AN ABSTRACT OF THE THESIS OF

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Abstract Approved:

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The possible effect of oxidation [processing with or without sulfur dioxide ($\pm SO_2$)] and of pre-ultrafiltration treatment of juices with enzymes and fining agents on flux, and on juice color, composition and stability was investigated. White Riesling juice was ultrafiltered with a Romicon system operated with a nominal molecular weight cut-off (MWCO) of 10,000 daltons. In addition, UF White Riesling juices processed $\pm SO_2$, were stored for 2 months (1985 vintage) and 12 months (1984 vintage) at 2 °C and 20 °C, and evaluated by a trained panel using descriptive analysis. Pre-UF treatment with enzymes and fining increased flux. Sediment formation and instability to heat testing of UF permeates processed $-SO_2$ was prevented with pre-fining. Up to 99% of protein, 90% of pectin, 84% of color and low variable phenolics were retained by the membrane of 10,000 dalton MWCO. During UF there is a significant increase in the soluble protein and water soluble pectin passing through the membrane with increasing volume concentration ratio (VCR, process time). It is concluded that it is not only the amount but the nature/state of compounds such as proteins, phenolics, pectins, and their interaction that results in instability. UF juices processed with minimum oxidation and stored for 12 months had lower intensity aroma (apple/apple cider, sweet, and honey/caramel) and overall intensity flavor by mouth descriptors than those

processed with oxidation. Moreover, juices processed with minimum oxidation and stored for two months (1985 vintage) had significantly lower intensity of apple/apple cider, sweet, honey/caramel aroma descriptors when compared to those processed with oxidation. There was no effect of temperature of storage on any of the aroma and flavor-by-mouth descriptors for the 1985 juice after two months of storage. Only one aroma descriptor (vegetative) was significantly increased for the 1984 White Riesling juice after 12 months at 20 °C. This indicates the possibility that UF juices may be stored at higher temperature (20 °C) for less cost with minimal changes in aroma and flavor.

White Riesling (WR) and Gewurztraminer (GEW) wines were ultrafiltered with Romicon and Millipore pilot-scale systems, respectively. The effect of ultrafiltration (UF), membrane MWCO from 10,000-50,000 daltons, and of VCR on composition and wine stability was investigated. The effect of 1) pilot-scale UF processing and Bentonite fining on WR and GEW wines, and 2) commercial-scale UF processing on GEW wine was sensorially evaluated by a trained panel using descriptive analysis. UF processing significantly reduced color (A_{420nm}), total phenol, protein and heat/cold test (HCT) haze of both WR and GEW wines. Stability to HCT haze formation was obtainable with MWCO of 10,000 daltons, but trace instability can remain. With increasing VCR (process time) there was a significant decrease in membrane retention of color (A_{420nm}), protein, and HCT haze formation in the WR wine and the color (A_{420nm}) of the GEW wine. UF processing of the WR wine significantly decreased the perception of overall aroma intensity, fruity, fresh fruity citrus, floral, sweet and honey/caramel character but it also increased the intensity of the vegetative aroma descriptor when compared to the control unfiltered WR wine. In addition, significant differences were detected for these descriptors between the bentonite-fined WR wine and the ultrafiltered WR wine except for fresh fruity citrus and honey/caramel which were less intense in the ultrafiltered WR

ultrafiltered WR wine. UF processing of GEW wine significantly decreased the intensity of fruity, fresh fruity aroma descriptors; and increased the chemical aroma descriptor compared to the control unfiltered GEW wine. However, no significant differences were detected for these descriptors between the bentonite fined GEW wine and the ultrafiltered GEW wine except for fresh fruity which was less intense in the ultrafiltered GEW wine. Commercial processing of GEW wine by UF did not have any significant effect on the aroma and flavor by mouth descriptors evaluated as compared to standard processing procedures.

Ultrafiltration of Fruit Juice and Wine.

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TABLE OF CONTENTS

Page

Introduction 1

Review of Literature 3

Grape Juice Composition 3
Proteins 3
Phenolic Substances 4
Pectins 5
Colloidal Constituents 6
Enzymes 6
Juice and Wine Instability 8
Stabilization of Juice and Wines 10
Conventional Procedures 11
Ultrafiltration 11
Sensory Evaluation of Wine 15
Effect of Ultrafiltration (UF) on Juice and Wine Quality 17

ULTRAFILTRATION (UF) OF WHITE RIESLING JUICE: EFFECT OF OXIDATION AND OF PRE-UF TREATMENT ON FLUX, COMPOSITION AND STABILITY 19

Abstract 21

Introduction 22

Materials and Methods 23
Preparation of Grape Juices 23
Pre-UF Treatments 23
Ultrafiltration Process 23
Determination of Total Phenol and Soluble Protein 24
Color (browning) and Heat Stability Test 24
Determination of Total Pectin 24
Data Analysis 25

Results and Discussion 26
Effect of Pre-UF Treatments and VCR on Flux 26
Effect of Pre-UF Treatments on Color (browning), Total Phenol, Soluble Protein, Water Soluble Pectin, and Heat/Cold Test Haze of the Juices 29
Effect of VCR on Color (browning), Total Phenol, Soluble Protein, Water Soluble Pectin, and Heat/Cold Test Haze of Ultrafiltered Juices 37
Stability of the Ultrafiltered Juices 41

Page

Conclusions 43

Literature Cited 44

ULTRAFILTRATION OF WHITE RIESLING JUICE: SENSORY EVALUATION OF JUICE PROCESSED WITH AND WITHOUT SULFUR DIOXIDE AND STORED AT 2° AND 20° C 47

Abstract 49

Introduction 50

Materials and Methods 51
Preparation of Grape Juices 51
Ultrafiltration Process 51
Sensory Procedures 51
Sensory Design and Data Analysis 52

Results and Discussion 54
White Riesling Juice 1984 Vintage 54
White Riesling Juice 1985 Vintage 57

Conclusions 60
Effect of Sulfur Dioxide (SO_2) 60
Effect of Storage Temperature 60

Literature Cited 61

ULTRAFILTRATION OF WINE: EFFECT OF ULTRAFILTRATION ON WHITE RIESLING AND GEWURZTRAMINER WINE QUALITY AND STABILITY 63

Abstract 65
Introduction 67

Materials and Methods 68
Preparation of Wines 68
Ultrafiltration of Wines 68
Bentonite fining 69
Determination of Total Phenol and Soluble Protein 69
Color (browning) and Heat Stability Test 69
Wine Processing Data Analysis 69
Sensory Analysis 70
Sensory Data Analysis 72

Results and Discussions 74
Effect of MWCO and VCR on Flux 74

Page

Effect of UF and MWCO on Color (browning),Total Phenol, Soluble Protein, and Heat/Cold Test Haze Formation of White Riesling and Gewurztraminer Wines 76
Practical Storage Stability of the Ultrafiltered Wines 82
Effect of VCR on Color (browning), Soluble Protein, and Heat/Cold Test Haze of Ultrafiltered Wines 84
Sensory Analysis 88
White Riesling (WR) Wine 88
Gewurztraminer (GEW) Wine 91
Commercial Gewurztraminer Wine 93

Conclusions 95
Processing 95
Sensory Analysis 96
Pilot Scale 96
Commercial Scale 96

Literature Cited 97

Bibliography 100

LIST OF FIGURES

FIGURE		Page
II.1	Effect of Pre-UF Treatment on Flux of White Riesling Juice	27
II.2	Effect of SO_2, Pre-UF Treatment, and UF on Color (browning) of White Riesling Juice	30
II.3	Effect of SO_2 and Pre-UF Treatment on Total Phenols of White Riesling Juice	32
II.4	Effect of SO_2, Pre-UF treatment, and UF on Soluble Protein of White Riesling Juice	33
II.5	Effect of SO_2 and UF on Water Soluble Pectin of White Riesling Juice	36
II.6	Effect of Volume Concentration Ratio on Color (browning) of White Riesling.Juice	38
II.7	Effect of Volume Concentration Ratio on Soluble Protein of White Riesling Juice	39
II.8	Effect of Volume Concentration Ratio on Water Soluble Pectin of White Riesling Juice	40
IV.1	Effect of Membrane Molecular Weight Cut-Off on Flux of White Riesling Wine	75
IV.2	Effect of UF and Membrane Molecular Weight Cut-Off on Color (browning) of White Riesling Wine	78
IV.3	Effect of UF and Membrane Molecular Weight Cut-Off on Soluble Protein and Heat/Cold Test Haze Formation of White Riesling Wine	79
IV.4	Effect of UF and Membrane Molecular Weight Cut-Off on Soluble Protein and Heat/Cold Test Haze Formation of Gewurztraminer Wine	80
IV.5	Effect of Volume Concentration Ratio and Membrane Molecular Weight Cut-Off on Color (browning) of White Riesling Wine	84
IV.6	Effect of Volume Concentration Ratio and Membrane Molecular Weight Cut-Off on Color (browning) of Gewurztraminer Wine	86

FIGURE Page

IV.7 Effect of Volume Concentration Ratio and Membrane Molecular Weight Cut-Off on Soluble Protein and Heat/Cold Test Haze Formation of White Riesling Wine..... 87

LIST OF TABLES

TABLE		Page
II.1	Analysis of Variance, Degrees of Freedom (DF), and F-ratios of UF Flux of White Riesling Juice	26
II.2	Analysis of Variance, Degrees of Freedom (DF), and F-ratios for Color (browning), Total Phenol, Soluble Proteins, and Water Soluble Pectin of White Riesling Juice	29
II.3	Analysis of Variance, Degrees of Freedom (DF), and F-ratios for Color (browning), Total Phenol, Soluble Proteins, and Water Soluble Pectin of White Riesling Juice During Ultrafiltration (process time, VCR)	37
II.4	Effect of Pre-UF Juice Treatment on Clarity and Stability of White Riesling Juice Before and After Ultrafiltration	42
III.1	Aroma and Flavor-by-Mouth Descriptors and Standards Developed for the Two Vintages of White Riesling Juice	53
III.2	Analysis of Variance of the Significant Aroma and Flavor-by-Mouth Descriptors for the 1984 White Riesling Ultrafiltered Juice	55
III.3	Mean Scores of the Statistically Significant Sensory Descriptors of the 1984 White Riesling Juice	56
III.4	Analysis of Variance of the Significant Aroma and Flavor-by-Mouth Descriptors for the 1985 White Riesling Ultrafiltered Juice	58
III.5	Mean Scores of the Statistically Significant Sensory Descriptors of the 1985 White Riesling Juice	59
IV.1	Aroma Descriptors and Standards Developed for White Riesling and Gewurztramier Wines	71
IV.2	Flavor-by-Mouth Descriptors Developed for White Riesling and Gewurztramier Wines	72

TABLE Page

IV.3 Analysis of Variance, Degrees of Freedom (DF), and F-ratios for UF Flux of White Riesling and Gewurztraminer Wines 74

IV.4 Analysis of Variance, Degrees of Freedom (DF), and F-ratios of the Effect of UF, Including MWCO, on Color (browning), Total Phenol, Soluble Proteins,and Heat/Cold Test Haze of White Riesling and Gewurztraminer Wines 77

IV.5 Results of Haze/Sediments Formation in Ultrafiltered Wines 83

IV.6 Analysis of Variance, Degrees of Freedom (DF), and F-ratios for Color (browning), Soluble Proteins, and Heat/Cold Test Haze during UF (Including VCR and MWCO) of White Riesling and Gewurztraminer Wines 85

IV.7 Analysis of Variance of the Significant Aroma Descriptors for White Riesling Wine 89

IV.8 Mean Scores of the Significant Aroma Descriptor Ratings for White Riesling Wine 90

IV.9 Analysis of Variance of the Significant Aroma Descriptors for Gewurztraminer Wine 92

IV.10 Mean Scores of the Significant Aroma Descriptor Ratings for Gewurztraminer Wine 93

ULTRAFILTRATION OF FRUIT JUICE AND WINE

Introduction

Current research and previous investigation in our laboratory over several vintages (Fombin, 1983; Heatherbell *et al.*, 1985) indicates that grape juice processed with oxidation (-SO_2) and clarified by Ultrafiltration (UF) tends to develop hazes and sediments upon storage. These observations have suggested the possibility of the formation of hazes from oxidized polymerized phenolics and proteins (Heatherbell, 1984; Lea, 1984; Matheis and Whitaker, 1984), although pectins, and to a lesser extent other polysaccharides, such as starch and arabans, may contribute to haze and sediment formation (Heatherbel, 1984; Schmitt, 1985).

A major interest of our research group has also been to evaluate the application of UF for the production of clarified, stable grape juice, 1) as a product in its own right, 2) blending into other beverages, and 3) use as "sweet" reserve for back-blending into wines. We have also been investigating the production (and storage) of clarified grape juice by UF with or without low concentrations of sulfur dioxide.

Moreover, in recent years there has been increasing interest internationally in the application of UF for wine processing (Gibson, 1986; Heatherbell *et al.*, 1985; Hsu *et al.*, 1987; Maglioli and Marchesini, 1985; Parrini and Mattera, 1984; Raugon and Bourdier, 1985; Serrano *et al.*, 1984; Wucherpfennig *et al.*, 1984). The possibility of using membrane UF for clarifying and protein stabilization of wine has been recognized (Drioli *et al.*, 1981; Gaillard, 1985; Gnekow *et al.*, 1983; Hsu *et al.*, 1987; Wucherpfennig *et al.*, 1984; Wysocki, 1977).

The three main objectives of this study were to investigate: the effect of oxidation (processing ±SO_2) and of juice pre-UF treatment with enzymes and fining agents on UF juice flux, color (browning), composition (proteins, phenolics, and

pectins) and stability; the sensory evaluation, by a trained panel using descriptive analysis, of the juices processed with and without SO_2, and to evaluate the effect of storage temperature (2°, 20° C) on these juices; and the effect of UF (MWCO) and VCR during processing on composition and stability on White Riesling and Gewurztraminer wines, and the effect of UF on the sensory qualities of these wines.

Literature Review

Grape Juice Composition

Considerable research has been conducted on the chemical composition of fruits commonly used in wines. The variability of fruit composition is well documented, especially for grapes. Factors known to influence the chemical composition include variety, maturity, soil, rootstock, climatic conditions, crop yield, and post harvest handling. Grape juice is the liquid expressed from suitably ripened fruit of the grape. Grape juice differs little in composition from the grapes except for the content of crude fiber and the oils which are primarily present in the seed. Only compositional components of specific interest to this study will be reviewed. These include proteins, pectins, enzymes, and phenolics, which can affect the quality and stability of clarified juices and wines, in particular browning, haze and sediment formation.

Proteins: In grapes, nitrogen containing compounds are found in the form of ammonium cations and as organic compounds - amino acids, peptides and proteins. Grape proteins are complex (Koch and Sajak, 1959) and the amino acids involved and their specific peptide linkages are still unknown. The soluble proteins in grapes and wines are globular, mainly albumins, the nature of which differ somewhat between varieties (Henning, 1970; Koch, 1963). For the White Riesling and Müller-Thurgau varieties they appear to be glucoproteins.

The amount of protein in grape juices and wines is quite variable and depends on several factors. Some of these factors are grape variety, nutritional status, conditions of maturation, time of harvest, season and region (Tarantola, 1971). Amerine *et. al.* (1980) reported that protein content ranges from 10 to 200 mg/L.

There is considerable variation in the molecular weight (MW) of protein fractions; they have been reported to range anywhere from 8,000 to greater than 1,000,000. The highest MW reported was in Australian wines. However, the higher MW fractions only account for a very small percentage of the total proteins (Heatherbell, *et. al.* 1985; Lee, 1986).

Phenolic Substances: Phenolic compounds have been particularly well studied in grapes, since they play an important role in the determination of the character of wines (Ribéreau-Gayon, 1964) to which they constitute the color, taste and "body" during aging.

Phenolics generally constitute derivatives of hydroxylated benzoic and hydrocinnamic acids, tannins and other nonflavonoids, flavonoids (catechins, antocyanogens or condensed tannins), anthocyanins, flavonols and flavones (Singleton and Esau, 1969).

Most grape phenolics in a gross sense are anthocyanogenic tannins, 80% being in the seeds and 20% in the skins. Most phenols of juice are chlorogenic-acid-like (Singleton *et al.*, 1966). On the average about 200 mg/kg phenolic cinnamic acid derivatives are found in the juice, most of the others are located in small amounts in the skins. Singleton and Esau in 1969, reported that the maximum average total phenolic contents for red and for white grape are 5500 and 4000 mg/kg, respectively. Red and white table wines average closer to 1400 mg/L and 250 mg/L, respectively (Singleton and Noble, 1976).

Polymers of catechin are reported to range in MW from 500 to 3,000. Goldstein and Swain (1963) reported MWs of 2,000-5,000 and even 50,000, whereas Ribereau-Gayon and Stone Street (1966a,b) reported 600 to 2,300.

Several factors effect the amount of total phenol found in a wine. Skin and seed contact time, ethanol concentration, fermentation temperature, agitation of juice and skins, intensity of pressing, and grape variety.

Pectins: Polyuronides, the pectic substances found in the cell wall function as an intercellular cement and are present in fruits as complex polymers of galacturonic acid or its methyl ester, with side chains of covalently linked neutral sugars, typically arabinose, rhamnose and galactose (Usseglio-Tomasset, 1959; Plink and Voragen, 1970). In many cases the hydrocolloid character of pectin is particularly important, as in beverages produced from fruits or vegetables, with regard to their viscosity, clarification, cloud and color stability, and flavor retention.

Three distinct classes of pectic substances have been recognized: protopectin, the water insoluble parent pectic substance; pectinic acids or high methoxyl pectins and pectinates; and pectic acids or low methoxyl pectins and pectates. Soluble pectin is found in the juice while insoluble protopectin is strongly bound in the cell wall of the tissue.

The pectin content of the ripe fruit of *Vitis vinifera* grape varieties is reported to range from 0.02 to 0.6% (Amerine *et al.*, 1972; Robertson, 1979). Pure pectin content ranged from 0.12-0.87 g/L, trace to 0.14 g/L, and 0.09-0.54 g/L in grape musts, white wines, and red wines, respectively (Peynaud 1952). The degree of esterification has been reported at an average of 31.4% in Swiss musts (Solms *et al.*, 1952). Contrary to the suggestion of Amerine *et al.*, (1972), Robertson *et al.*, in 1980 reported that the total pectic substances decreased during maturation. In the unhydrolyzed form their MW may be very high. The MW of soluble pectin in grape juice has been reported to range from 6,500 to 500,000.

Colloidal Constituents: Colloidal phenomena is important to understand because it is relative to the stabilization of juice and wine. Grape and wine contain colloids, which are composed of polysaccharides, proteins, and polyphenolics (Nilov *et al.*, 1975; Usseglio-Tomasset and Stefano, 1977). Wine colloids are mainly composed of neutral polysaccharides. These neutral polysaccharides come from three major sources; grapes, yeasts, and *Botrytis cinerea*.. The major polysaccharides from grapes are mainly arabans, galactans, and arabinogalactans; those from yeast are mainly mannans; and when the grapes are infested with *B. cinerea*, a beta glucan can also be found (Villettaz, 1984). Colloids content of 167-324 mg/L in white wines and 963 mg/L in red wine have been reported (Wucherpfennig and Dietrich, 1983).

Enzymes: Certain enzymes in grapes may be resposible for juice and wine disorders such as clouding, darkening (browning), or an oxidized taste. A variety of enzymes have been reported in grape: peroxidase, polyphenol oxidases, catalases, invertase, tannases, ascorbase, dehydratase, esterase, and pectolytic and proteolytic enzymes (Gallander, 1974; Amerine *et al.*, 1980). Of particular interest to this study are polyphenol oxidase and pectolytic enzymes.

Polyphenol oxidase (PPO) catalyzes two quite different types of reactions, both of which involve phenolic compounds. These reactions involve hydroxylation of monophenols to give *o*-diphenols, and the removal of hydrogens from *o*-diphenol to give an *o*-quinone (Scott, 1975; Matheis and Whitaker, 1984). The oxidation of an o-diphenol produces yellow- or red-colored quinones. These in turn react with more oxygen to produce brown-colored condensation products.

Although enzymatic browning may occur in wine, enzymes play a minor role in the darkening of wines. However, this is contrary to fresh juice where enzymatic browning usually occurs rapidly, and its control is important in producing a high

quality product, particularly for white wines. Browning can be prevented or minimized by materials that form complexes with quinones. The most commonly used inhibitor is sulfite, either in the form of SO_2 or $NaHSO_3$. Browning may also be prevented by heat inactivation of the enzyme or by exclusion of atmospheric oxygen.

Ivanov reported that PPO activity was high in grape skins and low in the juice of mature grapes (Gallander, 1974). He also showed that varieties varied in the amount of PPO activity. This was latter supported by Cassignard and Poux (Gallander, 1974). The MW of grape PPO has not been reported. However, the MW for each of the three fractions of PPO in apple is of 30,000-40,000, 60,000-70,000 and 120,000-130,000 (Harel and Mayer, 1968). True isozymes have been reported to occur in grapes (Harel *et al.*, 1973).

Practices which reduce the undesirable effects of PPO include selecting the proper variety, obtaining sound and mature fruit, pressing not too long or hard, centrifuging or settling, and bentonite fining.

The occurrence of pectolytic enzymes (pectin methyl-esterase, polygalacturonase) has been demonstrated in grapes (Marteau, 1967). However, Hobson in 1962 could not demonstrate polygalacturonase activity in grapes, which may have been due to its inactivation during extraction or the presence of inhibitors (Dilley,1970). The pectin methyl-esterase activity is generally twice as high in the skin as in the pulp, but is much less in the seeds. The activity of pectin methyl-esterase and polygalacturonase steadily increases during ripening (Peynaud and Ribéreau-Gayon, 1971). The action of pectin methyl-esterase is to remove the methoxyl groups from methylated pectic substances (pectin). The galacturonases hydrolyze the $\alpha(1\text{-}4)$ linkages between D-galacturonic acid units.

Enzymes are widely used commercially for the treatment of fruit pulps to expedite handling, in particular to facilitate extraction and pressing, to increase juice yield and to clarify the juice or wine obtained from the fruit (Neubeck, 1975,

1981). The commercial enzyme preparations commonly used in the processing of fruits are mostly mixtures of pectinases, cellulases and hemicellulases (Voragen and Plink, 1981). Therefore, the effectiveness of commercial pectinase preparations in some applications is influenced by the "side activities" of other enzymes present such as cellulases and hemicellulases.

The first report on the use of pectolytic enzymes for the clarification of fruit juices was by Kertesz in 1930; Since then a relatively large number of commercial pectic enzyme preparations have been used successfully for the clarification of apple juice as well as other juices.

Initially pectic enzymes were added to wines in order to obtain more rapid clarification or as a desperate attempt to clarify wines that did not respond to clarification utilizing bentonite or gelatin. Pectic enzymes are now widely used, but they have not been universally adopted for clarification of wines. Observers generally agree that wines prepared from pectic enzyme-treated grapes clarify more rapidly, and in addition, most of the workers report increases in free run juice or wine as well as in total juice or wine.

Juice and Wine Instability

Koch and Sajak (1959) reported that heat-formed sediment as well as protein haze in wines mainly contained protein, but also contained pectin, tannins and inorganic constituents. Ribereau-Gayon *et. al.* (1976) described that protein haze contains 5-12% nitrogen (of which 50-80% is protein), 1-15% ash, and the remainder being divided between adsorbed phenolics (2-5%) and polysaccharides (12-14%). However, higher proportions of the protein component (40-50%) have been reported by Moretti and Berg (1965); and Usseglio-Tomasset (1978). Usseglio-Tomasset (1978) also indicated that protein haze in wine is mainly due to the interactions between proteins, tannins, carbohydrates and pectins.

Hazes and precipitates often form in foods because of changes that decrease the solubility of one or more of the solutes in the fluid. The most common factor resulting in haze or precipitation is a decrease in temperature. However, changes in pH such that the isoelectric point (pI)of a protein(s) is approached result in a decrease in solubility. It has been shown that wines with a pH close to 3.0 are more likely to be heat stable than wines of higher pH value (Moretti and Berg, 1965; Bayly and Berg, 1967). The pI of proteins from various wines have been reported to fall in the range of 2.5-8.7 (Lyubarevich *et al.*, 1975; Anelli, 1977; Yokotsuka *et al.*, 1977; Luis, 1983; Heatherbell *et al.* 1985; Lee, 1986). In general, the wine protein which precipitates first is likely to be the largest, most denatured, and nearest to its pI. Its precipitation may be induced by metals or oxidized phenolics (Singleton, 1974).

