THE IMPACT OF FINING ON THE CHEMICAL AND SENSORY PROPERTIES OF WASHINGTON STATE CHARDONNAY AND GEWÜRZTRAMINER WINES

By

MELISSA SANBORN

A thesis submitted in partial fulfillment of
the requirements for the degree of

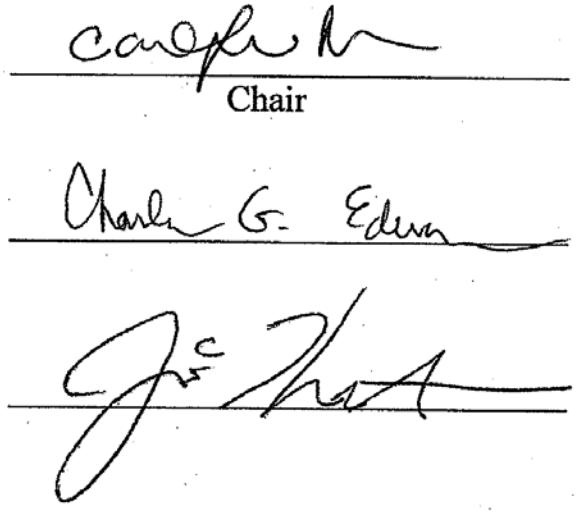
MASTER OF SCIENCE IN FOOD SCIENCE

WASHINGTON STATE UNIVERSITY
Department of Food Science

AUGUST 2008

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of MELISSA SANBORN find it satisfactory and recommend that it be accepted.



Chair

ACKNOWLEDGEMENTS

I would like to thank Dr. Carolyn Ross for giving me the opportunity to study wine and for introducing me to sensory science. I appreciate her patience and guidance during my education, and her dedication to do good science. My extended thanks to Dr. Charles Edwards for his encouragement and advice with thesis revisions and to Dr. James Harbertson for his suggestions on this project.

This degree could not have been obtained without the help of Karen Weller, Scott Mattinson, Dr. Jeff Bohlscheid, Coco Umiker, and Frank Younce. Their advice and help during my research was invaluable.

My appreciation goes out to the faculty and staff of the Food Science department for their assistance the past 2 years, and a special thank you to Jodi Anderson for keeping me on task and putting up with my utter procrastination and absentmindedness.

This work could not have been completed without the financial support of the Washington Wine Commission, and without the generous wine donation from Columbia Crest Winery.

I am genuinely grateful to have befriended such wonderful peers during my education. A special thanks to Josie Landon for her companionship during our time at WSU, and to the *Ross Group,* Andrea, Luan, Tina, Medy, Laura, and CJ. In addition, I am indebted to all fellow students of whom I was fortunate to work with, and whose encouragement and laughter never went unappreciated.

My sincere gratitude to my parents for not only supporting me emotionally throughout my education, but for their help in bottling nearly a thousand cases of wine for my project and ensuring that not a drop was wasted.

To my husband, for playing me old Tom Waits records and feeding me tomato sandwiches during long nights of studying, and for his undivided support in all I do.

And to my dear friend Charlotte Zyskowski, for her strength and optimism, and who gave me the idea in the first place.

THE IMPACT OF FINING ON THE CHEMICAL AND SENSORY PROPERTIES OF WASHINGTON STATE CHARDONNAY AND GEWÜRZTRAMINER WINES

Abstract

by Melissa Sanborn, M.S.
Washington State University
August 2008

Chair: Carolyn F. Ross

The impact of fining on the sensory and chemical properties of Washington State white wine was investigated. Unfined, commercially-prepared Chardonnay and Gewürztraminer wines were treated with bentonite (1000 mg/L), isinglass (60 mg/L), Sparkalloid (360 mg/L), activated charcoal (450 mg/L), whole milk (500 mg/L), or wheat gluten (400 mg/L). Ethyl dodecanoate was the only volatile compound to significantly differ between Chardonnay treatments, which was highest in the control (0.031 mg/L) and lowest in Chardonnay treated with bentonite (0.017 mg/L). Conversely, a number of volatile compounds varied significantly between Gewürztraminer treatments. Ethyl acetate was significantly highest in the activated charcoal treatment (25.4 mg/ L), while lowest in the Sparkalloid treatment (22.1 mg/L). In addition, Gewürztraminer treated with activated charcoal contained high concentrations of higher alcohols. Wheat gluten significantly decreased the concentrations of 1-hexanol, 3-methyl-1-butanol acetate, and 2-methyl-1-butanol. Benzeneethanol was significantly lower in the Sparkalloid, wheat gluten, and bentonite treatments. Conversely, benzeneethanol was highest in the isinglass (85.2 mg/L) and activated charcoal (74.7 mg/L) treatments. 2-phenylethyl acetate and linalool were lowest in Gewürztraminer fined with bentonite. No significant differences were found between treatments for either varietal when the wines were subjected to difference

