Traphagen, F.W., and Burke, E. 1903. Occurrence of salicylic acid in fruits. J. Am. Chem. Soc. 25:242-244.

Webb, A.D. 1962. Present knowledge of grape and wine flavors. Food Technol. 16:56-59.

Winkler, A.J., and Amerine, M.A. 1938. Color in California wines. I. Methods for measurement of color. Food Res. 3: 429-438.

Yamamoto, A., Omori, T., and Yasui, H. 1966. Flavers in sake. IX. The precursor of p-hydroxybenzaldehyde and p-hydroxybenzoic acid in sake. Nippon Nogeikagaku Kaishi 40, 152-160. Chem. Abstr. 64:18357(19669).