Denatured proteins are less soluble than native proteins because their hydrophobic groups, in the interior of the native protein, are exposed to the aqueous solvent. This leads to aggregation of the denatured molecules, resulting in decreased solubility. Limited proteolytic enzyme hydrolysis (1-2% of peptide bonds) of proteins often leads to decreased solubility. This is probably due to a disruption of the tertiary structure, leading to more exposure of the hydrophobic side chain residues of the amino acids. Large peptide fragments then aggregate, with a resulting decrease in solubility. Further proteolysis increases the solubility since the smaller peptides cannot form aggregates large enough to be insoluble (Whitaker, 1984).

Simpler phenolics, such as chlorogenic acid, have little affinity for proteins, but under the influence of heat, oxygen, or simply storage of juice and wine, can polymerize (tannins) and thereby contribute to turbidity on their own or by complexing with proteins (Heatherbell, 1984; Lea, 1984; Van Buren, 1978, 1983).

Protein-tannin complexation often leads to decreased solubility and precipitation. The formation of polymeric compounds due to the oxidation of a protein-tannin complex can easily lead to flocculation and haze formation (Heatherbell, 1984). Tannins can compete with proteins for the water solvation. The phenolic hydroxyl group of tannin, through hydrogen bonding, dislodges the hydrophilic moiety of the protein and is sufficient to bring about precipitation. It has also been proposed that the positive charge of the proteins is neutralized by the negative charge of the tannins, resulting in the formation of a complex which flocculates and forms a haze (Ferenczy, 1966; Somers and Ziemelis, 1973a, Loomis, 1974; Thoukis, 1974).

The involvement of pectins in haze and sediment formation is well established. The protective colloidal action of pectins causes small particles to remain suspended and results in cloudy juice or wine. Yamasaki *et. al.* (1967) demonstrated that pectin surrounds central positively charged core material including cation, protein-phenolic complexes, leaving the carboxyl negative charge of the pectin oriented to the outside. Mutual charge repulsion of these particles retains them in suspension.

Stabilization of Juice and Wines

Stability of juice and wine is a very relative term and may denote various things to different people. Today's demand is for brilliantly clear juice (if clear type) and wines. The presence of sediment or the development of haze during marketing is generally considered by consumers as evidence of incipient spoilage or instability.

Clarification is defined as the process to ensure the production of juice or wine products sufficiently clear for consumer acceptance and which will stay clear. Although this product may not be completely free of sediment, fermentation should be prevented and certain pathogenic micro-organisms suppressed or eliminated without affecting the aroma or flavor of the product. Stabilization, is theproduction of a product which will withstand exposure to the temperature of household

refrigerators, the hot air of retail stores, and the light and heat of the sun when the product is displayed in windows (Joslyn and Amerine, 1964).

Conventional Procedures: Several processes may be employed in clarification and they can be divided into the following categories: 1) Physical processes: including settling, centrifugation, and filtration; 2) Biochemical processes-enzymes: including pectinases, amylases, proteases, arabinases, and glucanases; 3) Chemical processes: fining (bentonite, gelatin, tannin, isinglass, casein, and egg white). Unfortunately, none of the above procedures alone has been found to be sufficient to clarify and stabilize juices or wines. Therefore, it is a necessity to use these processes in a complementary manner, with a judicious application of fining agents in conjunction with prior enzymatic juice treatment for removal of colloidally dispersed haze forming compounds and particles (Heatherbell, 1984).

Ultrafiltration: Traditional methods of juice and wine production involve several batch operations which are labor and time consuming. Therefore, a simpler means of producing clarified juice and wine is desirable and ultrafiltration (UF) provides a reasonable solution. UF is a pressure-driven membrane filtration process used for the separation and/or purification of dissolved or suspended particles. The pore size of a UF membrane is typically in the range of 10-200 Angstroms (0.001-0.02 microns). This is sufficient to produce economical throughputs while filtering out all suspended material as well as bacteria, yeast, and some haze precursors. An important feature of UF is that it is a cross-flow process. This technique - wherein unfiltered juice or wine flows parallel or tangential to the membrane surface - promotes a sweeping action that keeps the surface clean and provides a high stable throughput. Recycling can be carried out, with some permeate passing though the membrane during each cycle, until a small volume of retentate contains most of the solids present in the original volume of juice or wine.

UF membranes are said to be "asymmetric" or "anisotropic". Such a membrane is non-uniform with a tight, extremely thin "skin" (0.1-1.0 microns) on its surface. This skin is the sole active membrane layer with the remaining structure, or substructure, serving primarily as a porous support. The pore size, or molecular weight cut-off (MWCO) rating, of an ultrafiltration membrane is determined experimentally by the rejection characteristics of the membrane when challenged with known test molecules. Ideally, a membrane will have a sharply defined MWCO. However, the membrane pores cannot be accurately measured due to their small size, so UF membranes are generally classified by their nominal MWCO.

In recent years UF has emerged as a versatile membrane separation process which is increasingly finding application in the beverage industry (Swientek, 1986). Beverage manufacturers employ UF to clarify or concentrate liquids and to remove impurities from the final product.

Its application as an alternative to conventional procedures for clarifying and preserving fruit juices was first demonstrated by Heatherbell *et. al.* in 1977. The process was recognized as a low energy-physical process with potential for substituting for several conventional batch operations including the use of enzymes, fining agents, centrifugation and filtration (Berezovsky, 1985; Milnes, 1984; Paulson *et al.*, 1985; Rösch, 1985; Swientek, 1986; Tump, 1983; Vrignaud, 1983; Wucherpfennig *et al.*, 1984).

The use of UF for the clarification and preservation of juices of kiwifruit (Wilson and Burns, 1983), pear (Hudgson, 1981; Kirk *et al.*, 1983), apple (Baumann *et al.*, 1986; Drake and Nelson, 1986; Glaser and Güngrich, 1986; Möslang, 1984; Nagel and Schobinger, 1985; Rösch, 1985; Vrignaud, 1983; Wucherpfennig *et al.*, 1984, 1985) and grape (Fombin, 1983; Heatherbell *et al.*, 1984; Garcia, 1984; Wucherpfennig and Neubert, 1977; Wucherpfennig *et al.*, 1984) has been demonstrated.

UF has recently been applied in the winemaking industry. UF units have been operating for the past 2-3 years in USA, Germany, France, Italy, Israel and Australia (Gibson, 1985; Gnekow *et al.*, 1983; Parrini *et al.*, 1985; Payne, 1985; Serrano *et al.*, 1984; Wucherpfennig, 1984). However, the commercial use of UF by the wine industry does not appear as advanced as in the juice industry.

UF can be used to improve the stability of the finished wine. Haze is a common problem in wines, especially white wines. Haze is due to proteins which are unstable or insoluble at the acidic pH of the finished wine. Haze has traditionally been removed by adding fining agents such as bentonite. Therefore, in most cases the application of choice for UF has been for the protein stabilization of wine without the use of bentonite.

UF has previously been reported as an effective technique for protein (heat) stabilization (Balanutse, 1983; Drioli *et al.*, 1981; Gaillard, 1984; Heatherbell *et al.*, 1985; Miller *et al.*, 1985; Poirier *et al.*, 1984; Wucherpfennig, 1978, 1980; Wysocki, 1977). Studies carried out by Gnekow *et al.*, (1983); and Luis, (1983) have shown that in both laboratory scale and production trials, a protein stable Sauvignon Blanc wine may be produced using 10,000 membrane nominal MWCO. However, Hsu *et al.*, (1987) presented evidence that even with membrane nominal MWCO as low as 10,000 daltons, small amounts of protein which can contribute to heat instability remain in UF permeates of White Riesling wine. Although stabilization is not always obtained, reductions of 80 to 95% of bentonite demand were achieved.

Polyphenols give wine its color and subtle, complex flavor. Normally, gelatin is added as a fining agent to remove polyphenols. However, gelatin is a colloidal protein, and the tannin-protein complex subsequently formed must be removed by decanting or filtration. Ultrafiltration can reduce the tannin concentration, but will not completely eliminate them since some of the polyphenols may pass through the

membrane. However, the enzymes (i.e. PPO) that cause the undesirable brown compounds to form are removed by UF, thus improving the color stability.

Yoshifumi *et al.*, (1980) confirmed that UF increased the resistance to browning of white table wine; polyphenols and nitrogenous compounds were also partially removed from the wine by UF. Heatherbell *et al.*, (1984) demonstrated that juice and wine color quality (and clarity) can be improved by utilizing the selective retention properties of the membranes of different MWCO, up to 99% retention of browning by membrane MWCO from 10,000 to 100,000 daltons. This was also demonstrated by Barillere *et al.*, 1985 and Moutounet *et al.*, 1985 for grape juice. However, Gaillard (1985); Meglioli and Marchesini, 1985; and Moutounet *et al.*, 1985 reported low retention of color (A_{420}) on wines.

Several authors have reported variable low percentages retention of phenolic compounds by UF, ranging from approximately 2-30% for grape juice and wine (Barillere *et al.*, 1985; Bastiali *et al.*, 1983; Fombin, 1983; Heatherbell *et al.*, 1984; Meglioli and Marchesini, 1985; Testaniere, 1985; Youval and Zisner, 1985).

UF has also been employed for tartrate removal from wines and musts (Berger, 1985; Bocker, 1981; Colagrande *et al.*, 1984; Staude *et al.*, 1976; Wucherpfennig, 1980). One method for accomplishing this involved concentration of the wine by UF to the point that the tartrate precipitated out and was subsequently removed by other filtration methods (Staude *et al.*, 1976). Wucherpfennig (1985), reported that tartaric acid separation in ultrafiltered wines occurs more rapidly - due to their low colloid content - than in conventional wines.

Although ultrafiltered juice and wine permeates are sparkling clear, studies on the retention of pectin have indicated considerable amounts of soluble pectin are passing through the membranes and remaining in solution in the permeate (Hodgson, 1981; Garcia, 1984; Gaillard, 1985; Wucherpfennig, 1985).

Sensory Evaluation of Wine

Sensory evaluation is an area of increasing interest in the evaluation of juice and wine quality. This is a scientific discipline involved in evoking, measuring, analyzing, and interpreting responses to properties of foods and materials as perceived by sight, smell, taste, touch and hearing. Sensory evaluation test methods have been divided into affective (subjective methods, i.e. acceptance or preferences tests) and discriminative (objective methods, i.e. simple difference, and descriptive tests).

Traditionally, each company had in its employ one or more individuals who where considered "experts". The judgement of the expert (or collective judgements of selected experts) was relied on for all manner of decisions, including which of several ingredients or products was most appropriate for the marketplace. Undoubtedly, an individual who has spent many years using his/her senses can make worthwhile contributions to his/her company's success, but there are limits to one person's abilities and availability.

The Arthur D. Little Company proposed The Flavor Profile as a means of dealing with the complex world of food flavor. The Flavor Profile method calls attention to the fact that a person can be trained to perceive and recognize individual flavor characteristics and, with the use of appropriate training aids, can reach an agreement with his or her fellow panel members on a total product impression. The panelists describe the product attributes, their intensities, and their order of detection (Amerine and Roessler, 1983; Stone and Sidel, 1985). However, this method involved a training period of up to six months and training was general, not for just one product. More recently, methods such as Quantitative Descriptive Analysis developed by Stone *et al.*, (1974), involve training individuals to identify and quantify, in order of occurrence, the sensory properties of a product or an

ingredient. This technique involves a much shorted period of training, and is specific to the particular product with which one is working.

Descriptive analysis involves the detection and description of a product's sensory characteristics by a well trained panel. It involves two major steps: 1) discriminative, involving the detection of flavor, aroma or texture (mouthfeel in juice or wine) characteristics, and the detection of differences and the amount of each characteristic present; 2) descriptive, involving the qualification of the sensory characteristic and then quantification of it by stating the amount of each characteristic present (McDaniel, 1987).

Moskowitz (1983) recommends a panel comprising 10 or more individuals, for two pragmatic reasons. 1) Individuals perceive and rate products differently, whether simple stimuli or complex foods. Thus, too small a panel can provide distorted ratings simply from lack of representation; and 2) A small panel critically depends on the participation of each of its members.

Training of a descriptive panel must be well-planned and conscientiously carried out. A panelist needs to be screened for basic taste acuity and aroma descriptive ability, selected on the basis of acuity, motivation, verbal communication skills, have a cooperative, non-domineering personality, be available, and finally trained to do the task at hand (Moskowitz, 1983).

This descriptive method requires that the panel develop a set of terms or words that form the basis for their scorecard or ballot. Subjects must agree as to the meaning of those words and in a quantitative test indicate the intensity of each sensory quality perceived. The language is developed through careful training and practice by the panel. However, the descriptor list always remains incomplete. During the course of the training, panelists will introduce new terms and eliminate some of the unimportant or redundant older terms. In order to develop a good frame

of reference and to facilitate specific training, many reference standards must be used.

Vocabulary for profiling specific wines has been developed for Zinfandel (Noble and Shannon, 1987), and for Cabernet Sauvignon (Heymann and Noble, 1987). McDaniel (1986) used descriptive analysis to evaluate Pinot Noir wines fermented with different strains of malolactic bacteria, while Noble *et. al.* (1983) applied it to Bordeaux wines.

The Analysis of Variance (ANOVA) model is the most appropriate statistical procedure for analyzing responses (Stone and Sidel, 1985). In descriptive analysis, the subjects evaluate all of the products on a dimension by dimension basis on more than a single occasion. In statistical terms, this represents a treatment-by-subject repeated measures design, which is extremely helpful in characterizing the reliability of response patterns, as well as in providing a larger data basis for analysis. Analysis is always conducted on an individual attribute basis. A statistical statement can therefore be made about every attribute on the ballot.

Effect of Ultrafiltration (UF) on Juice and Wine Quality: The sensory evaluation of the aroma and flavor of ultrafiltered juice and wine products have shown interesting results.

Wilson, *et. al.* (1984) did not detect significant differences in the sensory assessment of the flavor of freshly processed kiwifruit juices by UF and conventional processing techniques. For apple juice, Drake and Nelson (1986), reported that the ultrafiltered apple juice was significantly rated lower in sensory flavor than the apple juice filtered by conventional plate and frame method.

Balanutse *et al.*, (1983) reported that ultrafiltration had a beneficial effect on the flavor of dry white wines. In the same year Sachs *et al.* reported that ultrafiltration did not affect wine taste, except for a slight reduction in the

bitterness of an astringent red wine. Similar results were reported by Gaillard (1985a). Serrano *et al.* (1984); and Berger (1985) stated that certain membranes profoundly modified the sensory character of red wines. Berger also reported that white wines seem to stand this filtration better. In a study by Meglioli and Marchesini (1985) with white wine, a sensory panel rated the controls as having a more pronounced odor and flavor initially than the UF wine (which nevertheless had a fine, delicate bouquet), but the controls showed slight oxidation after 4 months and considerable oxidation after 12 months. Sensory evaluation of white musts showed a preference for the ultrafiltered samples (Gaillard, 1985b). Testaniere, (1985) reported that in the sensory evaluation of ultrafiltered white wines and its bentonite fine treated control, the ultrafiltered sample had a cleaner aroma.

Although research has been carried out using sensory evaluation to study the effect of ultrafiltration on the quality of juice and wine products, no studies were found concerning the evaluation of this effect by descriptive analysis.

TITLE: ULTRAFILTRATION (UF) OF WHITE RIESLING JUICE: EFFECT OF OXIDATION AND OF PRE-UF JUICE TREATMENT ON FLUX, COMPOSITION AND STABILITY.

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RUNNING HEAD: ULTRAFILTRATION OF WHITE RIESLING JUICE.

Technical Paper No. _____ from the Oregon Agricultural Experiment Station.

ULTRAFILTRATION (UF) OF WHITE RIESLING JUICE: EFFECT OF OXIDATION AND OF PRE-UF JUICE TREATMENT ON FLUX, COMPOSITION AND STABILITY.

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Abstract

Investigations over 3 vintages indicated that White Riesling juice processed without SO_2 and clarified by UF tended to develop sediments upon storage. This study investigates the possible effect of oxidation [processing with or without sulfur dioxide ($\pm SO_2$)] and of pre-UF treatment of juices with enzymes and fining agents on flux, and on juice color (browning), composition, and stability. White riesling juice was ultrafiltered with a Romicon Lab-5 pilot scale hollow fiber unit operated in a batch mode with membrane of nominal molecular weight cut-off (MWCO) of 10,000 daltons. Grapes were processed $\pm SO_2$ and the effect of treatment of settled press juice with Rohapect VR Super (VRS, mainly pectinase and protease), and of bentonite, gelatine, silica sol fining before UF, investigated. Juice parameters evaluated included total protein, pectin, phenol, colour (A_{420nm}), and stability to heat/cold testing. Pre-UF treatment with enzymes and fining increased flux. Sediments were found to contain large amounts of proteins, phenolics, and trace amounts of pectin and neutral polysaccharides. Sediment formation and instability to heat testing of UF permeates processed $-SO_2$ was prevented with pre-fining. Up to 99% of protein, 90% of pectin, 84% of color and low variable phenolics were retained by the membrane of 10,000 dalton MWCO. However, pre-UF enzyme treatment can increase protein and pectin in permeates. During UF there is a significant increase in the soluble protein and water soluble pectin passing through the membrane with increasing volume concentration ratio (VCR, process time). It is concluded that it is not only the amount but the nature/state of compounds such as proteins, phenolics, pectins, and their interaction that results in instability.

Introduction

Current research and previous investigations in our laboratory over several vintages (4, 7) indicates that grape juices processed with oxidation (without SO_2) and clarified by ultrafiltration (UF) tend to develop hazes and sediments upon storage. In addition, an earlier study on the clarification of apple juice by UF (5) observed that the juice processed without pre-UF pasteurization developed sediments upon storage. These observations have suggested the possibility of the formation of hazes from oxidized polymerized phenolics and proteins (6, 14, 15), although pectins, and to a lesser extent other polysaccharides, such as starch and arabans, may contribute to haze and sediment formation (6, 23). Our observations have been supported by periodic reports of haze and sediment formation in juices commercially processed by UF. Nagel and Schobinger (17) have also recently postulated the possibility of oxidation and polyphenols contributing to turbidity reported in concentrates of ultrafiltered apple and pear juices.

Clearly, the effect of pre-UF treatment of juice including oxidation may be important to the subsequent quality and stability of juices clarified by UF. This study investigated the effect of oxidation (processing $\pm SO_2$) and of juice pre-UF treatment with enzymes and fining agents on UF juice flux, color (browning), composition (proteins, phenolics, and pectins) and stability.

Materials and Methods

Preparation of Grape Juices: White Riesling grapes were processed with and without the addition of 50 ppm of sulfur dioxide (± SO_2) into juices (20.4° Brix, pH 3.13) by conventional procedures in the Oregon State University experimental winery. Settled press juices (overnight settling at 18° C) were filtered through a 200-μ mesh filter bag (Filter Specialists, Inc., MI), the SO_2 adjusted to 25-30 mg/L free SO_2, and then frozen until the pre-ultrafiltration (pre-UF) treatments were applied.

Pre-UF Treatments: Juices processed with (adjusted to 25-30 mg/L free SO_2) and without SO_2 were subjected to the following pre-UF treatments:

1) Enzyme treatment. Reacted for up to 6 hrs at room temperature (20° to 23° C) with 50 mg/L Rohapect VRS super (mainly pectinase and protease. ROHM Tech., Inc. Malden, MA, subsidiary of ROHM GmbH, Darmstadt, Fed. Rep. Germany). Pectin was degraded until no longer detected by the alcohol test (21).

2) Fining with Bentonite (sodium bentonite, Volclay), Gelatin (SCOTT Labs., San Rafael, CA) and Silica Sol (Clarifying Agent C-2, ROHM Tech., Inc. Malden, MA) at equivalent rates of 50 g/Hl, 10 g/Hl, and 30 mL/Hl, respectively. Approximately 20 min reaction time was allowed between fining treatments.

3) Enzyme followed by Fining treatment, as described previously in 1) and 2).

Ultrafiltration Process: The HF-Lab-5 Ultrafiltration system (Romicon Inc., MA) equipped with a PM-10 cartridge (membrane nominal molecular weight cut-off of 10 000 daltons; membrane area, 0.46 m^2) was used in this study. The unit was operated in the batch mode under standardized operating conditions (ΔP_T = 1.23 kg/cm2 , 18° to 25° C, to VCR = 6.7), which were previously determined (7).

For operation in the batch mode, 20-L of juice were ultrafiltered, and no feed was added to the recirculation loop. Permeate samples for analysis were obtained from the permeate exit pipe, which was covered with aluminum foil to prevent airborn contamination. Final ultrafiltered juices were aseptically bottled (containing ≈25 mg/L of free SO_2) in a sterile hood using 800 mL glass bottles and crown caps. Retentate samples were obtained from the feed tank. At the completion of an experiment the UF unit was immediately drained and flushed with water at 45° C. The cartrige was subsequently cleaned and sanitized following the procedure described in the Romicon manual (22). Familiarity with the following terms is necessary to interpret the results discussed in the tables and figures:

Volume Concentration Ratio (VCR) = $V_I / (V_I - V_P) = V_I / V_R$

were V_I = initial (feed) volume

V_R = retentate volume

V_P = permeate volume.

ΔP_T = average transmembrane pressure = $(P_{inlet} + P_{outlet})/2$

were P = pressure.

Determination of Total Phenol and Soluble Protein: Total phenol, soluble protein determination and electrophoresis of proteins were performed as previously described (9).

Color (browning) and Heat Stability Test: Juice browning was measured as optical density at 420-700 nm, against a water blank. The heat stability of the juices was determined using the procedure recommended by Pocock and Rankine (19), the haze formation being measured as previously described (10).

Determination of Total Pectin: The pectin substances in grape juice were fractionated following the procedure described by Robertson (20). Uronic acid content of each fraction was determined using an adaptation of the automated version by Thibault (25) of the m-hydroxydiphenyl method (2). Water soluble pectin was determined.

Data Analysis: All analytical determinations were carried out in duplicate on each processing trial replicate. All statistical analysis were performed using SAS (Statistical Analysis Systems, Cary, NC). A completely randomized factorial design and a completely randomized factorial design with repetitive measurements during processing (VCR) were used. Analysis of Variance was calculated using the PROC GLM to test the effect of:

1) SO_2, pre-UF treatments, and UF on color (browning), total phenol, soluble protein, and water soluble pectins.
2) SO_2, pre-UF treatments, and VCR on permeation rate (flux), color (browning), total phenol, soluble protein, and water soluble pectins.

Results and Discussion

UF operating conditions chosen for this study had been standardized in previous studies (7). A membrane with a nominal MWCO of 10,000 daltons was chosen to permit maximum possible retention of proteins, phenolics and pectins, previous studies having indicated less retention with membranes of MWCO 30, 50, and 100,000 daltons (4, 7, 11)

The pre-UF treatments with enzyme and fining agents were chosen to have a major possible effect on juice unstable components of potential importance: the enzyme (Rohapect VR Super) containing pectinase and protease activity; the fining with bentonite, gelatin, and silica sol being a comprehensive fining for attempted removal of unstable proteins and phenolics (6).

Effect of Pre-UF Treatment and VCR on Flux: A summary of the Analysis of Variance for Flux is shown in Table II.1.

Table II.1 Analysis of Variance, Degrees of Freedom (DF), and F-values of UF Flux of White Riesling Juice.

SOURCE	DF	F-values
SO_2 (S)	1	0.01
PRE-UF TREATMENT (P)	3	295.60***
S X P	3	83.97***
MEAN SQUARE ERROR I	4	13.33
VOLUME CONCENTRATION RATIO (VCR)	6	11.77***
S X VCR	6	2.96*
P X VCR	18	2.28*
S X P X VCR	17	0.66
MEAN SQUARE ERROR II	24	1.29

*, **, *** indicates significant at $p < 0.05$, < 0.01, < 0.001, respectively.