testing (duo-trio) by an untrained panel ($p \geq 0.05$). No differences were found between Gewürztraminer treatments evaluated by a trained panel, whereas differences in spicy aroma and floral/honey flavor were observed between Chardonnay treatments ($p \geq 0.05$). This study demonstrated the impact fining can have on the chemical and sensory properties of wine and confirmed the importance of selecting the appropriate type and concentration of fining agent in order to maintain wine quality.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Fining is critical towards the consumer acceptance of white wines as bottle haze may eventually lead to consumer rejection and economic loss to the winery (Lopez et al., 2001). With racking and filtration, fining agents improve clarity and increase shelf life. Fining alters the chemical composition (*i.e.,* protein or polyphenol) of wine (Ough, 1960; Sims et al., 1995; Zoecklein et al., 1995; Boulton et al., 1996; Gómez-Plaza et al., 2000; Castillo-Sánchez et al., 2006; Girotti et al., 2006; Ribéreau-Gayon et al., 2006). Fining may also impact the sensory quality of wines, the extent depending on the chosen agent and wine (Razmkhab et al., 2002).

Fining refers to the deliberate addition of materials to a wine followed by the precipitation of components (Boulton et al., 1996). One agent, bentonite, is commonly used to reduce protein content and aids in achieving a heat-stable wine. Proteinaceous fining agents help reduce browning and astringency by removing tannins and polymeric phenols. Synthetic substances, such as polyvinylpolypyrrolidone (PVPP) can be added to reduce polyphenols, whereas carbon agents decolorize and deodorize.

Fining agent performance can be unpredictable and may result in overfining, excessive lees production, and a loss in wine quality. Therefore, bench trials are essential in selecting agent concentration. Additionally, the rising concern of wine additives and labeling regulations has forced winemakers to seek out alternatives to animal-based products.

The primary objective of this study was to evaluate the impact of fining on the sensory and chemical properties of white wines, specifically Washington State

Chardonnay and Gewürztraminer wines. These agents were selected based on industry demand and lack of published data with regards to their sensory impact on wine. Additionally, the efficacy of new fining agents was investigated. It was hypothesized that 1) fining would affect the chemical properties of protein concentration, turbidity, and color, as well as sensory properties such as aroma, taste, and flavor of white wine, and 2) differences would be observed based on the fining agent.

LITERATURE REVIEW

A consumer's initial contact with wine is visual. Wine clarity is essential for quality, particularly in white wine (Amerine and Roessler, 1976; Dupin et al., 2000; Ribéreau-Gayon et al., 2006). According to Ribéreau-Gayon (2006), the only acceptable deposit in a wine is in older red wines, and such sediment should be easily removed by decanting. For this reason, the removal of sediments and haze forming material is essential in white wine production.

Clarification and stabilization is one of the main reasons for fining (Zoecklein et al., 1995; Bird, 2000; Salazar et al., 2006; Cosme et al., 2008). Fining is defined as the intentional addition of materials to a wine with the purpose of altering chemical and/or sensory properties. The type and concentration of fining agent is dependent on the wine and the overall goal of the winemaker.

Not only does haze hinder the visual quality of wine, aroma and flavor can also be affected by turbidity (Karagiannis and Lanaridis, 2002). For example, Ancín et al. (1996) showed that unfined must produced wines with higher isoamyl and isobutyl alcohol levels than musts that had been clarified prior to fermentation. Here, the degree of must clarification played a significant role in the formation of higher alcohols which, in turn, affected aroma. It is therefore of no surprise haze prevention in bottled white wine has become of utmost significance in the wine industry (Saywell, 1934; Zoecklein et al., 1988; Moio et al., 2004).