From the analysis of variance (ANOVA) of flux (Table II.1), the main factors Pre-UF treatment ($p< 0.001$), and VCR ($p< 0.001$) had a significant effect on flux. However, their interactions were also significant (Table II.1). Flux was significantly increased when the juice was processed with oxidation ($-SO_2$), compared to the processing with minimal oxidation ($+SO_2$) (Fig. II.1). This can be attributed to the inhibitory effect of SO_2 on Polyphenol oxidase (PPO). PPO is mainly responsible for the polymerization of phenols and protein cross-linking, with the subsequent formation of large molecules which settle out rapidly and co-precipitate compounds which can limit filtration rate (6). The application of pre-UF clarifying treatment significantly increased the flux of both $\pm SO_2$ juices (Fig. II.1). Wucherpfennig et al (28, 30) have also demonstrated that pre-UF juice treatment with enzyme and fining agents can increase UF flux of apple juice.

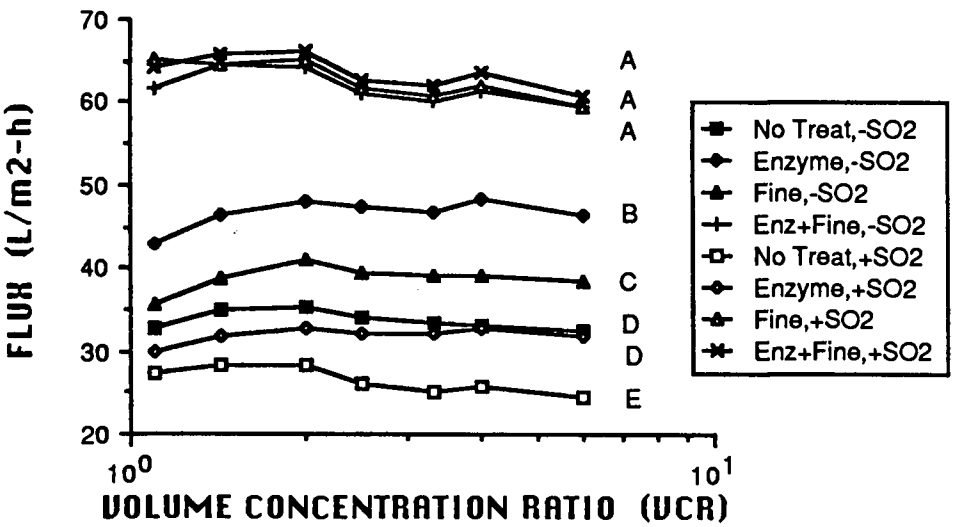

Fig. II.1 Effect of Pre-UF Treatment on Flux of White Riesling Juice. Romicon HF-Lab 5 system operated with PM-10 cartridge at ΔP_T=1.23 kg/cm^2, 18-25°C, VCR 6.7. Means designated with the same letter are not significantly different at p = 0.05.

Highest fluxes were obtained when juices processed ±SO_2 were pre-UF treated with Enzyme+Fining (Fig. II.1). Cloudy or turbid juices are complex colloidal systems containing molecules/particles ranging in size from approximately 0.001μ to 1000μ in diameter. To remove this finely dispersed colloidal material and precursors of haze formation the judicious application of fining agents in conjunction with prior enzymatic juice treatment is usually necessary (6, 31). This may be due to the fact that soluble pectin acts as a protective colloid and that the partial hydrolysis of this pectin permits insoluble and finely divided particles to flocculate and to be exposed to fining (18). This is necessary to reduce viscosity and remove compounds which limit permeation rate (6, 31).

Interestingly, equally high fluxes were obtained with the juice pre-UF fine treated in the presence of SO_2 but without prior enzyme treatment (Fig. II.1). In this study there may have been sufficient endogenous pectinase activity present in the juice processed with SO_2 to permit optimal fining without the addition of commercial pectinases (in comparison with the natural enzyme activity in juices processed without SO_2 which may have been less due to inactivation/co-precipitation of enzymes by oxidized polymerized phenolics).

From the ANOVA in Table II.1, VCR had a significant effect ($p< 0.001$) on flux, however its effect was not independent, as VCR significantly interacted with SO_2 and pre-UF treatment ($p< 0.05$). Of the eight different pre-UF treatments (Fig. II.1) VCR only had a significant effect on three (the pre-UF enzyme, and enzyme+fine treated juices processed without SO_2 and the juice not pre-UF treated processed with SO_2). The initial increase in flux and surprising apparent lack of flux decline can be attributed to the slight increase in temperature during processing (from 18 to 25°C.). The rate of flux decrease with VCR is relatively small in this study compared with what we have observed previously (5, 7, 12). The decrease in flux is considered to be related to an increase in the concentration of pectin and other

macromolecules such as polyphenols and proteins within the retentate, which cause an increase in the gel polarization layer. However, the juice utilized for these experiments contained relatively low concentration of proteins and pectins (Fig. II.4, II.5). In addition the juice had low suspended solids (well settled) and was apparently free from polysaccharide colloids, originating either from the fruit or from microbial contamination that can block filters and accelerate flux decline in juices and wines (3, 6, 27, 29).

Effect of Pre-UF Treatments on Color (browning), Total Phenol, Soluble Protein, and Water Soluble Pectin of the Juices: A summary of the Analysis of Variance of color (browning), total phenol, soluble protein, and water soluble pectin is shown in Table II.2.

Table II.2. Analysis of Variance, Degrees of Freedom (DF), and F-values for Color (browning), Total Phenol, Soluble Proteins, and Water Soluble Pectin of White Riesling Juice.

SOURCE	DF	F-values			
		COLOR (BROWNING)	TOTAL PHENOL	SOLUBLE PROTEIN	PECTIN1
SO_2 (S)	1	518.55***	291.71***	33.53***	3.26
PRE-UF TREATMENT. (P)	3	34.51***	3.59	38.44***	1.10
S X P	3	7.78**	5.54*	29.22***	0.17
ULTRAFILTRATION (UF)	1	379.88***	0.77	181.98***	70.01***
S X UF	1	206.29***	4.83	11.29**	7.15*
P X UF	3	31.49***	0.33	32.63***	2.70
S X P X UF	3	9.69**	3.17	22.90***	0.28
MEAN SQUARE ERROR	10	0.0013	556.88	6.52	2046.99

1 Water Soluble Pectin.
*, **, *** indicates significant at $p < 0.05$, < 0.01, < 0.001, respectively.

Sulfur dioxide, pre-UF treatment, and UF had a highly significant effect ($p < 0.001$) on color (browning). Their interaction effects were also significant ($p < 0.01$, Table II.2). The results for the effect of these factors on juice browning are illustrated in Fig. II.2.

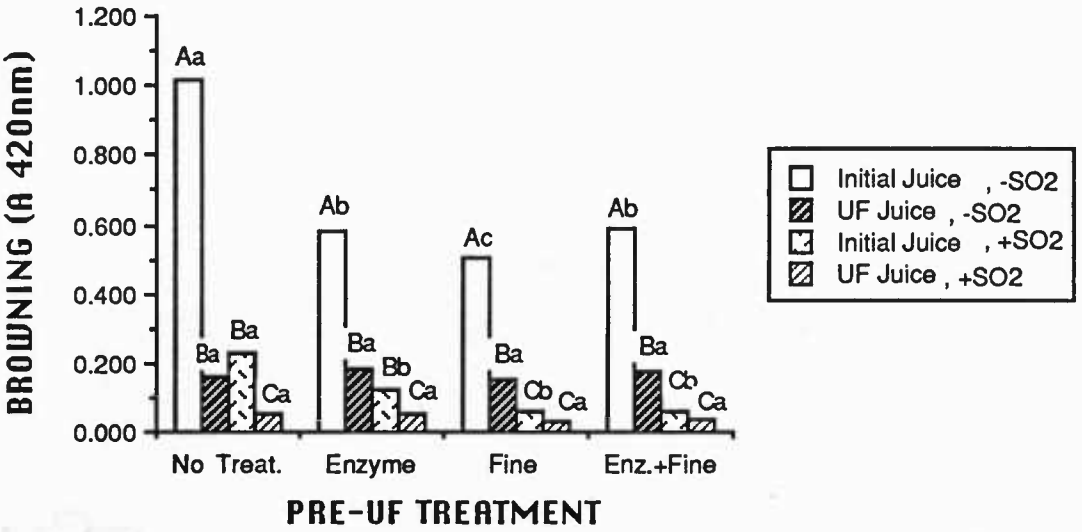

Fig. II.2 Effect of SO_2, Pre-UF Treatment, and UF on Color (browning) of White Riesling Juice. Romicon HF-Lab 5 system operated with PM-10 cartridge at ΔP_T=1.23 kg/cm2, 18-25°C, VCR 6.7. Capital letters indicate mean separation at each Pre-UF treatment, and small letters indicate mean separation between Pre-UF treatment. Means designated with the same letter are not significantly different at p = 0.05.

Processing juices with SO_2 significantly decreases browning in both non-ultrafiltered (77.3%) and ultrafiltered (67.4%) juices. This is mainly due to the inhibitory effect of SO_2 on PPO which oxidize *o*-dihydroxy phenolic compounds to form *o*-quinones that react with each other or proteins to form brown-colored products (15).

Ultrafiltration significantly reduced brown color in juices by 77% in the presence of SO_2 and by 83.9% in the absence of SO_2. UF reduced brown color in

juices processed without SO_2 to the same level obtained by processing with SO_2 without UF. This supports our previous studies indicating that browning complexes in the juice are of a relatively high molecular weight, similar retentions being obtained with membranes MWCO up to 100,000 daltons (7). This conclusion is further supported by French workers (1) who have reported similar reductions in juice browning with microfiltration.

Pre-UF treatment (enzyme, fine, and enzyme+fine) significantly decreased the color (browning) of the non-ultrafiltered juices processed $\pm SO_2$. However, once colored compounds have been removed by pre-UF treatment there is no further reduction in color by UF, whether processed $\pm SO_2$.

Ultrafiltration did not have a significant effect on total phenol content of the juices processed in this study (Table II.2). Previous studies in our laboratory have indicated considerable variation in retention of phenolics by UF membranes; 3%, and up to 30% being retained in White Riesling and Thompson seedless, respectively, when juices were processed $-SO_2$ (4, 7). Other authors have also reported similar variations in reduction of juice phenolics by UF in juices and wines (1, 17, 24, 32).

Sulfur dioxide significantly affected ($p< 0.001$) the total phenol content of the juices. However SO_2 significantly interacted ($p< 0.05$) with Pre-UF treatment (Table II.2). In Fig. II.3 the effect of SO_2 and Pre-UF treatment on total phenols is plotted. Comparison of the non pre-UF treated juices processed with and without SO_2 indicates that the total phenol content of the juice is significantly reduced (33%) when oxidation has occurred ($-SO_2$). This can also be explained by the oxidation of *o*-diphenols to *o*-quinones by PPO. Quinones then become irreversibly bound to the pomace and a large percent are removed from the juice by settling (13). Phenols may also polymerize and precipitate out of solution, in particular by co-precipitating proteins (6, 14, 26).

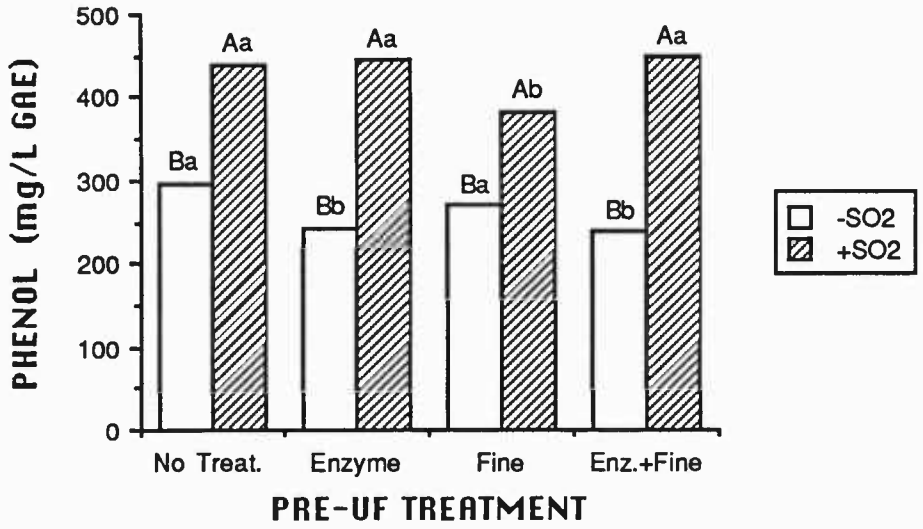

Fig. II.3 Effect of SO_2, and Pre-UF Treatment on Total Phenols of White Riesling Juice. Romicon HF-Lab 5 system operated with PM-10 cartridge at ΔP_T=1.23 kg/cm^2, 18-25°C, VCR 6,7. Capital letters indicate mean separation at each Pre-UF treatment, and small letters indicate mean separation between Pre-UF treatments. Means designated with the same letter are not significantly different at p = 0.05.

In the juices processed without SO_2, pre-UF treatment with enzyme, and enzyme+fine significantly reduced (≈19%) the total phenol content when compared to the non pre-UF treated sample. In the absence of SO_2 oxidation will proceed as previously described and the polymerized phenolic compounds could bind the enzyme protein, reducing its activity and thus reducing the concentration of these compounds in the juice (13).

Pre-UF fining significantly reduced the level of phenols in juice processed with SO_2 (13%). Gelatin reacts with the soluble polyphenols and co-precipitates them from solution (6, 14, 15).

Therefore, in general, for juices investigated in this study, oxidation and pre-UF treatment reduce total phenol content in the juices. Subsequent UF treatment of

oxidized and pre-UF treated juice did not further reduce the total phenol content of the juices.

From Table II.2, Sulfur dioxide, Pre-UF treatment, and UF, had a highly significant effect ($p< 0.001$) on the concentration of soluble proteins in juices. However, their interaction was significant ($p< 0.001$, Table II.2). The effect of SO_2, pre-UF treatment and UF on the concentration of the juice soluble proteins are presented in Fig. II.4.

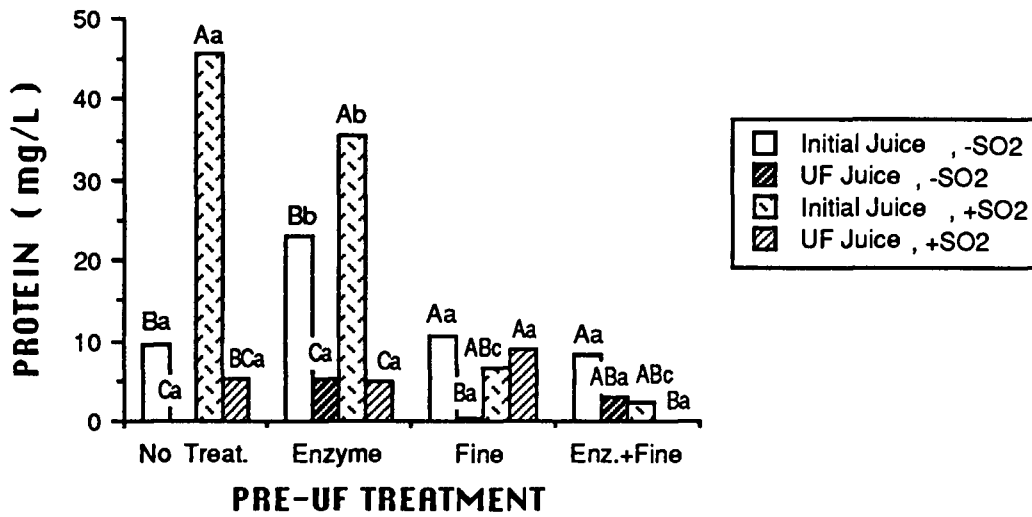

Fig. II.4 Effect of SO_2, Pre-UF Treatment, and UF on Soluble Protein of White Riesling Juice. Romicon HF-Lab-5 system operated with PM-10 cartridge at ΔP_T=1.23 kg/cm^2, 18-25°C, VCR 6.7. Capital letters indicate mean separation at each Pre-UF treatment, and small letters indicate mean separation between Pre-UF treatment. Means designated with the same letter are not significantly different at p = 0.05.

Processing of juice with oxidation (-SO_2) significantly decreased (79%) the concentration of soluble proteins present in settled juice compared to when juices were processed with SO_2 (Fig. II.4). This is attributed to PPO activity in the

absence of SO_2 causing their precipitation by cross-linking or by polymerized phenolics (7, 15).

Ultrafiltration significantly reduced the protein content of both juices processed $+SO_2$ (89%) and $-SO_2$ (99%) (Fig. II.4). Similar results have already been reported for juices processed with SO_2 (11). Although the protein content of the ultrafiltered juice processed $\pm SO_2$ were not significantly different, one would anticipate a higher membrane retention of proteins in juices processed $-SO_2$ due to complexing with polymerized phenolics.

Pre-UF enzyme treatment of the initial juices significantly decreased the soluble protein content (22%) in the juices processed with SO_2, however the concentration of soluble protein was significantly increased (59%) in the juices processed in the absence of SO_2. In the juice processed with SO_2 the amounts of protein complexed with polymerized phenolics or surrounded by protective colloids is limited, therefore enzymatic degradation of the proteins by the proteolytic fraction of the enzyme preparation may occur. In addition, protease activation by SO_2 has to be considered (7, 16). The increase in protein caused by the enzyme in the juices processed without SO_2 may be due to the release of proteins from cellular debris and protein complexes left after crushing and pressing. Likewise, pre-UF fining and enzyme+fine of the non-UF juices significantly decreased (85%, 95% respectively) the levels of protein in the presence of SO_2 but they did not have any significant effect in the juices processed without SO_2.

Ultrafiltration of juices pre-UF treated with the enzyme preparation significantly reduced the protein content in the presence (86%) or absence (77%) of SO_2. Ultrafiltration significantly reduced the protein content of the pre-UF fine treated juice without SO_2 (97%). Apparently in the absence of SO_2 and after fining, protein complexes are still present which are large enough to be retained by UF. However, ultrafiltration of pre-UF fine treated, and enzyme+fine treated juices did

not have a significantly effect on the soluble protein content of the juices processed $+SO_2$ and $\pm SO_2$, respectively. Therefore, it is concluded that although apparent reduction in total proteins occurs pre-UF treatment of juices processed $\pm SO_2$ did not have a statistically significant effect on the soluble protein content of the UF permeate juices. The small changes in total protein which coincide with changes in juice stability may not be statistically significant but may be significant to juice stability (refer stability of UF juices).

From the ANOVA on Table II.2, UF had a significant effect ($p< 0.001$) on the concentration of water soluble pectin content (WSP) of the juices, but its effect interacted significantly ($p< 0.05$, Table II.2) with SO_2. The different Pre-UF treatments did not have a significant effect in the concentration of WSP of the juices (Table II.2). Processing with SO_2 significantly reduced the contents of WSP in the non-ultrafiltered juices (Fig. II.5). Furthermore, UF significantly reduced the WSP content of both juices processed with and without SO_2 by 89% and 77%, respectively. However, there was no significant differences in the soluble pectin content of these juices. Endogenous pectolytic enzymes present in the juice are affected by whether, among other factors, the juice was processed with or without SO_2. In the absence of SO_2, oxidation products could bind to the enzymes and co-precipitate them from solution, thus reducing enzyme activity.

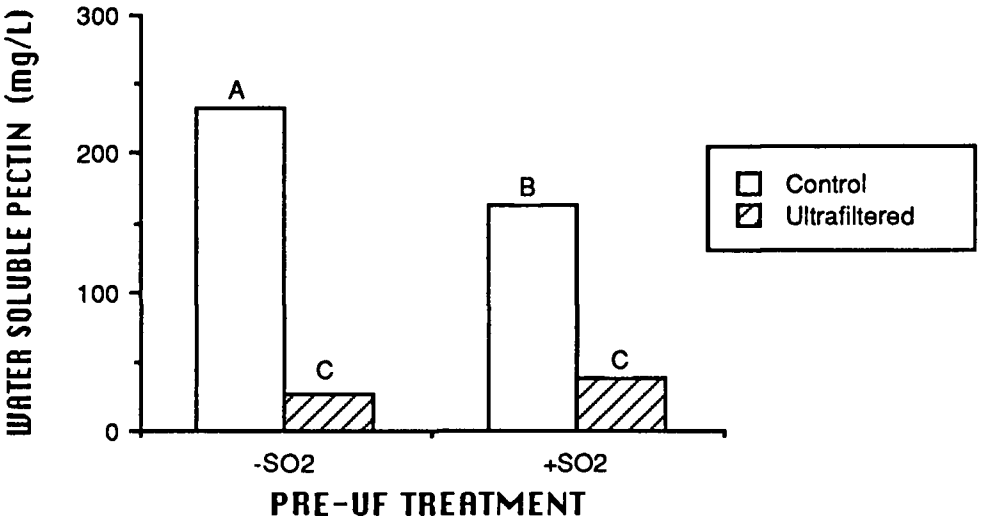

Fig. II.5 Effect of SO_2, and UF on Water Soluble Pectin of White Riesling Juice. Romicon HF-Lab 5 system operated with PM-10 cartridge at ΔP_T=1.23 kg/cm^2, 18-25°C, VCR 6.7. Means designated with the same letter are not significantly different at p = 0.05.

High retention of pectic substances by UF have also been reported for apple, grape, and pear juice (5, 7, 8). This may be explained by the fact that although values given for molecular weight of pectins in the literature are very broad, they are usually higher than 30 000 MW. The pectin content remaining in the permeate may have resulted form partial endogenous enzymatic degradation of juice pectins before UF.

Effect of VCR on Color (browning), Total Phenol, Soluble Protein, and Water Soluble Pectin of Ultrafiltered Juices: A summary of the Analysis of Variance for color (browning), total phenol, soluble protein, and water soluble pectin is shown in Table II.3.

Table II.3 Analysis of Variance, Degrees of Freedom (DF), and F-ratios for Color (browning), Total Phenol, Soluble Proteins, and Water Soluble Pectin of White Riesling Juice During Ultrafiltration (process time, VCR).

		F-values			
SOURCE	DF	COLOR (BROWNING)	TOTAL PHENOL	SOLUBLE PROTEIN	PECTIN[1]
SO_2 (S)	1	193.76***	202.71***	12.82*	4.36
PRE-UF TREATMENT (P)	3	1.91	1.99	12.26**	6.83*
S X P	3	1.53	2.54	19.97**	6.19*
MEAN SQUARE ERROR I	5	14.2x10-4	1052.37	3.77	164.29
VOLUME CONCENTRATION RATIO (VCR)	2	7.17*	0.44	40.52***	58.53***
S X VCR	2	2.52	0.83	8.42**	25.93***
P X VCR	6	0.19	1.03	15.00***	20.91***
S X P X VCR	6	0.13	2.18	2.69	28.68***
MEAN SQUARE ERROR II	10	35.2x10-5	493.38	0.31	10.19

1 Water Soluble Pectin.
*, **, *** indicates significant at $p < 0.05$, < 0.01, < 0.001, respectively.

Although Pre-UF treatment did not have an effect on the retention of color (browning), SO_2 ($p < 0.001$) and VCR ($p < 0.05$) did have a significant effect on it (Table II.3). Processing with SO_2 significantly decreased color (browning) on all the

juices regardless of the pre-UF treatment (Fig. II.6). Although pre-UF treatment of the juices processed $\pm SO_2$ had no effect on the color (browning) at low VCR (VCR 2), with increasing VCR differences were obtained on the juices processed without SO_2 (Fig. II.6). For example, as the VCR increased the color of the pre-UF enzyme treated juice differed significantly from the non pre-UF treated, and the pre-UF fine treated juices.