Factors leading to wine turbidity

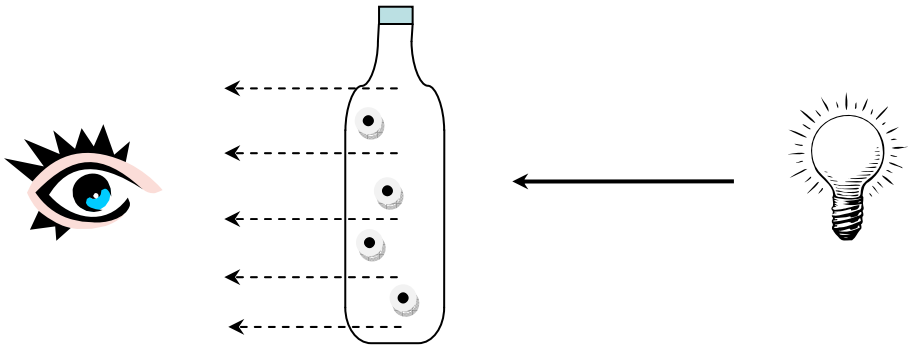
A wine will appear clear when light is allowed to transmit through the wine bottle. Conversely, when light is deflected or halted from its direct path due to the

presence of suspended particles, the wine will appear turbid; this is known as the Tyndall effect (Figure 1) (Ribéreau-Gayon et al., 2006). As particles combine and/or agglomerate, light passage through the wine is hindered and the wine appears turbid, appearing cloudy or opaque to the eye. Suspensions containing particles approximately 100 μm or greater in diameter are reported to be visibly turbid (Ribéreau-Gayon et al., 2006). Optical instruments are helpful in assessing the effectiveness of clarification treatments. A nepheleometer, for example, measures the amount of light diffused perpendicular to the incident of light, expressed in nephelometric turbidity units (Boulton et al., 1996; Maury et al., 2003).

Colloidal phenomena are the conditions under which suspended particles grow in size, flocculate, and eventually form sediment. Some colloidal particles remain stable and pose no threat to wine clarity. On the contrary, unstable colloids tend to amass and form large particles, causing haze. Some will naturally settle from wine, whereas others will remain in suspension. These particles must be removed by the winemaker to prevent clouding in the bottle.

One of the most common and necessary methods of achieving clarity and stabilization is through protein removal. Both grape and autolyzed yeast proteins contribute to the total protein content in wine (Moretti and Berg, 1965; Bayly and Berg, 1967; Zoecklein, 1988; Dupin et al., 2000; Achaerandio et al., 2001). About half of these large and insoluble proteins will eventually precipitate with or without the addition of heat (Zoecklein, 1988). The remaining proteins, derived mostly from the grape, can contribute to wine haze (Murphey et al., 1989).

a)



b)

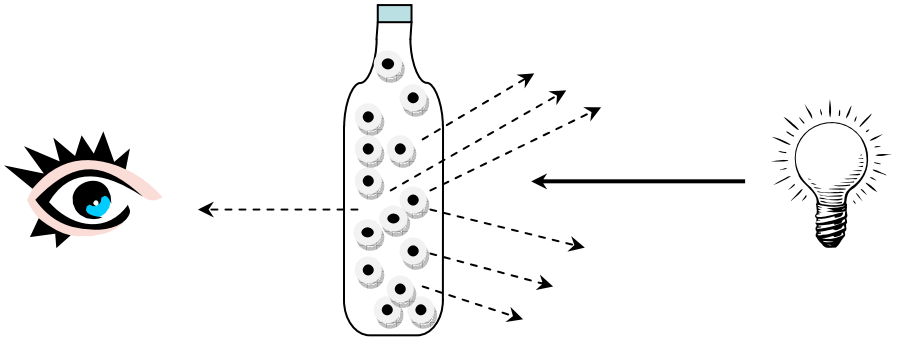


Figure 1. The Tyndall effect as observed in wine. a) Light transmittance through the wine. b) Light deflected by the wine.

Protein instability can vary between wines as a result of several factors, including grape variety, terrior, processing methods, and conditions like pH, ionic strength, and ethanol concentration (Bayly and Berg, 1967; Sarmento et al., 2000; Achaerandio et al., 2001; Mesquita et al., 2001). After bottling and during storage, wines may experience changes in protein stability due to alterations in extrinsic variables such as temperature (Hsu and Heatherbell, 1987; Boulton et al., 1996; Sarmento et al., 2000).

Other colloidal substances known to cause wine turbidity include yeasts, bacteria, tartrate precipitations, and metal precipitations such as ferric ferrocyanide and copper sulfate (Bird, 2000; Jackson, 2000; Ribéreau-Gayon et al., 2006).