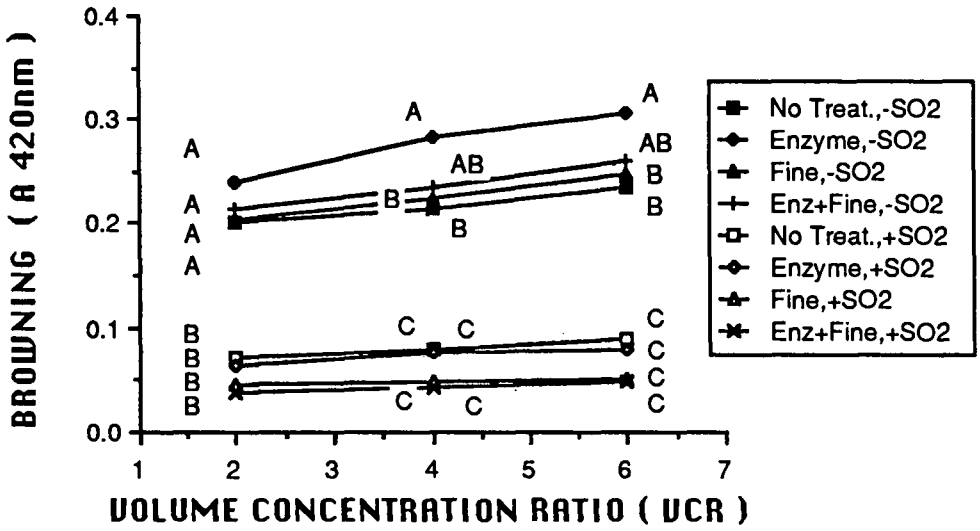

Fig. II.6 Effect of Volume Concentration Ratio on Color (browning) of White Riesling Juice. Romicon HF-Lab 5 system operated with PM-10 cartridge at ΔP_T=1.23. kg/cm^2, 18-25°C. Capital letters indicate mean separation at each VCR, and small letters indicate mean separation between VCR. Means designated with the same letter are not significantly different at p = 0.05.

Data in table II.3 indicates that Sulfur dioxide, Pre-UF treatment, and VCR had a highly significant effect on the soluble protein content of the ultrafiltered juices. However, Pre-UF treatment significantly interacted with SO_2 ($p< 0.001$) and with VCR ($p< 0.05$). A significant increase in the levels of total protein from VCR 2 to VCR 6 was found for the non pre-UF treated (33%) and the pre-UF enzyme treated

(75%) juices processed with SO_2, and the pre-UF enzyme treated (38%)juices processed without SO_2 (Fig. II.7).

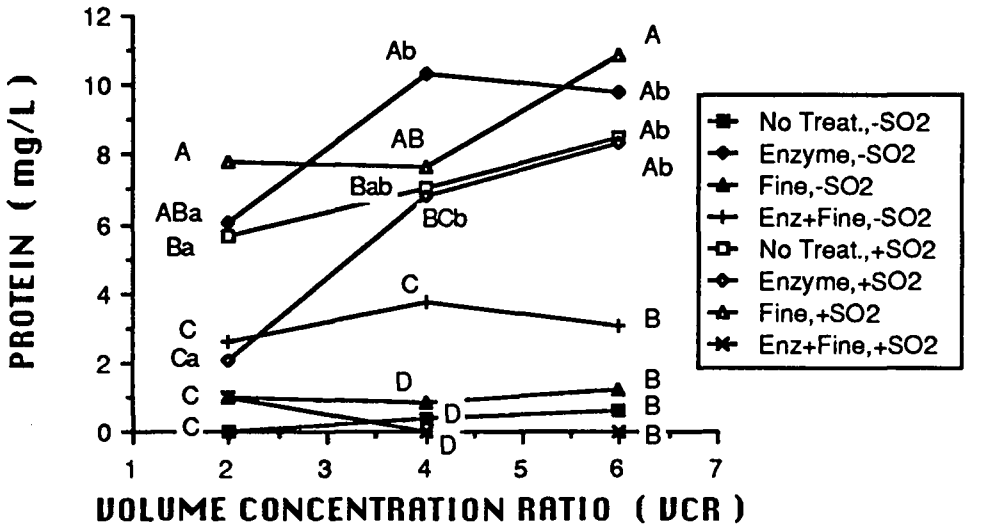

Fig. II.7 Effect of Volume Concentration Ratio on Soluble Protein of White Riesling Juice. Romicon HF-Lab 5 system operated with PM-10 cartridge at ΔP_T=1.23 kg/cm^2, 18-25°C. Capital letters indicate mean separation at each VCR, and small letters indicate mean separation between VCR. Means designated with the same letter are not significantly different at p = 0.05.

Taking the concentration effect into consideration, the enzyme added prior to ultrafiltration may continue to work in the retentate thus releasing more protein form particulate matter still present in the juice.

The differences found between the juice processed with and without SO_2 are probably due to loss of enzyme activity due to the oxidation effect caused by the absence of SO_2 and the additional oxidation by the continual recycling of the juice in the ultrafiltration system.

Although ANOVA of WSP indicates that only Pre-UF treatment, and VCR had a significant effect on WSP, the interaction effect S x P x VCR was highly significant

($p < 0.001$, Table II.3). The WSP content of the juices processed with SO_2 and pre-UF fine, and enzyme+fine treated significantly increased from VCR 2 to VCR 4 by 53% and 64%, respectively (Fig II.8). Heatherbell et al., and Hodgson have also demonstrated an increase in permeate pectin content by increasing VCR (5, 8). Once again, pre-UF treatment did not have an effect on WSP at low VCR, however with increasing VCR differences were obtained.

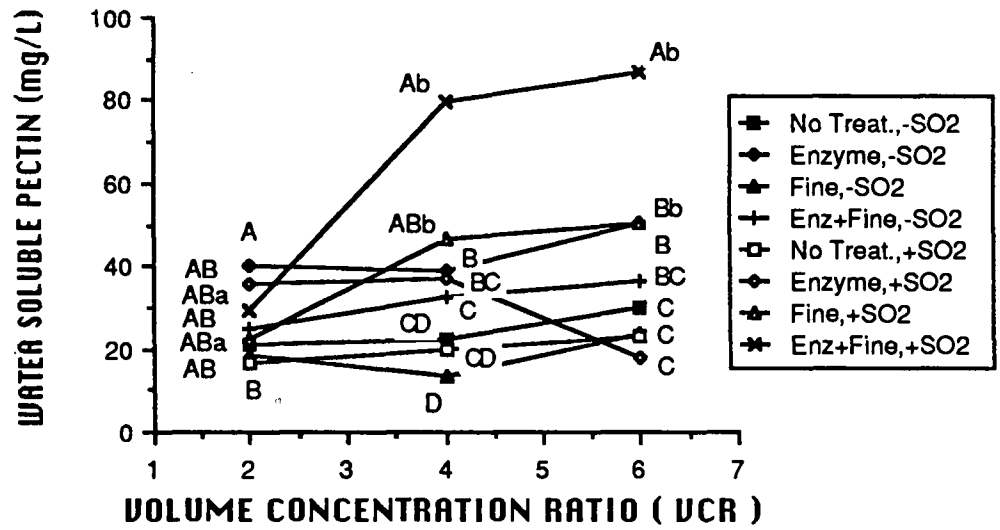

Fig. II.8 Effect of Volume Concentration Ratio on Water Soluble Pectin of White Riesling Juice. Romicon HF-Lab 5 system operated with PM-10 cartridge at ΔP_T=1.23 kg/cm2, 18-25°C. Capital letters indicate mean separation at each VCR, and small letters indicate mean separation between VCR. Means designated with the same letter are not significantly different at p = 0.05.

In general, the decrease in the efficiency of retention may probably be a result of an increase in the concentration polarization, which would result in leakage of the compressed pectic material into the permeate. In addition, the presence of endogenous and added enzymes in the juices has to be considered. These enzymes are presumably retained by the membrane and, therefore, concentrated as the

processing progresses, producing partially degraded lower molecular weight pectic material which may no longer be retained by the membrane.

Stability of the Ultrafiltered Juices: Although no hazes were detected after UF processing, small amounts of secondary hazes and sediments were developed after heat/cold testing, and storage (Table II.4). The formation of hazes and sediments was associated with juices processed with oxidation ($-SO_2$). These sediments were found to contain large amounts of proteins, phenolics, and trace amounts of pectin and neutral polysaccharides (reported in detail elsewhere). Of interest, proteins fractions found in these sediments are of lower molecular weight (12,000 - 30,000 range) thus supporting previous work reported by Hsu et al (10, 11). Haze formation was minimized by reducing the amount of oxidation ($+SO_2$), and by the use of pre-UF treatments with enzyme, fine, and enzyme+fine of the juices. Sediment formation and instability to heat testing of UF permeates processed $-SO_2$ was prevented with pre-UF fining.

The observation that very small reductions in the concentration of total protein, phenol and pectins which are not always significant, coincide with stabilization of juices to haze and sediment formation indicates that it is not only the amount but the nature/state of compounds such as protein, phenolics, pectins and their interaction that results in instability. Further research with more better sensitive analytical techniques is required at the molecular level.

Table II.4 Effect of Pre-UF Juice Treatment on Clarity and Stability of White Riesling Juice Before and After Ultrafiltration.

PRE-UF TREATMENT OF JUICE 2	INITIAL JUICE 1		ULTRAFILTERED JUICE			
	HEAT/COLD TEST			HEAT/COLD TEST		STORAGE (20°C,1yr)
	HUNTER HAZE	VISUAL CLARITY	VISUAL CLARITY	HUNTER HAZE	VISUAL CLARITY	VISUAL CLARITY
-SO_2	7.21	++	-	4.19	++	+++
-SO_2, ENZYME	30.50	+++	-	14.10	++	++
-SO_2, FINE	0.03	-	-	1.25	-	+
-SO_2, ENZYME+FINE	0.04	-	-	1.60	-	-
+SO_2	8.49	-	-	0.74	-	-+
+SO_2, ENZYME	N. D.	-	-	N. D.	-	-
+SO_2, FINE	0.00	-	-	0.80	-	-
+SO_2, ENZYME+FINE	N. D.	-	-	N. D.	-	-

1 Pre-UF treated juice, settling overnight at 18°C, filtered through 0.45µ millipore membrane before subjecting to heat/cold test.

2 Juices processed with oxidation (-SO_2) and with minimal oxidation (+SO_2).

Hunter haze reading after heat/cold test (80°C, 6h; 4°C, 12h) and measured by Hunter meter.

Visual Clarity: - = clear, -+ = Trace haze, + = Slight haze, ++= Moderate haze, +++ = Strong haze, ++++ = Extreme haze.

N. D. : Not determined.

Conclusions

The conclusions obtained in this study can be summarized as follows:

1.- Pre-UF treatment with enzyme and fining increased UF flux.

2.- Processing with and without oxidation (± SO_2) affected color, composition and stability of ultrafiltered juices.

3.- Post clarification sediment formation is associated with oxidation.

4.- Sediments contained proteins, phenolics and neutral polysaccharide (trace pectin).

5.- Membranes do not retain all proteins, pectins, phenolics. e.g. up to 99% of pectins, 90% of pectins, and low variable percentage of phenolics were retained by membrane MWCO 10,000 daltons.

6.- Pre-UF treatment with enzyme and fining removed compounds (or precursors) including proteins and phenolics which passed through membranes and caused sediment formation, even in juices processed without SO_2.

7.- During UF, there is significant increase in the concentration of soluble protein and water soluble pectin passing through the membrane with increasing VCR (process time).

8.- It is not only the amount but the nature/state of compounds such as proteins, phenolics, pectins, and their interaction that results in instability. More research is required at the molecular level.

Literature Cited

1. Barillere J. M., J. L. Escudier, M. Moutounet, and P. Benard. Microfiltration tangentielle de mouts et de cins lere partie: compte-rendu des essais 1984. *In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 179-195. Institut Technique de la Vigne et du Vin. 21, rue Francois 1er 75008 Paris (1985).

2. Blumenkrantz, M., and G. Asboe-Hansen. New method for qualitative determination of uronic acids. Anal. Biochem. 54:484-9 (1973).

3. Feuillat, M., and P. Benard. Influence de la filtration tangentielle de mouts et de vins. Quelques essais industriels conduits en 1984. *In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 225-250. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris (1985).

4. Fombin, J. F. Ultrafiltration of Grape Juice: Effects on composition, quality, and preservation. Master Thesis, Oregon State University. (1983).

5. Heatherbell, D. A., J. L. Short, and P. Stauebi. Apple juice clarification by ultrafiltration. Confructa 22:157-169 (1977).

6. Heatherbell, D. A. Fruit juice clarification and fining. Confructa 22:192-7 (1984).

7. Heatherbell, D. A., P. Nagaba, J. Fombin, B. Watson,Jr., Z. Garcia, J. Flores, and J. Hsu. Recent developments in the application of ultrafiltration and protease enzymes to grape juice and wine processing. *In:* Proceedings of the International Symposium on Cool Climate Viticulture and Enology. D. A. Heatherbell, P. B. Lombard, F. W. Bodyfelt, and S. F. Price, Eds. pp 418-45. Oregon State University, Corvallis, OR. (1985).

8. Hodgson, J. Ultrafiltration of Pear Juice: Chemical and physical analysis. Master Thesis, Oregon State University. (1981).

9. Hsu. J. C., and D. A. Heatherbell. Isolation and characterization of soluble proteins in grape juice, and wine. Am. J. Enol. Vitic. 38:6-10 (1987).

10. Hsu, J. C., and D. A. Heatherbell. Heat-unstable proteins in wine. I. Characterization and removal by bentonite fining and heat treatment. Am. J. Enol. Vitic. 38:11-16 (1987).

11. Hsu, J. C., D. A. Heatherbell, J. H. Flores, and B. T. Watson. Heat-unstable proteins in grape juice and wine. II. Characterization and removal by ultrafiltration. Am. J. Enol. Vitic. 38:17-22 (1987).

12. Kirk, D. E., M. W. Montgomery, and M. G. Kortekaas. Clarification of pear juice by hollow fiber ultrafiltration. J. Food Sci. 48:1663-6 (1983).

13. Lea, A. G. H., and C. F. Timberlake. The phenolics of ciders: Effect of processing conditions. J. Sci. Fd. Agric. 29:484-492 (1978).

14. Lea, A. G. H. Tannin and colour in English cider apples. Flussiges Obst 8:356-361 (1984).

15. Matheis, G., and J. R. Whitaker. Modification of proteins by polyphenol oxidase and peroxidase and their products. J. Food Biochem. 8:137-162 (1984).

16. Nagaba-Mbiakop, P. R. Investigation of methods for determination and prevention of protein instability in wines. Ph. D. Thesis, Oregon State University. (1981).

17. Nagel, C. W., and U. Schobinger. Investigation of the origen of turbidity in ultrafiltered apple and pear juice concentrate. Confructa 29:16-22 (1985).

18. Neubeck, C. E. Fruits, fruits products and wines. *In:* Enzymes in Food Processing. 2nd Editon. G. Reed, Ed. pp 397-438. Academic Press. New York, NY. (1975).

19. Pocock, K. F., and B. C. Rankine. Heat test for detecting protein instability in wine. Aust. Wine. Brew. Spirit Rev. 91:42-3 (1973).

20. Robertson, G. L. The fractional extraction and quantitative determination of pectic substances in grapes and musts. Am. J. Enol. Vitic. 30:182-186 (1979).

21. Rohm Tech. Inc. (Subsidiary of Rohm GmbH, Darmstadt, W. Germany): Technical information in alcohol test for residual pectin in fruit juices. New York, NY. March. (1982).

22. Romicon, Inc. Operating instructions Romicon model HF-LAB-5 ultrafiltration system with reverse flow. Woburn, MA (1984).

23. Schmitt, R. Turbidity in apple juice - arabanes ?. Confructa 29:22-26 (1985).

24. Testaniere, D. Resultats d'essai d'ultrafiltration de vin blanc appareil industriel: Millipore.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 131-140. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris (1985).

25. Thibault, J. F. Automatisation du dosage des substances pectiques par la methode au meta-hydroxydiphenyl. Lebensm. Wiss. u -Technol. 12:247-51 (1979).

26. Van Buren, J. P. Haze in bottled apple juice. *In:* Processed Apples-Research Report 1983. D. L. Dowing, Ed. New York state agricultural experiment station, Geneva, N. Y. November. (1983).

27. Villetaz, J. C., D. Steiner, and H. Trogus. The use of a beta glucanase as an enzyme in wine clarification and filtration. Am. J. Enol. Vitic. 35(4):253-256 (1984).

28. Wucherpfennig, K., H. Dietrich, and K. Bazinger. Use of micro- and ultrafiltration on fruit juice and wine (trans). Der Duetsche Weinbau 22/26:1144-56 (1984).

29. Wucherpfennig, K., H. Dietrich, and R. Fauth. The influence of polysaccharides on clarification and filterability of wine with special reference to Botrytis glucan (trans). Deutsche Lebensmittel-Rundschau 80(2):38-44 (1984).

30. Wucherpfennig, K., H. Dietrich, and R. Scholz. Influences of enzyme treatment and fining of apple juice on fluxrate on ultrafiltration systems. Fluss. Obst. 6:324-330 (1985).

31. Wucherpfennig, K., H. Dietrich, H. Schmitt, and K. Weber. Effect of different clarification processes with regard to their absorptive capacity for colloidal juice components. Fluss. Obst. 53:527-538 (1986).

32. Youval, A., and E. Zisner. Les materiels, les materiaux et les systemes d'ultrafiltration et de microfiltration. tangentielle.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 61-70. Institut Technique de la Vigne et du Vin. 21, rue Francois 1er 75008 Paris (1985).

TITLE: ULTRAFILTRATION OF WHITE RIESLING JUICE: SENSORY EVALUATION OF JUICE PROCESSED WITH AND WITHOUT SULFUR DIOXIDE AND STORED AT 2° AND 20°C.

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RUNNING HEAD: ULTRAFILTRATION OF WHITE RIESLING JUICE.

Technical Paper No. _____ from the Oregon Agricultural Experiment Station.

ULTRAFILTRATION OF WHITE RIESLING JUICE: SENSORY EVALUATION OF JUICE PROCESSED WITH AND WITHOUT SULFUR DIOXIDE AND STORED AT 2° AND 20°C.

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Technical Paper No._____ from the Oregon Agricultural Experiment Station.

Abstract

Clarified White Riesling juices processed by Ultrafiltration (UF) with SO_2 (with minimum oxidation) and without SO_2 (with oxidation), and stored for 2 months (1985 vintage) and 12 months (1984 vintage) at 2°C and 20°C, were evaluated by a trained panel using descriptive analysis. Of the 22 descriptors evaluated, five were significantly different among treatments for the 2-month-old juice, and six were for the 12-month-old juice. UF juices processed with minimum oxidation and stored for 12 months had lower intensity aroma (apple/apple cider, sweet, and honey/caramel) and overall flavor intensity than those processed with oxidation. Moreover, juices processed with minimum oxidation and stored for two months (1985 vintage) had significantly lower apple/apple cider, sweet, honey/caramel aroma intensity when compared to those processed with oxidation. There was no effect of temperature of storage on any of the aroma and flavor-by-mouth descriptors for the 1985 juice after two months of storage. Only one aroma descriptor (vegetative) was significantly increased for the 1984 White Riesling juice after 12 months at 20°C. This indicates the possibility that UF juices may be stored at higher temperature (20°C) for less cost with minimal changes in aroma and flavor .

Introduction

Conventional procedures used for the clarification and stabilization of grape juice are labor, time and energy consuming. Typically, several batch operations including the use of enzymes, bentonite, centrifugation and filtration, are followed by thermal processing and/or expensive refrigeration and/or storage in the presence of high concentrations of SO_2. In recent years UF has emerged as a versatile membrane separation process which is increasingly finding application in the beverage industry (11). A major interest of our research group has been to evaluate the application of UF for the production of clarified, stable grape juice, as a product in its own right, for blending into other beverages, and for use as "sweet" reserve for back-blending into wines.

Sulfur dioxide (SO_2) is one of the most important additives used in winemaking, and it has been used as a preservative in wines and other food for centuries (9). Recently SO_2 has been shown to be harmful to asthmatics. Therefore, we have been investigating the production (and storage) of clarified stable grape juice by UF with or without low concentrations of sulfur dioxide (2).

Descriptive analysis is a sensory technique which involves the detection and description of a product's sensory characteristics by a well trained panel. Descriptive analysis has found considerable application in the field of beverage flavor research, including wines (4, 5, 7, 8), beer (6), cider (13), and fruit juices (1, 12). The main objective of this study was the sensory evaluation, by a trained panel using descriptive analysis, of the juices processed with and without SO_2, and the evaluation of the effect of storage temperature (2° and 20°C) on these juices.

Material and Methods

Preparation of Grape Juices: Two vintages of White Riesling grapes were processed with and without the addition of 50 ppm of sulfur dioxide (± SO_2) into juices by conventional procedures in the Oregon State University experimental winery. Settled press juices (overnight settling at 18°C) were filtered through a 200-μ mesh filter bag (Filter Specialists, Inc., MI), with the SO_2 adjusted to 25-30 ppm free SO_2, before ultrafiltration (UF).

Ultrafiltration Process: The HF-Lab-5 Ultrafiltration system (Romicon Inc., MA) equipped with PM-10 cartridge [membrane molecular weight cut-off (MWCO) of 10 000 daltons; membrane area, 0.46 m^2] was used in this study. The unit was operated in the batch mode under standardized operating conditions (ΔP_T = 1.23 kg/cm2 , 18-25°C, to VCR = 6.7), which were previously determined (3). For operation in the batch mode, 20-L of juice were ultrafiltered, and no feed was added to the recirculation loop. Final ultrafiltered juices were aseptically bottled in a sterile hood using 800 mL glass bottles and crown caps. The juices were stored for 2 months (1985 vintage) and 12 months (1984 vintage) at 2°C and 20°C before subjection to sensory analysis. At the completion of an experiment the UF unit was immediately drained and flushed with water at 45°C. The cartridge was subsequently cleaned and sanitized following the procedure described in the Romicon manual (10).

Sensory Procedures: Ten Oregon State University Food Science students were selected on the basis of availability and interest to participate in a juice panel. The selected panel met for nine sessions, where they were exposed to a range of White Riesling juices. In the initial training sessions, the panel was encouraged to individually provide aroma and flavor terms that described the juices. In subsequent sessions the panel was provided with standards to aid in the development of a set of aroma and flavor descriptors. In the three final training sessions the panel practiced

evaluating actual test samples with a ballot and with standards available in order to test and refine the descriptive terms. In using the ballot, panelists were asked to first evaluate the sample for overall intensity. Then the sample was rated for its more general characteristics progressing to the more specific terms. The aroma and flavor-by-mouth terms selected for the two vintage White Riesling juices are listed in Table III.1, with the composition of the reference standards used to define them. The meaning of terms not having reference standards were agreed upon through discussion by the panel.

At the beginning of each test session, the judges smelled the reference aroma standards and then scored the intensity of each of the aroma terms for the juices on a nine-point intensity scale (1= none, to 9= extreme). Once the aroma was evaluated, the samples were re-coded and returned to the judges, and the juices were then scored on the intensity of flavor-by-mouth descriptors. Panelists were seated in individual testing booths with red lighting to mask color differences. The study was divided in two parts, 1) evaluation of the vintage 1984 White Riesling juice; and 2) evaluation of the vintage 1985 White Riesling juice. The four treatments ($\pm SO_2$ at 2°C and 20°C) were evaluated at each session. Twenty-five mL samples were presented at ambient temperature (20°C), in a random order in three digit coded, black 250 mL (8.5 oz) tulip-shaped glasses, which were covered with watch glasses.

Sensory Design and Data Analysis: A randomized block design for a factorial treatment set was used to evaluate the 4 juices of each vintage in triplicate in order to evaluate the effect of SO_2 (minimum and maximum oxidation) and temperature storage (2°C and 20°C) on ultrafiltered White Riesling juice. Analysis of variance was conducted for each term rated by the judges. Fisher's least significant difference was calculated for each attribute which differed across wines.

Table III.1 Aroma and Flavor-by-Mouth Descriptors and Standards Developed for the Two Vintages of White Riesling Juice.

TERM	STANDARD[1]
AROMA	
Overall Intensity	
Fruity	
Apple/A. Cider	Fresh, sliced apple - 1/4 cup Fresh press apple juice - 1/4 cup
Grape	
Apricot/Peach (nectar)	Canned apricots in heavy syrup - 1/4 cup Canned peaches in heavy syrup - 1/4 cup
Vegetative	
Stem/Seeds	Thompson grape stems - 3 in. crushed grape seeds - crushed
Asparagus/Green Beans	Canned sparagus - 1/4 cup Canned green beans - 2 tbs.
Sweet	
Honey/Caramel	Clover honey - 1 tbs.
Chemical	
Sulfur dioxide	Sulfur dioxide - 40 ppm & 10% citric ac. in H_2O - 1 tbs.
Metallic	
FLAVOR BY MOUTH	
Overall Intensity	
Sweet	
Sour	
Fruity	
Apple/Apple Cider	
Grape	
Apricot/ Peach (nectar)	
Mouth feel:	
Viscosity	
Astringency	

1 Standards served as described in 8 1/2 ounce wine glasses covered with watch glasses.

Results and Discussion

White Riesling Juice 1984 Vintage: Of the 22 descriptors available for describing the aroma and flavor-by-mouth of the WR juices, only 5 aroma and 1 flavor-by-mouth descriptors showed significant differences (Table III.2). A summary of the Analysis of Variance (ANOVA) of the effect of sulfur dioxide and storage temperature on these significant aroma and flavor-by-mouth descriptors is shown in Table III.2.

SO_2 had a significant effect on ratings of five aroma descriptors (apple/apple cider, vegetative, sweet, honey/caramel and sulfur dioxide) and the overall intensity descriptor for flavor-by-mouth (Table III.2). UF juices processed with SO_2 and stored for 12 months were rated lower in aroma intensity for apple/apple cider, sweet, and honey/caramel and lower in overall flavor-by-mouth intensity than those processed without SO_2 (Table III.3). However, the vegetative and sulfur dioxide aroma intensities were found to be significantly higher in the juice processed with SO_2.

Storage temperature only had a significant effect on the vegetative aroma descriptor. After 12 months of storage, the intensity of the vegetative aroma descriptor on the juice stored at 20°C (2.9) was found to be significantly higher than the intensity of the juice stored at 2°C (2.4).

From the ANOVA table III.2, three aroma descriptors (apple/apple cider, sweet, and honey/caramel) were found to have a significant judge by treatment (J x T) effect. Inspection of each judge's ratings as compared to the panel's average rating can help explain the source of the interaction. Close examination of the data showed that for the sweet aroma descriptor only one panelist rated the juices processed with SO_2 higher, while the rest of the panel rated the juices processed without SO_2

Table III.2 Analysis of Variance of the Significant Aroma and Flavor-by-Mouth Descriptors for the 1984 White Riesling Ultrafiltered Juice: F-values and Degrees of Freedom.

DESCRIPTORS	F - values										MEAN SQUARE
				TREATMENT (T)							
	JUDGES (J)	REPLI. (R)	J x R	TOTAL	SULFUR DIOXIDE (S)	TEMPER-ATURE (TEMP)	S x TEMP	J x T	T x R	ERROR	
AROMA:											
APPLE/APPLE CIDER	2.04*	1.21	1.03	1.99	4.44*	0.99	0.52	1.86*	0.75	1.892	
VEGETATIVE	4.04**	1.63	0.73	2.98*	3.88*	4.34*	0.71	1.09	0.40	1.472	
SWEET	1.45	0.49	1.46	5.01**	13.70**	0.93	0.38	1.93*	0.79	1.064	
HONEY/CARAMEL	4.44**	0.39	1.35	4.78**	13.35**	0.60	0.42	2.69**	0.59	0.985	
SULFUR DIOXIDE	4.70**	0.83	0.88	3.04*	7.40*	0.63	1.09	0.92	0.74	0.665	
FLAVOR BY MOUTH:											
OVERALL INTENSITY	4.57**	2.51	1.16	2.42*	4.48*	1.27	1.51	0.78	0.28	0.898	
Degrees of Freedom	9	2	18	3	1	1	1	27	6	54	

*,**, indicate significance at $p < 0.05$, $p < 0.01$, respectively.

higher. Therefore, this significant judge by treatment effect is a result of a crossover type effect by only one panelist.

Table III.3 Mean Scores* of the Statistically Significant Sensory Descriptors of the 1984 White Riesling Juice.

DESCRIPTORS	SULFUR DIOXIDE	
	PRESENCE	ABSENCE
AROMA		
APPLE/APPLE CIDER	2.15b	2.97a
VEGETATIVE	2.85a	2.37b
SWEET	2.87b	4.00a
HONEY/CARAMEL	1.83b	3.15a
SULFUR DIOXIDE	1.78a	1.28b
FLAVOR BY MOUTH		
OVERALL INTENSITY	5.88b	6.22a

*Scored on a nine-point intensity scale (1=none, to 9=extreme).
Note: Means within the same row designated by the same letter are not significant different at $p< 0.05$.

Similar results were found for the aroma descriptors apple/apple cider and honey/caramel. However, the numbers of panelists having a crossover effect was higher. For the apple/apple cider and honey/caramel aroma descriptors 3 and 2 judges, respectively, rated the juices processed with SO_2 higher on these aroma intensities while the rest of the panel rated higher the juices processed without SO_2.

Therefore, the majority of the panelists agree on the pattern of response to these treatments and the significant judge by treatment effect may be the result of only 1 to 3 judges with a different pattern of response from the rest of the panel.

White Riesling Juice 1985 Vintage: Of the 22 descriptors available for describing the aroma and flavor-by-mouth of the WR juices, only 3 aroma and 2 flavor-by-mouth descriptors showed significant differences (Table III.4). A summary of the Analysis of Variance (ANOVA) of the effect of sulfur dioxide and storage temperature on these significant aroma and flavor-by-mouth descriptors is shown in Table III.4. SO_2 had a significant effect on the ratings of three aroma descriptors (apple/apple cider, sweet, and honey/caramel) and two flavor-by-mouth (overall intensity, and apple/apple cider) descriptors (Table III.5). UF juices processed with SO_2 and stored for 2 months had, once again, lower intensity aroma (apple/apple cider, sweet, and honey/caramel) and flavor-by-mouth (overall intensity, apple/apple cider) than those processed without SO_2 (Table III.5).

Storage temperature did not have a significant effect on any aroma or flavor-by-mouth descriptor evaluated in this study.

All the significant aroma and flavor-by-mouth descriptors, except overall intensity flavor-by-mouth, had a significant judge by treatment effect (Table III.4). From these, the panelist ratings for the aroma apple/apple cider and honey/caramel descriptors had 2, and 1 (respectively) out of 10 panelist who rated the juices processed with SO_2 higher than the juices processed without SO_2. From the remaining panelists, one panelist on each descriptor did not detect any difference between $\pm SO_2$. Once again, the significant judge by treatment effect is mainly the result of a crossover type effect by one or two panelists.

The results for the other two descriptors (sweet aroma, and apple/apple cider flavor-by-mouth) should be interpreted cautiously since 4 out of 10 panelist had different response patterns than the remaining judges. This may be an indication of inadequate training for these descriptive terms or real differences in the way they are perceived.

Table III.4 Analysis of Variance of the Significant Aroma and Flavor-by-Mouth Descriptors for the 1985 White Riesling Ultrafiltered Juice: F-values and Degrees of Freedom.

	F - values									MEAN SQUARE
				TREATMENT (T)						
DESCRIPTORS	JUDGES (J)	REPLI. (R)	J x R	TOTAL	SULFUR DIOXIDE (S)	TEMPER-ATURE (TEMP)	S x TEMP	J x T	T x R	ERROR
AROMA:										
APPLE/APPLE CIDER	1.17	1.04	1.03	3.72*	9.35**	1.04	0.78	5.37**	0.85	0.956
SWEET	2.93**	2.29	0.74	3.54*	9.82**	0.32	0.48	2.98**	1.15	0.684
HONEY/CARAMEL	2.04*	1.24	1.45	6.14**	16.69**	0.48	1.25	4.01**	0.55	0.710
FLAVOR BY MOUTH:										
OVERALL INTENSITY	8.03*	0.93	1.43	1.98	3.92*	1.09	0.95	0.80	0.32	0.635
APPLE/APPLE CIDER	2.56*	1.21	1.43	2.31	6.13*	0.33	0.45	2.78**	0.59	1.060
Degrees of Freedom	9	2	18	3	1	1	1	27	6	54

*,**, indicate significance at $p < 0.05$, $p < 0.01$, respectively.

Table III.5 Mean Scores* of the Statistically Significant Sensory Descriptors of the 1985 White Riesling Juice.

DESCRIPTORS	SULFUR DIOXIDE	
	PRESENCE	ABSENCE
AROMA		
APPLE/APPLE CIDER	5.35b	5.68a
SWEET	2.70b	3.65a
HONEY/CARAMEL	1.62b	2.95a
FLAVOR BY MOUTH		
OVERALL INTENSITY	5.70b	6.00a
APPLE/APPLE CIDER	2.13b	2.97a

*Scored on a nine-point intensity scale (1=none, to 9=extreme).
Note: Means within the same row designated by the same letter are not significant different at $p< 0.05$.

Conclusions

The panel selected 22 terms to describe White Riesling juice character. Very few of these descriptors were found to show significant differences among the treatments tested (six for 1984, and five for 1985 juices). The other terms described aroma and flavor notes which did not change due to processing and storage.

Effect of Sulfur Dioxide (SO_2): Juices processed without SO_2 (oxidation) were higher in apple/apple cider, sweet, and honey/caramel character. These aroma qualities may be highly desirable in some juice applications such as back-blending into wines. For example the honey character is a late harvest age character in some wine styles which may be achievable in a young wine by back-blending the juice as sweet reserve.

Effect of Storage Temperature: There was no effect of temperature of storage on any of the aroma and flavor-by-mouth descriptors for the 1985 juice after two months of storage. Only one aroma descriptor (vegetative) was significantly increased for the 1984 White Riesling juice after 12 months at 20°C. This indicates the possibility that UF juices may be stored at higher temperature (20°C) for less cost with minimal changes in aroma and flavor.

Literature Cited

1. Dürr, P. Development of an odour profile to describe apple juice essences. Lebensm.-Wiss. u.-Technol. 12;23-26 (1979).

2. Flores, J. H., D. A. Heatherbell, J. C. Hsu, and B. T. Watson. Ultrafiltration (UF) of White Riesling juice: Effect of oxidation and of pre-UF juice treatment on flux, composition and stability. Submitted for publication Am. J. Enol. Vitic. (1987).

3. Heatherbell, D. A., P. Nagaba, J. Fombin, B. Watson,Jr., Z. Garcia, J. Flores, and J. Hsu. Recent developments in the application of ultrafiltration and protease enzymes to grape juice and wine processing. *In:* Proceedings of the International Symposium on Cool Climate Viticulture and Enology. D. A. Heatherbell, P. B. Lombard, F. W. Bodyfelt, and S. F. Price, Eds. pp 418-45. Oregon State University, Corvallis, OR. (1985).

4. Heymann, H., and A. C. Noble. Descriptive analysis of commercial Cabernet Sauvignon wines from California. Am. J. Enol. Vitic. 38(1):41-44 (1987).

5. McDaniel, M. R., L. A. Henderson, B. T. Watson, and D. A. Heatherbell. Sensory panel training and screening for descriptive analysis of the aroma of Pinot noir fermented by several strains of malolactic bacteria. J. of Sensory Studies, in press. (1987).

6. Mecredy, J., J. Sonnemann, and S. Lehmann. Sensory profiling of beer by a modified QDA method. Food Technol., 28(11):36-39 (1974).

7. Noble, A. C., A. A. Williams, and S. P. Langron. Descriptive analysis and quality of Bordeaux wines. *In:* Sensory Quality in Food and Beverages. definition, measurement and control. A. A. Williams, and R. K. Atkin, Eds. pp 324-334. Verlay Chemie International, Inc., Florida. (1983).

8. Noble, A. C., and M. Shannon. Profiling Zinfandel wines by sensory and chemical analysis. Am. J. Enol. Vitic. 38(1):1-5 (1987).

9. Ough, C. S. Sulfur dioxide and sulfites. *In:* Antimicrobials in Foods. A. L. Branen, and P. M. Davidson, Eds. p. 177. Marcel Dekker, Inc. New York. (1983).

10. Romicon, Inc. Operating instructions Romicon model HF-LAB-5 ultrafiltration system with reverse flow. Woburn, MA (1984).

11. Swientek, R. J. Ultrafiltration's expanding role in food and beverage processing. Food Processing 47; 71-83 (1986).

12. Von Sydow, E., H. Moskowitz, H. Jacobs, and H. Meiselman. Odor-taste interaction in fruit juices. Lebensm.-Wiss. u.-Technol. 7;18-24 (1974).

13. Williams, A. A. Development of a vocabulary and profile assessment method for evaluating the flavour contribution of cider and perry aroma constituents. J. Sci. Fd. Agric. 26;567-582 (1975).

TITLE: **ULTRAFILTRATION OF WINE: EFFECT OF ULTRAFILTRATION ON WHITE RIESLING AND GEWURZTRAMINER WINE QUALITY AND STABILITY.**

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RUNNING HEAD: ULTRAFILTRATION OF WINE.

Technical Paper No. _____ from the Oregon Agricultural Experiment Station.

ULTRAFILTRATION OF WINE: EFFECT OF ULTRAFILTRATION ON WHITE RIESLING AND GEWURZTRAMINER WINE QUALITY AND STABILITY.

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Abstract

White Riesling (WR) and Gewurztraminer (GEW) wines were ultrafiltered with Romicon and Millipore pilot-scale systems, respectively. The effect of ultrafiltration (UF), membrane nominal molecular weight cut-off (MWCO) from 10,000-50,000 daltons, and of volume concentration ratio (VCR) on composition and wine stability were investigated. The effect of 1) pilot-scale UF processing and bentonite fining on WR and GEW wines stored for 9 and 13 months, respectively, at 13° to 15°C, and 2) commercial-scale UF processing on GEW wine which had been stored at 13° to 15°C for 9 months was sensorially evaluated by a trained panel using descriptive analysis. UF processing significantly reduced color (A_{420nm}), total phenol, protein and heat/cold test (HCT) haze of both WR and GEW wines. Stability to HCT haze formation was obtainable with MWCO of 10,000 daltons but trace instability can remain. With increasing VCR (increasing process time) there was a significant decrease in membrane retention of color (A_{420nm}), protein, and HCT haze formation in the WR wine and the color (A_{420nm}) of the GEW wine. UF processing of the WR wine significantly decreased the overall aroma intensity, and fruity, fresh fruity citrus, floral, sweet and honey/caramel aromas but it also increased the intensity of vegetative aroma when compared to the control unfiltered WR wine. In addition, significant differences were detected for these descriptors between the bentonite-fined WR wine and the ultrafiltered WR wine except for fresh fruity citrus and honey/caramel aromas which were less intense in the ultrafiltered WR wine. UF processing of GEW wine significantly decreased the intensity of fruity, fresh fruity aroma descriptors; and increased the chemical aroma descriptor compared to the control unfiltered GEW wine. However, no significant differences were detected for these descriptors between the bentonite fined GEW wine and the ultrafiltered GEW wine except for fresh fruity which was less intense in the

ultrafiltered GEW wine. Commercial processing of GEW wine by UF did not have any significant effect on the aroma and flavor-by-mouth descriptors evaluated.

Introduction

In recent years there has been increasing interest internationally in the application of cross-flow filtration (UF and microfiltration) for wine processing (5,8,12,16,17,20,21,28). Its application as an alternative to conventional processes for clarification and preserving fruit juices was demonstrated as early as 1977 by Heatherbell et al.(1977) for apple juice. The possibility of using membrane ultrafiltration for clarifying and protein stabilizing grape juice and wines has been recognized (1,3,6,28,29). Recently we reported on the application of UF for removing unstable proteins from grape juice and wine (12). The three main objectives of this study were to investigate: 1) effect of UF (MWCO) on composition and stability; 2) effect of VCR during processing on composition and stability; and 3) effect of UF on the sensory properties of the wines.

Materials and Methods

Preparation of Wines: White Riesling (WR) and Gewurztraminer (GEW) grapes were processed into wines by conventional procedures in the Oregon State University experimental winery. Settled young wines (after two rackings) were used for this study.

Ultrafiltration of Wines: The UF units used in pilot plant trials in this study were: Romicon HF-Lab-5 ultrafiltration system with reverse flow (Romicon, Inc., MA) with PM-10, and PM-50 cartridges [membrane molecular weight cut-off (MWCO), 10 000, 50 000 daltons, respectively; membrane area, 0.46 m^2], and a Millipore Pellicon cassette system (Millipore, Co., MA) with PTGC-10 and PTTK-30 cassettes (membrane MWCO, 10 000 and 30 000 daltons, respectively; membrane area, 0.46 m^2). The units were operated in the batch mode under standardized operating conditions as previously determined (9) and as specified under tables and figures. For operation in the batch mode of the Romicon and Millipore units, 20-L and 4-L, respectively, of wine were ultrafiltered to the VCR specified, and no feed was added to the recirculation loop. Permeate samples for analysis were obtained from the permeate exit pipe, which was covered with an aluminum foil to prevent airborn contamination. Retentate samples were obtained from the feed tank. Ultrafiltered wines were analyzed within 2 weeks. WR, and GEW pilot plant wine trials were bottled in 1 gal. glass bottles and stored for 9, and 13 months, respectively, at 13° to 15°C before subjection to sensory analysis. At the completion of an experiment the UF unit was immediately drained and flushed with water at 45°C. The cartridge was subsequently cleaned and sanitized following the procedure described in the Romicon manual (19). Commercially processed wine which had been ultrafiltered with a Millipore commercial PUF (process ultrafiltration) system with spiral-wound cartridges (polysulfone membrane with MWCO 10 000 daltons) was also evaluated.

In the commercial trial, 5500 gallons of GEW wine was ultrafiltered at 10° to 13°C to VCR=92. Commercial GEW wine trials were stored for 9 months, at 10° to 15°C before subjection to sensory analysis. Familiarity with the following terms is necessary to interpret the results discussed in the tables and figures:

Volume Concentration Ratio (VCR) = $V_I / (V_I - V_P) = V_I / V_R$

were V_I = initial (feed) volume
V_R = retentate volume
V_P = permeate volume.

ΔP_T = average transmembrane pressure = $(P_{inlet} + P_{outlet})/2$

were P = pressure.

Bentonite fining: A proportion of the WR and GEW pilot plant wine trials were also bentonite-fined (sodium bentonite, Volclay) at 80 g/hL and 30 g/hL, respectively, the minimum dosage required for protein stability.

Determination of Total Phenol and Soluble Protein: Total phenol, soluble protein determination and electrophoresis of proteins were performed as previously described (10).

Color (browning) and Heat Stability Test: Wine color (browning) was measured as optical density at 420-700 nm, against a water blank. The heat stability of the wines was determined using the procedure recommended by Pocok and Rankine (18). The formation of haze was measured as previously described (11).

Wine Processing Data Analysis: All analytical determinations were carried out in duplicate on each processing trial replicate. All statistical analysis were performed using SAS (Statistical Analysis Systems, Cary, NC). A completely randomized factorial design and a completely randomized factorial design with repetitive measurements during processing (VCR) were used. Analysis of Variance was calculated using the PROC ANOVA to test the effect of:

1) MWCO and UF on color (browning), total phenol, soluble protein, and Heat/Cold test (HCT) haze formation.

2) MWCO and VCR on flux, color (browning), total phenol, soluble protein, HCT haze formation.

Sensory Analysis: Ten Oregon State University Food Science students were selected on the basis of availability and interest to participate in a wine panel. The selected panel met for nine sessions, where they were exposed to a range of WR and GEW wines. In the initial training sessions, the panel was encouraged to individually provide aroma and flavor terms that described the wines. In subsequent sessions the panel was provided with standards to aid in the development of a set of aroma and flavor descriptors. In the three final training sessions the panel practiced evaluating actual test samples with a ballot and with standards available in order to test and refine the descriptive terms. In using the ballot, panelists were asked to first evaluate the sample for overall intensity. Then the sample was rated for its more general characteristics progressing to the more specific terms. The aroma and flavor-by-mouth terms selected for WR and GEW wines are listed in Table IV.1 and IV.2, respectively, with the composition of the reference standards used to define them. The meaning of terms not having reference standards were agreed upon through discussion by the panel.

At the beginning of each test session, the judges smelled the reference aroma standards and then scored the intensity of each of the aroma terms for the wines on a nine-point intensity scale (1= none, to 9= extreme). Once the aroma was evaluated, the samples were re-coded and returned to the judges, the wines were then scored on the intensity of flavor-by-mouth descriptors. Panelist were seated in individual testing booths with red lighting This study was divided in two parts, 1) evaluation of White Riesling wines (pilot-scale trial); and 2) evaluation of the

Table IV.1 Aroma Descriptors and Standards Developed for White Riesling[1] and Gewurztraminer Wines.

TERM	STANDARD[2]
Overall Intensity	
Fruity	
Fresh Fruit	
Citrus	
Grapefruit	Fresh grapefruit segments - 1/4 cup
Orange	Fresh orange segments - 1/4 cup
Lemon	Fresh lemon segments - 1/4 cup
Peel	
Berry	
Apple/A. Cider	Fresh, sliced apple - 1/4 cup Fresh press apple juice - 1/4 cup
Grape	
Cooked Fruit	
Apricot/Peach (nectar)	Canned apricots in heavy syrup - 1/4 cup Canned peaches in heavy syrup - 1/4 cup
Spicy	
Floral	
Vegetative	
Fresh Vegetative	
Stem/Seeds	Thompson grape stems - 3 in. crushed grape seeds - crushed
Cooked Vegetative	
Asparagus/Green Beans	Canned asparagus - 1/4 cup Canned green beans - 2 tbs.
Sweet	
Honey/Caramel	Clover honey - 1 tbs.
Vanilla	Vanilla extract - 1/2 tsp.
Chemical	
Pungent	
Sulfur dioxide	Sulfur dioxide - 40 ppm & 10% citric ac. in H_2O - 1tbs.
Ethanol	Ethanol - 10% in H_2O- 1 tbs.
Lactic acid	Lactic ac. - 10% in H_20- 1 tbs.
Acetaldheyde	Acetaldheyde - 50 ppm. in wine - 1 tbs.

1 Same as above but exclude Berry and Spicy term.

2 Standards served as described in 8 1/2 ounce wine glasses covered with watch glasses.

Gewurztraminer wines (pilot-scale; and commercial trials). In order to evaluate the pilot-scale trials WR and GEW wines and the commercial-scale GEW wines, four, three and two samples, respectively, were evaluated at each session. Twenty-five mL samples were presented at ambient temperature (20°C), in a random order in three digit coded, black 250 mL (8.5 oz) tulip-shaped glasses, which were covered with watch glasses.

Table IV.2 Flavor-by-Mouth Descriptors Developed for White Riesling[1] and Gewurztraminer Wines.

Overall Intensity
Sweet
Sour
Bitter
Fruity
Fresh Fruit
Citrus
Grapefruit
Orange
Lemon
Citrus Peel
Apple/A. Cider
Grape
Cooked Fruit
Apricot/Peach (nectar)
Vegetative
Mouth feel:
Viscosity
Astringency
Tingling

1 Same as above but exclude Vegetative term.

Sensory Data Analysis: A randomized block design was used to evaluate the different wines of each trial in triplicate in order to evaluate the effect of bentonite-fining and pilot-scale UF on WR and GEW wines; and the effect of commercial-scale UF on GEW wine. Analysis of variance was executed for each term

term rated by the judges. Fisher's least significant difference was calculated for each attribute which differed across wines.

Results and Discussion

The main objectives of this study were to investigate 1) the effect of UF (including MWCO and VCR) on the composition and stability of WR and GEW wine from pilot-scale trials; and 2) the effect of UF on the quality of these two pilot-scale wines as well as a commercial scale UF-GEW wine. No attempt is made to make comparisons between UF equipment in this report. Different wines were used in each study, and some variation in the percent retention of different molecules has been observed between different wines (or juices) filtered with the same UF system and MWCO.

Effect of MWCO and VCR on Flux: A summary of the Analysis of Variance for Flux is shown in Table IV.3.

Table IV.3 Analysis of Variance, Degrees of Freedom (DF),and F-values for UF Flux of White Riesling and Gewurztraminer Wines

SOURCE	WHITE RIESLING		GEWURZTRAMINER	
	DF	F-value	DF	F-value
MOLECULAR WEIGHT CUT-OFF (MWCO)	1	6.26	1	0.63
MEAN SQUARE ERROR I	2	48.62	2	9.46
VOLUME CONCENTRATION RATIO (VCR)	8	192.48***	3	105.69***
MWCO x VCR	8	1.66	3	0.58
MEAN SQUARE ERROR II	16	0.31	6	0.03

*, **, *** indicates significant at $p < 0.05$, < 0.01, < 0.001, respectively.

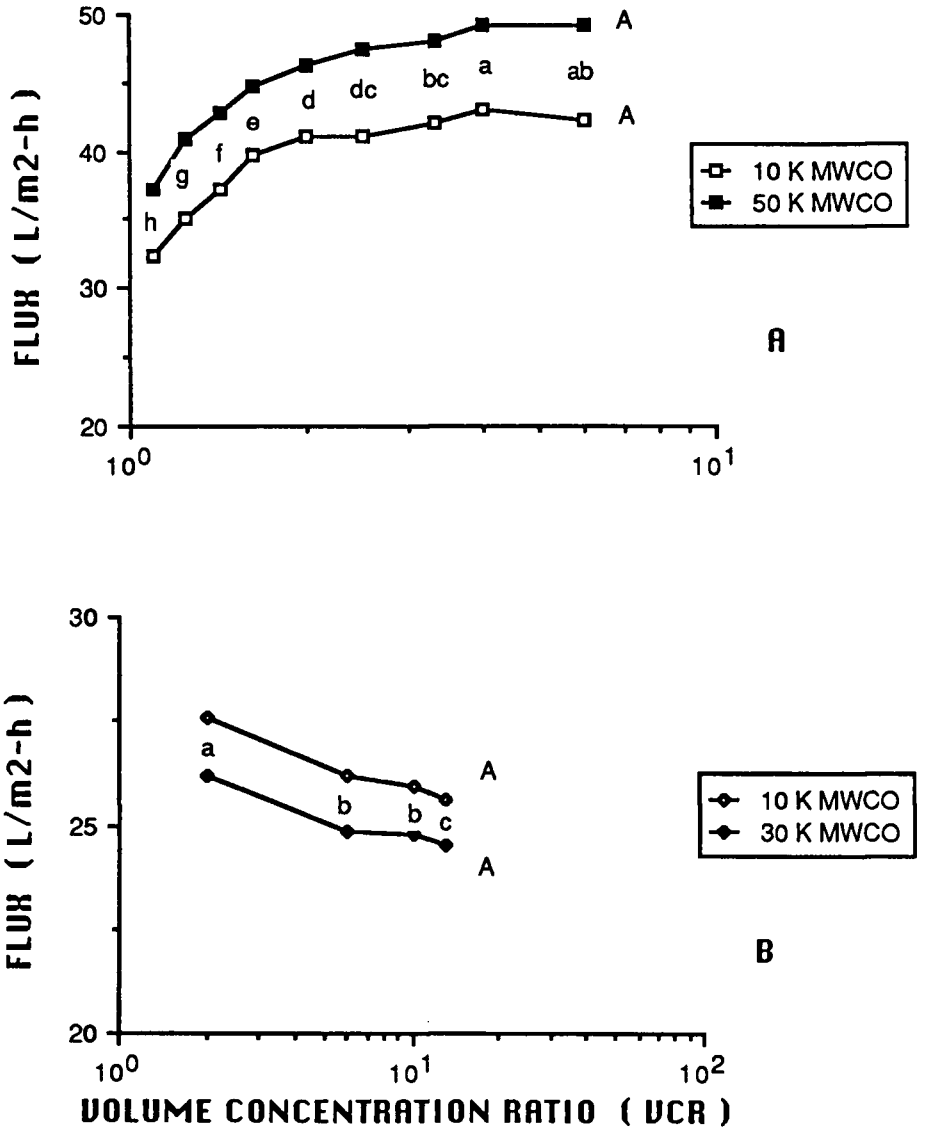

Fig. IV.1 Effect of Membrane Molecular Weight Cut-Off on Flux of **A** -White Riesling Wine (Romicon HF-Lab 5 system operated with at ΔP_T=1.23 kg/cm^2,18-27°C, VCR=6.7), and **B** -Gewurztraminer Wine (Millipore Pellicon cassette system operated with PTGC-10, and PTTK-30 cassettes at ΔP_T=1.23 kg/cm2,18-21°C, VCR=13). Capital letters indicate mean separation between different membrane MWCO, and small letters indicate mean separtion between VCR's (during UF). Means designated with the same letter are not significantly different at p = 0.05.

Flux of WR and GEW wines was significantly effected ($p < 0.001$ for both) by VCR (Table IV.3), regardless of membrane MWCO. Flux was significantly increased for the WR wine but decreased for the GEW wine as the VCR (process time) increased (Fig. IV.1). We have observed considerable variation in the fluxes achieved for different juices and wines. In this instance the increase of the WR wine flux is attributed to the increase in temperature from 18° to 27°C during processing. In contrast, the smaller temperature increment during processing (from 18° to 21°C) of the GEW wine had a relatively small effect on permeate flux in comparison with the effect of VCR, resulting in flux decline with increasing VCR. In general in our studies wine flux has not declined at the same rate juice flux has particularly at higher VCR's.

Effect of UF and MWCO on Color (browning), Total Phenol, Soluble Protein, and Heat/Cold Test Haze Formation of White Riesling and Gewurztraminer Wines: A summary of the Analysis of Variance of the effect of UF and MWCO on color (browning), total phenol, soluble protein, and HCT haze formation is shown in Table IV.4.

UF ($p < 0.001$), and MWCO ($p < 0.01$) significantly effected the color (browning) of the WR wine. However, there was a significant interaction effect between them ($p < 0.01$, Table IV.4). UF significantly reduced the color (browning) in the WR wine with both PM-10 (27%) and PM-50 (24%) MWCO membranes (Fig. IV.2).

However, no significant differences were found between the different membrane MWCOs, which indicates the nominal nature of MWCO and that brown complexes in these wines appear to be of relatively high molecular weight as has been observed previously (9).

Table IV.4 Analysis of Variance, Degrees of Freedom (DF), and F-values of the Effect of UF, Including MWCO, on the Color (browning), Total Phenol, and Soluble Proteins of White Riesling and Gewurztraminer Wines.

SOURCE	DF	F - values							
		WHITE RIESLING WINE				GEWURZTRAMINER WINE			
		COLOR (BROWNING)	TOTAL PHENOL	SOLUBLE PROTEIN	H/C T HAZE	COLOR (BROWNING)	TOTAL PHENOL	SOLUBLE PROTEIN	H/C T HAZE
ULTRAFILTRATION (UF)	1	5202.0***	105.8***	113.4***	235.5***	1626.8***	736.8***	919.0***	6407.3***
MOLECULAR WEIGHT CUT-OFF (MWCO)	1	50.0**	1.8	2.3	19.4*	1.0	1.1	9.1*	0.0
MWCO X UF	1	32.0**	57.8**	3.2	51.7**	2.8	5.1*	8.3*	0.0
MEAN SQUARE ERROR	4	25 X 10^{-8}	0.625	4.846	3.736	11.3 X 10^{-7}	1.606	4.859	0.263

*, **, *** indicates significant at $p < 0.05$, < 0.01, < 0.001, respectively.

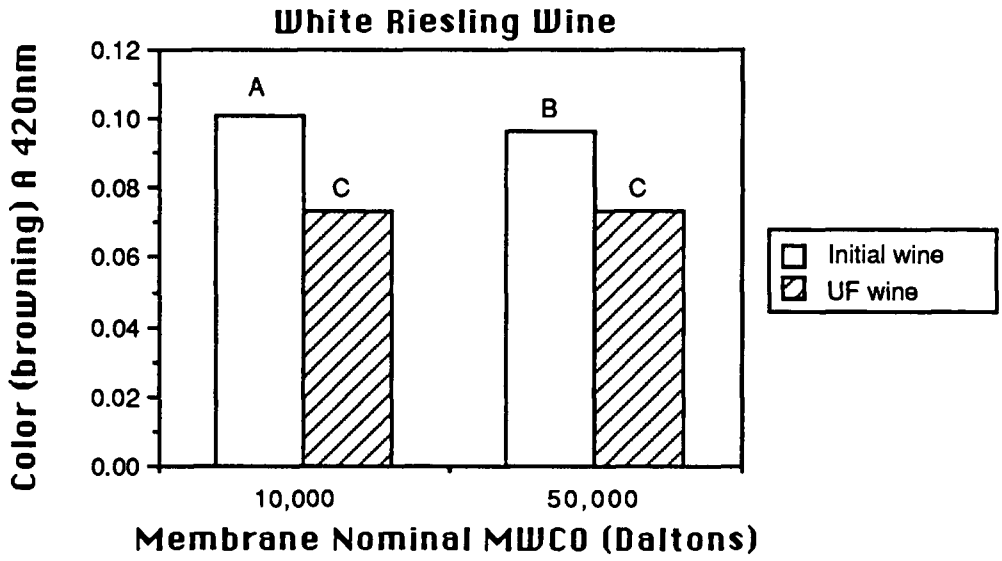

Fig. IV.2 Effect of UF and Membrane Molecular Weight Cut-Off on Color (browning) of White Riesling Wine. Romicon HF-Lab-5 system operated with PM-10, and PM-50 at ΔP_T=1.23 kg/cm2, 18-27°C, VCR=6.7. Means designated with the same letter are not significantly different at $p < 0.05$.

The effect of UF on the color (browning) of GEW wine was found to be significant ($p < 0.01$, Table IV.4). However, MWCO had no significant effect on the retention of browning compounds. Color was significantly reduced, from 0.078 to 0.048 (39%), by UF regardless of the membrane MWCO. Similar results were found for grape juice and wine in previous studies (2, 4).

UF significantly affected ($p < 0.001$) the total phenol content of the WR wine, and its effect interacted significantly ($p < 0.01$) with MWCO (Table IV.4). The amount of total phenols in this wine were significantly reduced by ultrafiltration, from 393 to 383 mg/L GAE (3%), when processed with the PM-10 MWCO membrane, but not with the PM-50 MWCO membrane. This indicates that the polymeric phenol complexes present in these wines are nominally of lower MWs than 50 000 daltons.

Likewise, UF had a significant effect ($p < 0.01$) on the total phenol content of the GEW wines (Table IV.4). A small significant reduction from 331 to 306 mg/L GAE

(7%) in the concentration of total phenols by the UF of Gewurztraminer wines was determined. However, no significant differences were detected between different MWCO membranes (Table IV.4). This again indicates the possibility that these complexes are nominally of relatively high MWs of greater than 30 000 daltons. However, consideration should also be given to the possibility that phenolics are being adsorbed to membranes. Other authors have also reported similar retentions of phenolics by UF in wines (4,23,24,30).

From the ANOVA of the effect of UF (including MWCO) on soluble protein concentration of the WR wine (Table IV.4), UF had a significant effect ($p < 0.001$) on the concentration of the soluble proteins. UF significantly decreases the concentration of soluble proteins in the WR wine by 54%, regardless of the MWCO (Fig. IV.3).

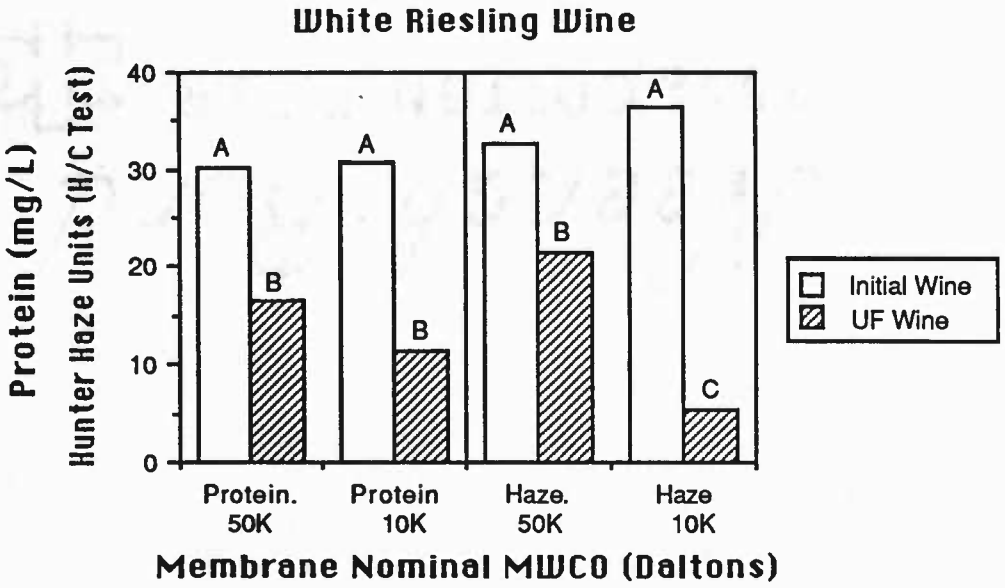

Fig. IV.3 Effect of UF and Membrane Molecular Weight Cut-Off on Soluble Protein and Heat/Cold Test Haze Formation of White Riesling Wine. Romicon system operated with PM-10, and PM-50 at ΔP_T=1.23 kg/cm2, 18-27°C, VCR=6.7. Means designated with the same letter are not significantly different at p = 0.05.

Although there is a decreasing retention of protein concentration as determined by the Bio-Rad dye-binding procedure with increasing MWCO from 10 000 to 50 000 daltons, this retention was not statistically significant. However, previous studies supported by electrophoresis of wine permeates demonstrated increased retention at 10,000 daltons (12).

Although, both UF and MWCO had a significant effect on the soluble protein content of GEW wine, and their interaction was significant (Table IV.4), the MWCO effect and UF x MWCO interaction effect accounts for less than 2% of the total source of variation while the UF effect accounts for 97%. Therefore, UF of this wine with both 10 000 and 30 000 MWCO membranes significantly reduced its protein content by 85% and 82%, respectively (Fig. IV.4).

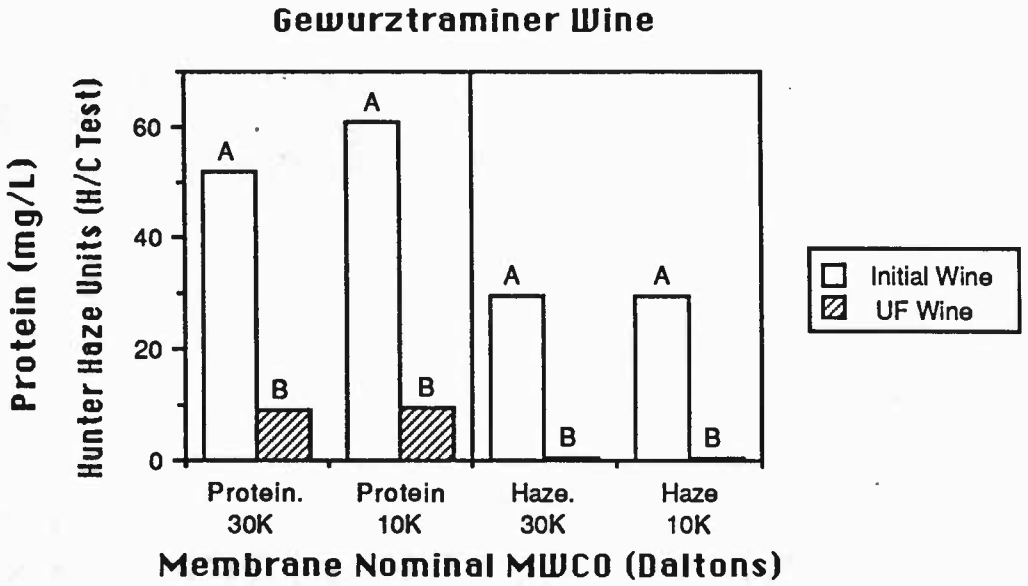

Fig. IV.4 Effect of UF and Membrane Molecular Weight Cut-Off on Soluble Protein and Heat/Cold Test Haze Formation of Gewurztraminer Wine.Millipore Pellicon cassette system operated with PTGC-10, and PTTK-30 cassettes at ΔP_T=1.23 kg/cm2,18-21°C, VCR=13. Means designated with the same letter are not significantly different at p = 0.05.

No significant differences were found between the two MWCO membranes in the retention of soluble proteins of GEW wine. Again., previous studies supported by electrophoresis demonstrated increasing retention by the 10 000 MWCO membranes. The effect of MWCO on the retention of above mentioned compounds is of particular significance to the heat/cold instability haze test. Table IV.4 demonstrates that the HCT induced haze of the WR wine was significantly affected by UF ($p < 0.001$) and MWCO ($p < 0.05$). However, these two factors significantly interacted ($p < 0.01$). HCT inducible haze was significantly reduced by ultrafiltration with PM-10 (85%) and PM-50 (32%) MWCO membranes (Fig. IV.3). Moreover, increase in the MWCO from 10 000 to 50 000 daltons significantly increased the HCT haze formation. Trace instability still remained for both WR wines ultrafiltered with Romicon membranes. Additional 5 g/Hl of bentonite was required for stabilization to HCT haze formation.

The HCT haze formation in GEW wine was significantly effected ($p < 0.01$) by ultrafiltration (Table IV.4). However, no significant differences were found between the 10 000 and the 30 000 MWCO membranes. UF significantly reduced (98%) the HCT induced hazed on GEW wines (Fig. IV.4).

Although HCT induced haze stability was achieved with the GEW wine ultrafiltered with the Millipore 10 000 MWCO membrane, trace instability still remained for the same wine ultrafiltered with Millipore 30 000-MWCO (Fig. IV.4). The protein content (as determined by the Bio-Rad dye-binding procedure) of these two wines is not significantly different (Fig. IV.4). However, an increase in protein at the higher MWCO is detectable by electrophoresis (12). Previous studies (9,12) have also indicated that very small changes in total protein (in the order of a few ppm), coincide with changes in stability to heat/cold testing.

It is also important to recognize that the behavior of the HCT induced haze may be influenced by several factors in addition to proteins, including pH and the extent of

of protein conjugation with oxidized, polymerized phenolics and carbohydrates (2,8,11,15,22). Indeed, hazes have been isolated from stored ultrafiltered WR juices which appear to contain oxidized polymerized phenolics with small amounts of proteins and neutral polysaccharides (2). In addition it is believed that oxidized polymerized phenolics themselves can contribute to haze and sediment formation (8,13,25).

Storage Stability of the Ultrafiltered Wines: UF wines as grape juice (2), were "sparkling" clear after UF processing. However, small amounts of secondary hazes were developed after heat/cold testing and after two years of storage under cellar conditions (Table IV.5). Therefore although UF is an effective mean for removing "protein" instability in wine, even with membrane MWCO as low as 10,000 daltons, there may be need for a small amount of bentonite fining (5 g/Hl) to guarantee stability. In addition to the presence of small amounts of hazes, tartrate crystals (brown from possible phenolic-protein compounds) were observed but only in the ultrafiltered wines and not in the non-UF wines. This may be due to the removal of colloid-stabilizing materials which may modify susceptibility to tartrate crystal formation. Of further importance is the observation that the use of the HCT as an index for stability to haze formation in wines correlates very well with the stability of wines under long-term storage conditions, in this case up to two years of storage.

Table IV.5 Haze/Sediment Formation in Ultrafiltered Wines.

WINE PROCESSED		HEAT/COLD TEST		STORAGE(13°C,2 yr)
	VISUAL * CLARITY	HUNTER HAZE	VISUAL CLARITY*	VISUAL CLARITY*
WHITE RIESLING				
INITIAL WINE	-	34.5	+++	++
ULTRAFILTERED PM-50[a]	-	21.4	+	+
ULTRAFILTERED PM-10[b]	-	5.6	+	-+
GEWURZTRAMINER				
INITIAL WINE	-	29.5	++	++++
ULTRAFILTERED PTTK-30[c]	-	0.5	+	+
ULTRAFILTERED PTGC-10[d]	-	0.5	-+	-+
COMMERCIAL GEWURZTRAMINER				
INITIAL WINE	-	N. D.	N. D.	++++
ULTRAFILTERED[e]	-	N. D.	N. D.	+

* Visual Clarity: - = clear, -+ = Trace haze, + = Slight haze, ++= Moderate haze, +++ = Strong haze, ++++ = Extreme haze.

Hunter haze reading after heat/cold test (80°C, 6h; 4°C, 12h) and measured by Hunter meter.

a, b Processed with a Romicon HF-Lab-5 system operated with membranes of 50,000 (PM-50), and 10,000 (PM-10) daltons, respectively, at ΔP_T= 1.23 kg/cm2, 18 -27°C, VCR=6.

c, d Processed with a Millipore Pellicon cassette system operated with membranes of 30,000 (PTTK-30), and 10,000 (PTGC-10) daltons, respectively, at ΔP_T= 1.23 kg/cm2, 18 -21°C, VCR=13.

e Processed with a Millipore commercial PUF system (MWCO 10 000 daltons), at 10 -13°C to VCR=92.

N. D. : Not determined.

Effect of VCR on Color (browning), Soluble Protein, and Heat/Cold Test Haze of Ultrafiltered Wines: Recognizing that compounds such as proteins, phenolics, and polysaccharides can contribute to the instability of juice or wine, it is important to investigate the effect of VCR (during processing) on some of these compounds. A summary of the analysis of variance for color (browning), soluble protein, and HCT Haze during UF (including VCR and MWCO) of WR and Gewurztraminer wines is shown in Table IV.6.

Although VCR had a significant effect ($p < 0.001$) on the color (browning) of the WR wine, MWCO did not have a significant effect on this parameter (Table IV.6). The color (browning) of the permeate significantly increased with increasing VCR, an overall increase of 17% from VCR 2 to VCR 6 was found (Fig. IV.5).

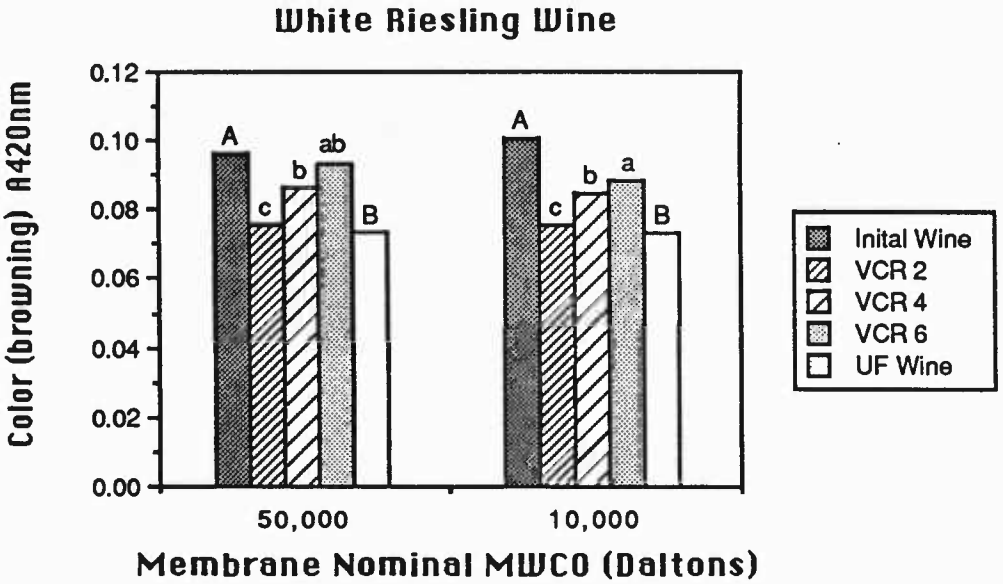

Fig. IV.5 Effect of Volume Concentration Ratio and Membrane Molecular Weight Cut-Off on Color (browning) of White Riesling Wine. Romicon system operated with PM-10, and PM-50 at ΔP_T=1.23 kg/cm2, 18-27°C, VCR=6.7. Capital letters indicate mean separation between Initial Wine and UF Wine, and small letters indicate mean separation between VCR's (during UF). Means designated with the same letter are not significantly different at p = 0.05.

Table IV.6 Analysis of Variance, Degrees of Freedom (DF), and F-ratios for Color (browning), Soluble Proteins, and Heat/Cold Test Haze during UF (Including VCR and MWCO) of White Riesling and Gewurztraminer Wines.

SOURCE	WHITE RIESLING WINE				GEWURZTRAMINER WINE			
		F - values				F - values		
	DF	COLOR (BROWNING)	SOLUBLE PROTEIN	H/C T HAZE	DF	COLOR (BROWNING)	SOLUBLE PROTEIN	H/C T HAZE
MOLECULAR WEIGHT CUT-OFF (MWCO)	1	1.11	11.97	59.79*	1	2.38	0.00	0.05
MEAN SQUARE ERROR I	2	$10.8x10^{-6}$	6.43	16.38	2	$8.5x10^{-6}$	2.72	5.72
VOLUME CONCENTRATION RATIO (VCR)	2	42.20**	36.31**	101.05***	3	36.93***	3.76	2.84
MWCO X VCR	2	0.98	0.24	1.82	3	0.07	0.20	0.55
MEAN SQUARE ERROR II	4	$5.3X10^{-6}$	2.01	6.57	6	$1.17X10^{-6}$	0.77	0.45

*, **, *** indicates significant at $p< 0.05$, < 0.01, < 0.001, respectively.

The decrease in the efficiency of retention is probably due to, among other factors, an increase in the concentration polarization which would result in a leakage of the compressed material to the permeate. In addition, this can also be due to an increase in the actual pigment concentration. Polymerized *o*-dihydroxyphenols may be contributing (13).

While VCR had a significant effect ($p<0.01$. Table IV.6) on the color (browning) of the GEW wines, VCR did not have any effect on the soluble protein concentration and HCT induced haze. An increase in color from VCR 2 to VCR 13 of 15% was found for GEW wines, regardless of the MWCO-membrane used (Fig. IV.6).

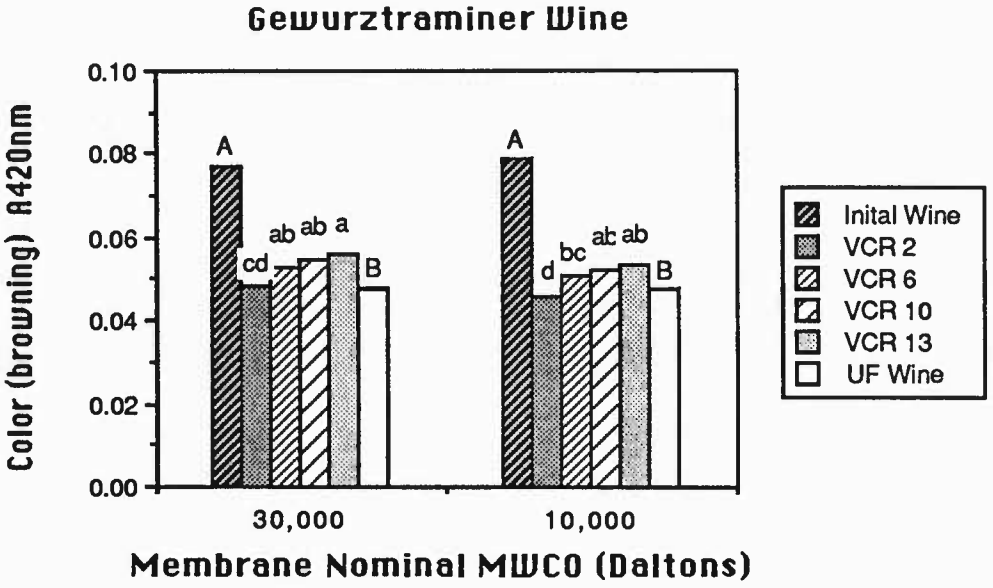

Fig. IV.6 Effect of Volume Concentration Ratio and Membrane Molecular Weight Cut-Off on Color (browning) of Gewurztraminer Wine. Millipore Pellicon cassette system operated with PTGC-10, and PTTK-30 cassettes at ΔP_T=1.23 kg/cm2,18-21°C, VCR=13. Capital letters indicate mean separation between Initial Wine and UF Wine, and small letters indicate mean separation between VCR's (during UF). Means designated with the same letter are not significantly different at p = 0.05.

Although no significant differences were found for color between the different membrane MWCO of the final combined permeates for the GEW wine, significant differences can be detected during the processing (VCR). {While in the final combined permeate there is a dilution factor, the concentration of a compound at a specific VCR is not changed by this dilution.}

VCR had a significant effect on the total protein content ($p < 0.01$, Table IV.6), and HCT induced haze ($p < 0.001$, Table IV.6) of the WR wines. Regardless of the MWCO, the total protein content of WR wine permeate significantly increased (39%) from VCR 2 to VCR 6 during the processing (Fig. IV.7). These trends are predicted to continue at higher VCR (in this study VCR was limited by sample size and hold-up volume).

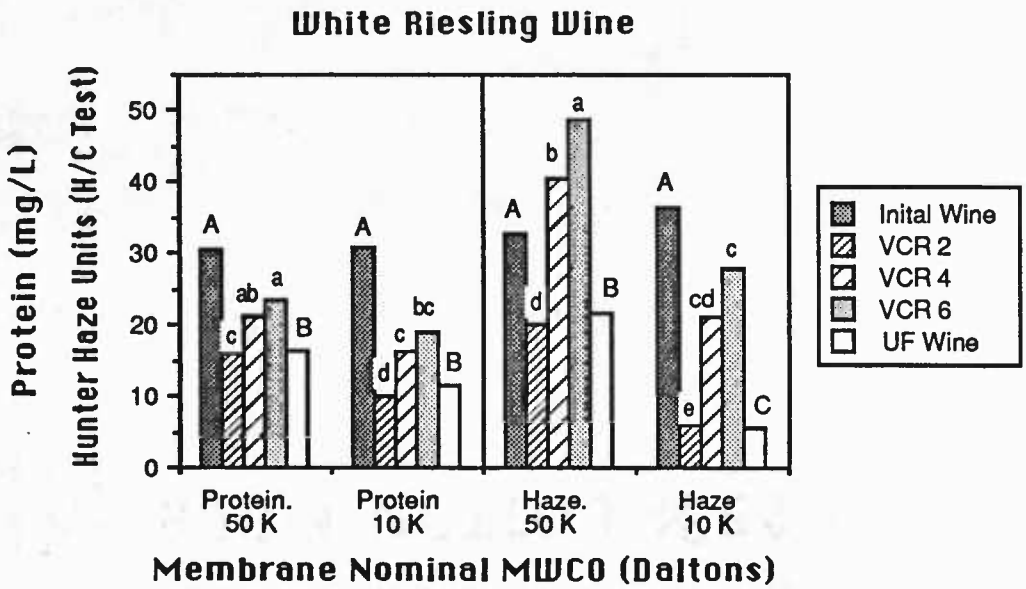

Fig. IV.7 Effect of Volume Concentration Ratio and Membrane Molecular Weight Cut-Off on Soluble Protein and Heat/Cold Test Haze Formation of White Riesling Wine. Romicon system operated with PM-10, and PM-50 at ΔP_T=1.23 kg/cm2, 18-27°C, VCR=6.7. Capital letters indicate mean separation between Initial Wine and UF Wine, and small letters indicate mean separation between VCR's (during UF). Means designated with the same letter are not significantly different at $p = 0.05$.

During processing, increasing VCR resulted in a significant increase of the HCT induced haze (66%) in the permeate of the WR wine (Fig. IV.7). In addition, decreasing membrane MWCO significantly decreased the HCT induced haze (30%) in the permeates (Fig. IV.7).

In general, attention should be given to the operation of the ultrafiltration unit in the batch mode which may result in a decrease in the efficiency of retention. This is probably due to an increase in the concentration polarization, which could result in a leakage of the compressed material into the permeate. This is important since the small amount of protein that is passing through during UF may be modified by conjugation with other compounds such as phenolics or carbohydrates. Phenolic compounds, if not polymerized or linked to macromolecules, pass the membrane, thus contributing to the formation of hazes, either independently or in association with other phenolic compounds and proteins (2,8,11,15,22).

In this study low phenolic retentions were obtained with membrane MWCO ≤ 30,000 daltons. Hsu et al., reported that although heat/cold induced haze formation progressively decreased with bentonite fining and protein reduction, bentonite addition had no effect on total phenol (11). Polymerized phenolics may be contributing to residual heat/cold induced haze that is difficult to remove by bentonite fining (11) and UF processing (2). Therefore, there is probably more than one type of haze involved in the instability of wines, ie. phenolic and phenolic-protein hazes.

Sensory Analysis: The effect of bentonite fining and pilot-scale UF on WR and GEW wines; and of a commercial-scale UF on GEW wine, on the aroma and flavor-by-mouth was evaluated by the use of descriptive analysis.

White Riesling (WR) Wine: Of the 46 descriptors used to describe the aroma and flavor-by-mouth of the WR wines, only 9 aroma descriptors showed

Table IV.7 Analysis of Variance of the Significant Aroma Descriptors for White Riesling Wine: F-values, Means, and Degrees of Freedom.

DESCRIPTORS	F - values						MEAN SQUARE
	JUDGES (J)	REPLI. (R)	J x R	TREAT-MENT (T)	J x T	T x R	ERROR
AROMA:							
OVERALL INTENSITY	6.63**	1.42	0.84	4.91**	1.04	0.45	0.517
FRUITY	3.83**	1.46	2.34	3.83*	1.14	0.95	0.737
F. F. CITRUS	4.78**	0.57	1.70	3.33*	1.22	0.68	1.119
FLORAL	1.87	0.56	1.27	5.94**	1.15	0.98	1.959
VEGETATIVE	3.24**	0.53	2.08*	4.13*	1.44	1.67	0.921
SWEET	2.22*	1.21	1.95**	4.08**	2.11**	0.40	0.905
HONEY/CARAMEL	1.40	0.89	1.90*	3.42*	1.54	0.40	0.893
CHEMICAL	2.03*	1.11	0.98	2.74*	0.46	0.30	1.221
PUNGENT	5.65**	1.07	1.10	2.21**	0.59	0.35	1.090
Degrees of Freedom	9	2	18	3	27	6	54

*,**, indicate significance at $p < 0.05$, $p < 0.01$, respectively.

significant differences. No flavor-by-mouth differences were noted. A summary of the Analysis of Variance for these descriptors is shown in Table IV.7.

A consistent decrease (control > bentonite > ultrafiltered) in the intensity of most of the significant aroma descriptors, except for vegetative, was detected (Table IV.8). UF processing of the WR wine significantly decreased the overall intensity, fruity, fresh fruity citrus, floral, sweet and honey/caramel descriptors but it also significantly increased the intensity of the vegetative aroma when compared to the control wine. However, no significant differences in these descriptors were detected between the control wine and the bentonite-fined wine.

Table IV.8 Mean Scores* of the Significant Aroma Descriptor Ratings for White Riesling Wine.

DESCRIPTORS	Control	Bentonite	UF 1	UF 2
OVERALL INTENSITY	5.97b	5.63b	5.33a	5.23a
FRUITY	4.83b	4.57b	3.93a	4.10a
F. F. CITRUS	3.47c	3.20cb	2.87ba	2.43a
FLORAL	3.63b	3.13b	1.83a	2.00a
VEGETATIVE	1.93a	1.87a	2.63b	3.13c
SWEET	3.40b	3.00b	2.37a	2.30a
HONEY/CARAMEL	2.43c	2.10cb	1.63ba	1.57a
CHEMICAL	4.07a	3.83a	3.97a	4.33a
PUNGENT	3.87a	3.67a	3.70a	4.10a

* Scored on a nine-point intensity scale (1 = none, to 9 = extreme).
Note: Means within the same row designated by the same letter are not significantly different at p = 0.05.

The results for the sweet term should be interpreted cautiously since significant judge x treatment interactions were found ($p < 0.01$, Table IV.7). Although the treatment effect of these attributes is highly significant ($p < 0.01$), the interaction effect accounts for up to 23% of the total source of variation while the treatments effect accounts for only 11%. Close inspection of the raw data presented evidence that less than 50% of the judges found differences on those attributes.

Despite the fact that the judges were well trained, significant judge x replication interactions were detected for the vegetative, sweet, and honey/caramel terms (Table IV.7). This is attributed to the inconsistent use of the scale by some panelists; in one replication they would use a different portion of the scale. However, for vegetative and honey/caramel, the interaction treatment replication was not significant, so the J x R interaction is of little importance.

Gewurztraminer (GEW) Wine: Of the 48 terms used to describe the aroma and flavor-by-mouth of the three GEW wines, only 4 aroma descriptors showed significant differences. A summary of the analysis of variance of the intensity ratings for these attributes is shown in Table IV.9.

UF processing of the GEW wine significantly decreased the intensity of fruity, and fresh fruity aroma descriptors, and significantly increased the chemical aroma intensity compared to the control unfiltered GEW wine (Table IV.10). In addition, significant differences were detected for cooked vegetative aroma between the bentonite-fined GEW wine and the ultrafiltered GEW wine.

The result for the fruity aroma attributes should be interpreted cautiously since significant judge x treatment interactions were found ($p < 0.05$, Table IV.9). Although the treatment effect of these attributes is significant, the interaction effect accounts for 16% of the total source of variation while the treatment effect accounts for only 4%. Therefore, the variability among judges in the use of this

Table IV.9 Analysis of Variance of the Significant Aroma Descriptors for Gewurztraminer Wine: F-values, Means, and Degrees of Freedom.

DESCRIPTORS	F - values						MEAN SQUARE
	JUDGES (J)	REPLI. (R)	J x R	TREAT-MENT (T)	J x T	T x R	ERROR
AROMA:							
FRUITY	3.17**	0.65	1.93*	3.06*	1.95*	0.24	1.103
FRESH FRUITY	2.77**	0.64	1.67	5.30**	1.68	0.26	1.475
COOKED VEG.	1.76	1.69	0.57	3.16*	1.04	0.62	2.017
CHEMICAL	2.03*	0.79	1.12	4.39*	1.40	0.47	2.214
Degrees of Freedom	11	2	22	2	22	4	44

*,**, indicate significance at $p < 0.05$, $p < 0.01$, respectively.

descriptor was of greater magnitude than the difference among the treatments. Very few judges found differences among the wines.

Table IV.10 Mean Scores* of the Significant Aroma Descriptor Ratings for the Gewurztraminer Wine.

DESCRIPTORS	Control	Bentonite	Ultrafiltered
FRUITY	5.22b	4.83ab	4.39a
FRESH FRUITY	4.61b	4.17b	3.39a
COOKED VEGETATIVE	1.97b	1.27a	2.22b
CHEMICAL	2.35a	2.33a	3.56b

* Scored on a nine-point intensity scale (1 = none, to 9 = extreme).
Note: Means within the same row designated by the same letter are not significantly different at p = 0.05.

Significant judge x replication interaction was detected for the fruity aroma descriptor ($p < 0.05$, Table IV.9). Once again this is attributed to the inconsistent use of the scale by some panelists.

Commercial Gewurztraminer Wine: Of the 48 descriptors used to describe the aroma and flavor-by-mouth of the two GEW wines, no significant differences were detected between the control and the ultrafiltered GEW wine.

Greater loss of aroma in OSU pilot scale trials as compared with commercial processing is attributed to the small scale operation, causing more "disturbance/oxidation" of the wine, and the fact that no precautions were taken to minimize this, for instance by operating at lower temperature, and under nitrogen conditions.

In comparing OSU ultrafiltered wine with the bentonite fined wine, consideration should be given to the fact that bentonite fined wine was not subjected to any filtration (as normally occurs commercially), before sensory evaluation.

Conclusions

PROCESSING:

1.- Membranes, even 10,000 dalton MWCO, do not retain all the proteins and retain a low variable percentage of phenolics.

2.- Stability to heat/cold test (HCT) haze formation was obtainable with 10,000 MWCO, but trace instability can remain (requires ca 5 g/hL bentonite for stability).

3.- There was a good correlation between HCT haze formation and haze/sediment formation upon storage (order of stability obtained with membranes: 10,000 > 30,000 > 50,000).

4.- During UF there was a significant decrease in membrane retention of color (A_{420nm}), protein and HCT haze formation in WR wine, and color (A_{420nm}) of the GEW wine. (Trends demonstrated are predicted to continue at higher VCR - consideration may need to be given to quality and stability effects of higher VCR fractions).

5.- Very small changes in total protein (and phenol) that are not always analytically significant, can coincide with changes in stability.

6.- It is not the amount, but nature/state of compounds such as proteins, phenolics, polysaccharides and their enviroment (for instance pH), and interaction, that results in instability (more research required at molecular level).

SENSORY ANALYSIS:

PILOT SCALE.-

7.- The panel detected significant differences among the treatments in several of the aroma descriptors for both WR and GEW wines. However, the panel did not detect any significant differences for the flavor-by-mouth descriptors.

8.- A consistent decrease of the intensity of most of the significant aroma descriptors across treatments (Intensity ratings: control unfiltered > bentonite fined > ultrafiltered) was detected for both wines. The only exception was "vegetative" which was higher in the ultrafiltered samples.

9.- This decrease of the aroma characters was statistically significant between the control and the ultrafiltered wines, but not between the control unfiltered and bentonite fined wines.

10.- In the comparison of bentonite-fined wines versus ultrafiltered wines, the ultrafiltered wines were generally statistically significantly lower in most aroma descriptors (except vegetative) for both wines.

11.- UF processing can modify the aroma attributes of wine, with the potential for being either a benificial or detrimental product modification.

COMMERCIAL SCALE.-

11.- Commercial processing of GEW wine by UF did not have any significant effect on the aroma and flavor-by-mouth descriptors evaluated.

Literature Cited

1. Drioli, E., G. Orlando, S. D'Ambra, and A. Amati. Membrane processing in must and wine. ACS Symposium Series 154, Synthetic Membranes, Vol. 2 pp. 17-26 (1981).

2. Flores, J. H., D. A. Heatherbell, J. C. Hsu, and B. T. Watson. Ultrafiltration (UF) of White Riesling juice: Effect of oxidation and of pre-UF treatment on flux, composition, stability and quality. Am. J. Enol. Vitic. Submitted for publication (1987).

3. Gaillard, M. Ultrafiltration et microfiltration en flux tangentiel. Les possibilités d'utilisation des processus membranaires en œnologie. Vignes Vis 329:26-33 (1984).

4. Gaillard, M. Résultats de l'observation du fonctionnement d'un appareil d'ultrafiltration industriel wine processor - Société I.D.E.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 109-120. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris. (1985).

5. Gibson, R. L. Cross flow membrane technology for the wine industry. The Australian Grapegrower & Winemaker pp 17-23. April. (1986).

6. Gnekow, B., P. M. Meir, D. Mezynski, and M. Mook. Protein stabilization of Sauvignon Blanc by ultrafiltration. Presented (abstract published) at the 34th Annual meeting of the ASEV, Reno, NV. June. (1983).

7. Heatherbell, D. A., J. L. Short, and P. Stauebi. Apple juice clarification by ultrafiltration. Confructa 22:157-169 (1977).

8. Heatherbell, D. A. Fruit juice clarification and fining. Confructa 22:192-7 (1984).

9. Heatherbell, D. A., P. Nagaba, J. Fombin, B. Watson,Jr., Z. Garcia, J. Flores, and J. Hsu. Recent developments in the application of ultrafiltration and protease enzymes to grape juice and wine processing. *In:* Proceedings of the International Symposium on Cool Climate Viticulture and Enology. D. A. Heatherbell, P. B. Lombard, F. W. Bodyfelt, and S. F. Price (Eds.). pp 418-45. Oregon State University, Corvallis, OR. (1985).

10. Hsu. J. C., and D. A. Heatherbell. Isolation and characterization of soluble proteins in grape juice, and wine. Am. J. Enol. Vitic. 38:6-10 (1987).

11. Hsu, J. C., and D. A. Heatherbell. Heat-unstable proteins in wine. I. Characterization and removal by bentonite fining and heat treatment. Am. J. Enol. Vitic. 38:11-16 (1987).

12. Hsu, J. C., D. A. Heatherbell, J. H. Flores, and B. T. Watson. Heat-unstable proteins in grape juice and wine. II. Characterization and removal by ultrafiltration. Am. J. Enol. Vitic. 38:17-22 (1987).

13. Lea, A. G. H. Tannin and colour in English cider apples. Flussiges Obst 8:356-361 (1984).

14. Lüdermann, A. Wine clarification with a crossflow microfiltration system. Am. J. Enol. Vitic., 38:228-235 (1987).

15. Matheis, G., and J. R. Whitaker. Modification of proteins by polyphenol oxidase and peroxidase and their products. J. Food Biochem. 8:137-162 (1984).

16. Maglioli, G., and C. Marchesini. Ultrafiltration: its winemaking applications (trans). Enotecnico 21(1):39-44 (1985).

17. Parrini, P., and A. Mattera. Ultrafiltration and reverse osmosis in winemaking (trans). Vignevini 11(3):29-33 (1984).

18. Pocock, K. F., and B. C. Rankine. Heat test for detecting protein instability in wine. Aust. Wine. Brew. Spirit Rev. 91:42-3 (1973).

19. Romicon, Inc. Operating instructions Romicon model HF-LAB-5 ultrafiltration system with reverse flow. Woburn, MA. (1984).

20. Rougon, H., and L. Bourdier, Eds. Ultrafiltration et microfiltration tangentielle en œnologie. Comte rendu des Journées Techniques organisées par l'ITV les 23-24 janvier 1985. Institute Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris. (1985).

21. Serrano M., S. B. Sachs, and P. Ribereau-Gayon. Utilization d'un microfiltre tangentiel en œnologie. Connaissance Vigne Vin 18(2): 135-154 (1984).

22. Somers, T. C. The inevitability of phenolic instability. *In:* Physical Stability of Wine. T. H. Lee (Ed.). pp 81-90. The Australian Wine Research Institute, Glen Osmond, S. A., Australia. (1986).

23. Testaniere, D. Resultats d'essai d'ultrafilration de vin blanc appareil industriel: Millipore.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 131-140. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris. (1985).

24. Trogus, H. The use of ultrafiltration in vinification (trans) Der Badische Winzer 1:360-364 (1984).

25. Van Buren, J. P. Haze in Bottled Apple Juice. *In:* Processed Apples-Research Report 1983. D. L. Dowing Ed. New York State Agricultural Experiment Station, Geneva, N. Y. November. (1983).

26. Wucherpfennig, K., and S. Neubert. Applicabilities of ultrafiltration for protein stabilization and of reverse osmosis for concentration of grape juice. Fluss. Obst. 44:46-9, 52-6 (1977)..

27. Wucherpfennig, K. Possibilities for the application of membranes for stabilizing wines. (ultrafiltration, reverse osmosis, electrodialysis). Ann. Technol. Agric. 27(1):319-331 (1978).

28. Wucherpfennig, K., H. Dietrich, and K. Banziger. Use of micro- and ultrafiltration on fruit juice and wine (trans). Der Duetsche Weinbau 22/26:1144-56 (1984).

29. Wysocki, G. Substance separation: Experience with KBM ultrafiltration equipment (trans). Ernaehrungswirtschaft/ Lebensmitteltechnik 6: 278-282 (1977).

30. Youval, A., and E. Zisner. Les materiels, les materiaux et les systémes d'ultrafiltration et de microfiltration tangentielle.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 61-70. Institut Technique de la Vigne et du Vin. 21, rue Francois 1er 75008 Paris. (1985).

Bibliography

Amerine, M. A., H. W. Berg, and W. V. Cruess. 1972. Technology of Wine Making, 3rd ed. AVI Publishing Co., Westport, Connecticut.

Amerine, M. A., H. W. Berg, R. E. Kunkee, C. S. Ough, V. L. Singleton, and A. D. Webb. 1980. The Technology of Wine Making. AVI Publishing Co., Westport, Connecticut.

Amerine, M. A., and E. B. Roessler. 1983. Wines their Sensory Evaluation. pp 289-304. W. H. Freeman and Company. New York.

Anelli, G. 1977. The proteins in mosts. Am. J. Enol. Vitic. 28:200-203.

Balanutse, A. O., G. F. Mustyatse, B. N. Kal'yan, and F. D. Kitii. 1983. Effect of ultrafiltration on content and fractional composition of protein in wine. Sadovodstvo, Vinogradorstvo, Vinodelie Moldavii 38(11):35-37. Abstract in: FSTA 8:H0129 (1985).

Barillere J. M., J. L. Escudier, M. Moutounet, and P. Benard. 1985. Microfiltration tangentielle de mouts et de cins lere partie: compte-rendu des essais 1984. *In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 179-188. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.

Bastioli, C., G. Gianotti, A. Mattera, and P. Parrini. 1983. Application of membrane process to enology. Vignevini 10(6):43-49.

Baumann, G., B. Strobel, and K. Gierschner. 1986. Microfiltration and ultrafiltration of apple juice - comparison of inorganic/organic membranes and conventional deep-bed filters. Flüssiges Obst. 53(5):246-261.

Bayly, F. C., and H. W. Berg. 1969. Grape and wine proteins of white wine varietals. Am. J. Enol. Vitic. 18(1):18-32.

Berezovsky, N. 1985. Future fruit juice technology. Review. Confructa :185-190.

Berger, J. L. 1985. Résultats analytiques et organoleptiques d'ultrafiltration pilote wine processor - Société I. D. E. *In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 121-124. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.

Blumenkrantz, M., and G. Asboe-Hansen. 1973. New method for qualitative determination of uronic acids. Anal. Biochem. 54:484-9.

Bocker, H. 1981. Control of acid content: An important measure for improving wine quality. Vignohrad 19(2):278-279. Abstract in: FSTA 2:H0272 (1983).

Colagrande, O., V. Mazzoleni, A. Silva, and S. Colm. 1984. Cold stabilization of wines to prevent potassium bitartrate precipitation. Industrie delle Bavande 13(74):459-468. Abstract in: FSTA 7:H0141 (1985).

Dilley, D. R. 1970. Enzymes. *In*: The Biochemistry of Fruit and their Products. A. C. Hulme ed., Vol. 1 p. 179-207. Academic Press. New York and London.

Drake, S. R., and S. W. Nelson. 1986. Apple juice quality as influenced by ultrafiltration. Journal of Food Quality 9:399-406.

Drioli, E., G. Orlando, S. D'Ambra, and A. Amati. 1981. Membrane processing in must and wine. ACS Symposium Series 154, Synthetic Membranes, Vol. 2 pp17-26.

Dürr, P. 1979. Development of an odour profile to describe apple juice essences. Lebensm.-Wiss. u.-Technol. 12;23-26.

Feuillat, M. and P. Benard.. 1985. Influence de la filtration tangentielle de moûts et de vins. Quelges essais industrieles conduits en 1984.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 225-250. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.

Flores, J. H., D. A. Heatherbell, J. C. Hsu, and B. T. Watson. 1987. Ultrafiltration (UF) of White Riesling juice: Effect of oxidation and of pre-UF juice treatment on flux, composition and stability. Am. J. Enol. Vitic., Submitted for publication.

Farenczy, S. 1966. Proteines et substances azotees. Bull. O. I. V. 39:1313-1336.

Fombin, J. F. 1983. Ultrafiltration of grape juice: Effects on composition, quality, and preservation. Master Thesis, Oregon State University.

Gaillard, M. 1984. Ultrafiltration et microfiltration en flux tangentiel. Les possibilités d'utilisation des processus membranaires en œnology. Vignes Vis 329:26-33.

Gaillard, M. 1985a. Résultats de l'observation du fonctionnement d'un appareil d'ultrafiltration industriel wine processor - Société I.D.E.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 109-120. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.

Gaillard, M. 1985b. Ultrafiltration et microfiltration tangentielle de moût de raisin sur membrane minérale Pilote Société S. F. E. C. *In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 141-151. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.

Gallander, J. F. 1974. Chemistry of grapes and other fruits as the raw materials involved in winemaking. *In:* Chemistry of Winemaking. A. D. Webb, ed. pp 11-49. American Chemical Society. Washington, D. C.

Garcia-Quintero, Z. H. 1983. Ultrafiltration of grape juice by hollow fiber membranes. Master Thesis, Oregon State University.

Gibson, R. L. 1986. Cross flow membrane technology for the wine industry. The Australian Grapegrower & Winemaker pp 17-23. April.

Glaser, W., and C. Güngerich. 1986. Fruit juice clarification by means of UF-Experience gained in USA. Flüssiges Obst. :136-138.

Gnekow, B., P. M. Meir, D. Mezynski, and M. Mook. 1983. Protein stabilization of Sauvignon Blanc by ultrafiltration. Presented (abstract published) at the 34th Annual meeting of the American Society of Enologist, Reno, NV, June.

Goldstein, J. L., and T. Swain. 1963. Methods for determining the degree of polymerization of flavans. Nature 198:587-588.

Harel, E., and A. M. Mayer. 1968. Interconversion of subunits of catechol oxidase from apple chloroplasts. Phytochemistry 7:199-204.

Harel, E., A. M. Mayer, and E. Lehman. 1973. Multiple forms of *Vitis vinifera* catechol oxidase. Phytochem. 12:2649-2654.

Heatherbell, D. A., J. L. Short, and P. Stauebi. 1977. Apple juice clarification by ultrafiltration. Confructa 22:157-169.

Heatherbell, D. A. 1984. Fruit juice clarification and fining. Confructa 22:192-7.

Heatherbell, D. A., P. Nagaba, J. Fombin, B. Watson,Jr., Z. Garcia, J. Flores, and J. Hsu. 1985. Recent developments in the application of ultrafiltration and protease enzymes to grape juice and wine processing. *In:* Proceedings of the International Symposium on Cool Climate Viticulture and Enology. D. A. Heatherbell, P. B. Lombard, F. W. Bodyfelt, and S. F. Price, Eds. pp 418-45. Oregon State University, Corvallis, OR.

Heymann, H., and A. C. Noble. 1987. Descriptive analysis of commercial Cabernet Sauvignon wines from California. Am. J. Enol. Vitic. 38(1):41-44.

Henning, K. 1970. Bilans de l'azote dans les mouts et les vins nouveaux en fermentation. Bull. O.I.V. 16:82-86.

Hsu, J. C., and D. A. Heatherbell. 1987. Isolation and characterization of soluble proteins in grape juice, and wine. Am. J. Enol. Vitic. 38:6-10.

Hsu, J. C., and D. A. Heatherbell. 1987. Heat-unstable proteins in wine. I. Characterization and removal by bentonite finning and heat treatment. Am. J. Enol. Vitic. 38:11-16.

Hsu, J. C., D. A. Heatherbell, J. H. Flores, and B. T. Watson. 1987. Heat-unstable proteins in grape juice and wine. II. Characterization and removal by ultrafiltration. Am. J. Enol. Vitic. 38:17-22.

Hodgson, J. 1981. Ultrafiltration of pear juice: Chemical and physical analysis. Master Thesis, Oregon State University.

Joslyn, M. A., and M. A. Amerine 1964. Desert, apperitizer and related flavored wines. Technology of their production. University of California, Division of Agricultural Science, Berkeley and Davis, CA.

Kirk, D. E., M. W. Montgomery, and M. G. Kortekaas. 1983. Clarification of pear juice by hollow fiber ultrafiltration. J. Food Sci. 48:1663-6.

Koch, J., and E. Sajak. 1959. A review and some studies on protein. Am. J. Enol. Vitic. 10:114-123.

Koch, J. 1963. Protéines des vins blancs. Traitements des précipitation protéiques par chauffage et á l'aide de la bentonite. Ann. technol. agr. 12(1):297-311.

Lea, A. G. H. 1984. Tannin and colour in English cider apples. Flussiges Obst 8:356-361.

Lee, T. H. 1986. Protein instability: Nature, characterization and removal by bentonite. T. H. Lee, Ed. Physical stability of wine. Preceding of a seminar, Oct. 10, 1985. Reynella, SA. Adelaide, SA. Australian Society of Viticulture and Oenology. Australian Wine Research Institute 1986:23-39.

Loomis, W. D. 1974. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. *In:* Methods in Enzymology. Vol. 31, S. Fleischer, and L. Parker, Eds. pp 528-544. Academic Press, New York.

Lüdemann, A. 1987. Wine clarification with a crossflow microfiltration system. Am. J. Enol. Vitic., 38:228-235.

Luis, E. S. 1983. A study of protein during grape maturation, juice preparation and wine processing. Ph. D. Thesis. University of New South Wales, Australia.

Lyubarevich, T. A., R. K. Mindadze, N. M. Paulenko, and E. N. Datunashvili. 1975. The proteins of wine grapes and their properties and stability in wine. Vinodel. Vinograd USSR 4:58-59. Abstract in: FSTA 8:3H365 (1976).

Marteau, G. 1967. Annls. Nutr. Aliment. 21(5):223-345.

Matheis, G., and J. R. Whitaker. 1984. Modification of proteins by polyphenol oxidase and peroxidase and their products. J. Food Biochem. 8:137-162.

McDaniel, M. R., L. A. Henderson, B. T. Watson, and D. A. Heatherbell. 1987. Sensory panel training and screening for descriptive analysis of the aroma of Pinot noir fermented by several strains of malolactic bacteria. J. of Sensory Studies. (in press).

McDaniel, M. R. 1987. Trained panel evaluation of wine. The Wine Advisory Board Research Report, Oregon Dept. of Agriculture. 4:11-13. Feb. 1987.

Mecredy, J., J. Sonnemann, and S. Lehmann. Sensory profiling of beer by a modified QDA method. Food Technol., 28(11):36-39. (1974).

Meglioli, G., and C. Marchesini. 1985. Ultrafiltration: its winemaking applications (trans). Enotecnico 21(1):39-44.

Miller, G. C., J. M. Amon, R. L. Gibson, and R. F. Simpson. 1985. Loss of wine aroma attributable to protein stabilization with bentonite or ultrafiltration. The Australian Grapegrower & Winemaker. pp 46-50. April.

Milnes, B. A. 1984. The application of ultrafiltration to apple juice process. Presented at the apple juice workshop. Special Report New York State Agricultural Experiment Station 54:33-38.

Moretti, R. H., and H. W. Berg. 1965. Variability among wines to protein clouding. Am. J. Enol. Vitic. 16(2):69-78.

Moskowitz, H. R. 1983. Derscriptive analysis of perseptions. *In:* Product Testing and Sensory Evaluation of Foods. Marketing and R&D Approaches. pp 20-78. Food & Nutrition Press, Inc. Westport, Conneticut.

Möslang, H. 1984. Ultrafiltration in the fruit juice industry. Confructa 28:219-224.

Moutounet, M., J. L. Escudier, J. M. Barillere, and P. Benard. 1985. Microfiltration tangentielle de moûts et de vins 2e partie: Données chromatiques. *In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 189-195. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.

Nagaba-Mbiakop, P. R. 1981. Investigations of methods for determination and prevention of protein instability in wines. Ph.D. Thesis. Oregon State University.

Nagel, C. W., and U. Schobinger.1985. Investigation of the origen of turbidity in ultrafiltered apple and pear juice concentrate. Confructa 29:16-22.

Neubeck, C. E. 1975. Fruit, fruit products, and wines. *In:* Enzymes in Food Processing. G. Reed, Ed. p. 397. Academic Press, Inc., New York.

Neubeck, C. E. 1981. Use of enzymes in fruit juice processing. *In:* Proceedings of the symposium on technological problems of fruit juice concentrates. Oregon Agric. Expt. Stn. Publication No. 6234. Corvallis, OR.

Nilov, V. I., G. M. Datunashvili, G. M. Zudova, V. N. Ezhov, and G. M. Anachekova. 1975. Effect of treatment with bentonite clays on the polysaccharide complex in wines. Sadov. Vinograd. Vinodel. Moldav. 30:25-27. Abstract in: FSTA 8:1H16, 1976.

Noble, A. C., A. A. Williams, and S. P. Langron. 1983. Descriptive analysis and quality of Bordeaux wines. *In:* Sensory Quality in Food and Beverages definition, measurement and control. A. A. Williams and R. K. Atkin, Eds. pp 324-334. Verlag Chemie International, Inc. Florida.

Noble, A. C., and M. Shannon. 1987. Profiling Zinfandel wines by sensory and chemical analysis. Am. J. Enol. Vitic. 39(1):1-5.

Ough, C. S. 1983. Sulfur dioxide and sulfites. *In:* Antimicrobials in Foods. A. L. Branen, and P. M. Davidson, Eds. p. 177. Marcel Dekker, Inc. New York.

Parrini, P., and A. Mattera. 1984. Ultrafiltration and reverse osmosis in winemaking (trans). Vignevini 11(3):29-33.

Paulson, D. S., R. L. Wilson, and D. D. Spatz. 1985. Reverse osmosis and ultrafiltration applied to the processing of fruit juices. ACS Symp. Ser. 281:325-344.

Payne, M. 1985. Wine processing using ultrafiltration. The Australian Grapegrower & Winemaker. pp 52-58. April.

Peynaud, E. 1952. Sur les matiéres pectiques des moûts de raisin et des vins. Ann. fals et fraudes 45:11-20.

Peynaud, E., and P. Ribéreau-Gayon. 1971. The Grape. *In:* The Biochemistry of Fruit and their Products. A. C. Hulme ed., Vol. 2 p. 171-205. Academic Press. New York and London.

Pilnik, W., and A. G. J. Voragen. 1970. Pectic substances and other uronides. *In:* The Biochemistry of Fruit and their Products. A. C. Hulme ed., Vol. 1 pp 63-87. Academic Press. New York and London.

Pocock, K. F., and B. C. Rankine. 1973. Heat test detecting protein instability in wine. Aust. Wine. Brew. Spirit. Rev. 91:42-3.

Poirier, D., F. Maris, M. Bennasar, J. Gillot, D. A. Garcera, and B. Tarodo de la Fuente. 1984. Clarification et stabilisation des vins par ultrafiltration tangentielle sur membranes minerales. Ind. Aliment. Agric. 101:481-490.

Raugon, H., and Bourdier, Eds. 1985. Ultrafiltration et microfiltration tangentielle en Œnologie. Compte rendo des Journées Techniques organisées par l'ITV les 23-24 janvier 1985. Institute Technique de la Vigne et du Vin. 21, rue François 1er - 75008 Paris.

Ribéreau-Gayon, J., E. Peynaud, P. Ribéreau-Gayon, and P. Sudraud. 1976. Sciences et techniques du vin. Tome 3. Vinifications. Transformations du Vin., pp 591-594. Dunod, Paris, Francis.

Ribéreau-Gayon, P. 1964. Les composés phénoliques du raisin et du vin. I. II. III. Ann. Physiol. Veg. 6:110-147, 211-242, 258-282.

Ribéreau-Gayon, P., and E. Stonestreet. 1966a. Vorkommen und bedeutung der catechine, leucoathocyanidine und der gerbstoffe in rotweinen. Deut. Lebensm. Rundschau. 62:1-5.

Ribéreau-Gayon, P., and E. Stonestreet. 1966b. Dosage des tannins du vin rouge ett determination de leur structure. Chem. Anal. 48:188-196.

Robertson, G. L. 1979. The fractional extraction and quantitative determination of pectic substances in grapes and musts. Am. J. Enol. Vitic. 30:182-186.

Robertson, G. L., R. Eschenbruch, and K. J. Cresswell. 1980. Seasonal changes in the pectic substances of grapes and their implication in juice extraction. Am. J. Enol. Vitic. 31(2):162-164.

Röhm Tech., Inc. 1982. (Subsidiary of Röhm GmbH, Darmstadt, W. Germany). Technical Information in alcohol test for residual pectin in fruit juices. New York, NY. March.

Romicon, Inc. 1984. Operating instructions Romicon model HF-LAB-5 ultrafiltration system with reverse flow. Woburn, MA.

Rösch, E. 1985. Experience with ultrafiltration in the 1984 season. Confructa 29(1):27-31.

Sachs, S. B., M. Gaillard, and R. Cassignard. 1983. Application of tangential filtration to wine processing (trans). Vignes et Vins. 320:23-30.

Schmitt, R. 1985. Turbidity in apple juice - arabans ?. Confructa 29:22-26.

Scott, D. 1975. Oxidoreductases. *In:* Enzymes in Food Processing. 2nd Edition. G. Reed, Ed: pp 219-254. Academic Press, New York.

Serrano M. S. B. Sachs, and P. Ribereau-Gayon. 1984. Utilization d'un microfiltre tangentiel en œnologie. Connaissance Vigne Vin 18(2): 135-154.

Singleton, V. L., S. E. Draper, and T. A. Rossi Jr. 1966. Paper chromatograph of phenols compounds from grapes, particularly seeds, and some variety-ripeness relationships. Am. J. Enol. Vitic. 17:206-217.

Singleton, V. L., and P. Esau. 1969. Phenolic substances in grapes and wine, and their significance. Supplement 1. Advan. Food Res., Academic Press, New York and London.

Singleton, V. L. 1974. Protein stability in wine. Proc. 7th Penn Wine Conf. Pennsylvania State University. pp 46-58. Dec. 5-6, 1974.

Singleton, V. L., and A. C. Noble. 1976. Wine flavor and phenolic substances. In: Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors, ACS Sym. Ser., American Chemical Society, Washington, D. C. 26:47-70.

Solms, J., W. Bücki, and H. Devel. 1952. Untersuchungen über den pektingehalt einiger. Traubenmoste. Mitt. Gebiete Lebensm. u. Hyg. 43:303-307.

Somers, T. C. 1986. The inevitability of phenolic instability. *In:* Physical Stability of Wine. T. H. Lee (Ed.). pp. 81-90. The Australian Wine Research Institute, Glen Osmond, S. A., Australia.

Somers, T. C., and G. Zimelis. 1973. Direct determination of wine proteins. Am. J. Enol. Vitic. 24(2):47-50.

Staude, E., K. Stenger, and J. Wildhardt. 1976. Precipitation of tartar by concentration of tartar containing solutions by ultrafiltration.(trans) Deutsche Lebensmittel Rundshau 72(6):189-193.

Stone, H., J. Sidel, S. Oliver, A. Woolsey, and R. C. Singleton. 1974. Sensory evaluation by quantitative descriptive analysis. Food Tech. 28(11):24-34.

Stone, H., and J. Sidel. 1985. Descriptive analysis. *In:* Sensory Evaluation Practices. pp 194-226. Academic Press, Inc. New York.

Swientek, R. J. 1986. Ultrafiltration's expanding role in food & beverage processing. Food Processing. 47:71-83.

Taranatola, C. 1971. Substances azotees du raisin et du vin. Bull. O. I. V. 44:47-54.

Testaniere, D. 1985. Resultats d'essai d'ultrafilration de vin blanc appareil industriel: Millipore.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 131-140. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.

Thibault, J. F. 1979. Automisation du dosage des substances pectiques par la methode au meta-hydroxydiphenyl. Lebensm. Wiss. u-Technol. 12:247-51.

Thoukis, G. 1974. Chemistry of wine stabilization: a review. *In:* Chemistry of Wine Making. A. D. Webb, Ed. pp 125-127. Advances in Chemistry Series. 137. American Chemical Society.

Trogus, H. 1984. The use of ultrafiltration in vinification (trans). Der Badische Winzer 1:360-364.

Tump, J. 1983. New ways of treating fruit juices by ultrafiltration. Flüssiges Obst. 50(10):550-551, 553.

Ussiglio-Tomasset, L. 1959. L'evoluzione delle sustanze colloidali dal mosto al vino. Ann. sper. agrar. (Rome) 13:375-404.

Ussiglio-Tomasset, L., and R. Stefano. 1977. Observations on nitrogenous constituents in colloids of musts and wines, and colloids formed by wine yeasts. Riv. Viticolt. Enol. 30:452-469. Abstract in: FSTA 11:1H56, 1979.

Ussiglio-Tomasset, L. 1978. Colloidal phenomena in musts and wines. Ann. Technol. Agric. 27:261-274. Abstract in: FSTA 11:8H1324, 1979.

Van Buren, J. P., and R. D. Way. 1978. Tannin hazes in deproteinized apple juice. J. Fd. Sci. 43(4):1235-1237.

Van Buren, J. P. 1983. Haze in bottled apple juice. *In:* Processed Apples-Research Report 1983. D. L. Dowing Ed. New York state agricultural experiment station, Geneva, N. Y. (November).

Villetaz, J. C., D. Steiner, and H. Trogus. 1984. The use of a beta glucanase as an enzyme in wine clarification and filtration. Am. J. Enol. Vitic. 35(4):253-256.

Von Sydow, E., H. Moskowitz, H. Jacobs, and H. Meiselman. 1974. Odor-taste interaction in fruit juices. Lebensm.-Wiss. u.-Technol. 7;18-24.

Voragen, A. G., and W. Pilnik. 1981. Specific enzymes actions during the liquefaction of apple cell wall preparations. Flüssiges Obst 262-264. August.

Vrignaud, Y. 1983. Recent techniques for clarifying fruit juices by ultrafiltration. Industries Alimentaries et Agricoles 100(4):245-250.

Williams, A. A. 1975. Development of a vocabulary and profile assessment method for evaluating the flavour contribution of cider and perry aroma constituents. J. Sci. Fd. Agric. 26;567-582.

Wilson, E. L., and D. J. W. Burns. 1983. Kiwifruit Juice Processing Using Heat Treatment Techniques and Ultrafiltration. J. Food Sci. 48:1101-1105.

Wilson, E. L., M. C. Hogg, and D. J. W. Burns. 1984. Sensory evaluation of clarified kiwifruit juices. Journal of Food Quality 7(1):43-57.

Whitaker, J. R. 1984. Review. Pectic substances, pectic enzymes and haze formation in fruit juices. Enzyme and Microbial Technology 6(8):341-349.

Wucherpfennig, K., and S. Neubert. 1977. Applicabilities of ultrafiltration for protein stabilization and of reverse osmosis for concentration of grape juice. Fluss. Obst. 44:46-9, 52-6.

Wucherpfennig, K. 1978. Possibilities for the application of membranes for stabilizing wines. (ultrafiltration, reverse osmosis, electrodialysis). Ann. Technol. Agric. 27(1):319-331.

Wucherpfennig, K. 1980. Possibilités d' utilisation de processus membranaires dans l'industrie des boissons (osmose inverse, ultrafiltration, electrodialyse). Bulletin de L'O.I.V., 53(589):187-208

Wucherpfennig, K., and H. Dietrich. 1983. Determination of the colloid content of wines (trans). Lebensmitteltechnik. 15:246, 248, 250, 252, 253.

Wucherpfennig, K., H. Dietrich, and K. Banziger. 1984. Use of micro- and ultrafiltration on fruit juice and wine (trans). Der Duetsche Weinbau 22/26:1144-56.

Wucherpfennig, K., H. Dietrich, and R. Fauth. 1984. The influence of polysaccharides on clarification and filterability of wine with special reference to Botrytis glucan (trans). Deutsche Lebensmittel-Rundschau 80(2):38-44.

Wucherpfennig, K., H. Dietrich, and R. Scholz. 1985. Influences of enzyme treatment and fining of apple juice on fluxrate on ultrafiltration systems. Flüssiges Obst 52(6):324-330.

Wucherpfennig, K., H. Dietrich, and K Weber. 1986. Effect of different clarification processes with regard to their absorptive capacity for colloidal juice components. Flüssiges Obst 53:527-538.

Wysocki, G. 1977. Substance separation: Experience with KBM ultrafiltration equipment (trans). Ernaehrungswirtschaft/ Lebensmitteltechnik 6: 278-282.

Yamasaki, M., S. Kato, S. Chur, and K. Ariman. 1967. Pectic enzymes in the clarification of apple juice. Part II. The mechanism of clarification. Agr. Bio. Chem. 31:552.

Yokotsuka, K., M. Yoshii, T. Aihara, and T. Kudhida. 1977. Isolation and characterization of proteins from juices, musts and wines from Japanese grapes. J. Ferment. Technol. 55:510-515.

Yoshifumi, A., K. Nakamura, T. Komuro, S. Kiribayashi, and M. Kagami. 1980. The control of browning of white table wine by ultrafiltration. Journal of the Institute of Enology and Viticulture. Yamanashi University 15:15-22.

Youval, A., and E. Zisner. 1985. Les materiels, les materiaux et les systemes d'ultrafiltration et de microfiltration tangentielle.*In:* ITV Proceedings: "Ultrafiltration et microfiltration tangentielle en Œnologie" pp 61-70. Institut Technique de la Vigne et du Vin. 21, rue François 1er 75008 Paris.