Achieving a stable wine

Clarity can be achieved several ways: spontaneous clarification (settling) by gravity, centrifugation, fluctuations in temperature and/or racking of the wine are common processing methods used in the wine industry (Zoecklein, 1988; Boutlon et al., 1996; Armada and Falqué, 2007). While eliminating suspended particles in a wine can help achieve clarity, such results are not always permanent. To prevent turbidity from developing during bottle aging, a wine must also be stabilized (Zoecklein, 1995; Boulton et al., 1996; Bird, 2000). Clarifying and stabilizing treatments carry different applications and some treatments are more practical/impractical than others. For example, racking, often used to remove sedimentation from a finished wine, does not always achieve wine clarity and several rackings are usually required to achieve a desired clarity level. In addition, racking does not necessarily stabilize a wine. Temperature can be manipulated to precipitate components, such as tartrates, from a wine. Spontaneous settling requires little equipment and occurs relatively fast in red and dry white wines, but

is less efficient in sweet white wines or wines that have a shorter aging period. In addition, factors such as wine viscosity, temperature, particle size, and the presence of protective colloids can all hinder spontaneous clarification. The goal of centrifugation is to accelerate settling by using speed to separate particles from solution. It is often used in place of filtration. However, centrifugation is expensive and is economically impossible for many small wineries. Additionally, centrifugation does not stabilize the wine against further haze. Hence, if the aim of the winemaker is to both clarify and stabilize the wine, fining would be a more appropriate treatment as it has the ability to accomplish both tasks (Ribéreau-Gayon et al., 2006; Armada and Falqué, 2007). The purpose of fining is to cause haze-forming particles to combine with additional agents, leading to flocculation, clarity, and stabilization.

Three major mechanisms of action of fining agents include charge-charge (electrical) interaction, bond formation, or absorption/adsorption. Wine components and the type of fining agent determine the mechanism of action. When compounds of opposite charges interact, larger particles form and settle. In the case of bond-formation, chemical bonds (*i.e.,* hydrogen bonds) form between fining agents and wine components. Absorption occurs when compounds are engulfed by the fining agent. Alternatively, when the substance is bound to the agent's surface, the substance is adsorbed.

Fining agent applications

Removing phenols

Fining agents are widely used to adjust levels of tannins or polymeric phenols (Ough, 1960; Sims et al., 1995; Boulton et al., 1996; Gómez-Plaza et al., 2000; Castillo-Sánchez et al., 2006; Girotti et al., 2006). These compounds, particularly oxidative

phenols, may inadvertently contribute to haze by reacting with residual protein fractions, eventually precipitating out of solution and causing turbidity (Saywell, 1934; Calderon et al., 1968; Somers and Ziemelis, 1973a; Zoecklein et al., 1995; Boulton et al., 1996; Jackson, 2000; Armada and Falqué, 2007).

Astringency is a significant component to red wine acceptability and quality. Phenols and tannins bind to and precipitate salivary proteins from the mouth, resulting in a loss of lubrication in the mouth and an increase in dry and puckering sensations (Amerine and Roessler, 1976; Naish et al., 1998; Maury et al., 2001). Tannins and phenols are responsible for the astringent mouthfeel perceived when consuming astringent wines, and specific fining agents help to reduce or soften highly astringent wines prior to bottling by reducing the tannin content (Rossi and Singelton, 1966; Naish et al., 1998; Sarni-Manchado et al., 1999; Gómez-Plaza et al., 2000; Maury et al., 2001; Harbertson, 2005).

In white wine production, oxidation of polyphenols such as catechins and proanthocyanidins cause a wine to brown (Boulton et al., 1996; Jackson, 2000; Spagna et al., 2000; Ribéreau-Gayon et al., 2006). Oxidation can also introduce unwanted aromas and flavors to a wine (*i.e.,* acetic acid) (Panagiotakopoulou and Morris, 1991; Sims et al., 1995; Boulton et al., 1996; Gómez-Plaza et al., 2000; Castillo-Sánchez et al., 2006), resulting in diminished sensory characteristics, a shorter shelf life, and a wine with impoverished quality (Spagna et al., 2000). By reducing the concentration of polyphenols in white wine prior to bottling, the winemaker increases the shelf potential and palatability of the wine.

Alternatively, it may be the winemaker's goal to decolorize or deodorize the wine without compromising other characteristics, such as astringency. Substances that target smaller polar phenolics and their derivatives can be applied to selectively alter certain properties of the wine (Jackson, 2000).

Main and Morris (1994) investigated fining agents as alternatives to sulfur dioxide (SO_2) in preventing the browning of Seyval blanc juice and wine. Here, bentonite, polyvinylpolypyrrolidone (PVPP), and kieselsol had little effect on quality attributes tested except color. Juice color was best maintained by bentonite (960 mg/L) and PVPP (719 mg/L) treatments, by SO_2 (100 mg/L), or by the combination of the three fining agents. Bentonite at a concentration of 960 mg/L performed similarly to 100 mg SO_2/L at preventing browning in the wine. Wine treated with Kieselsol (719 mg/L) was more yellow than the control, indicating browning. The authors conclude that bentonite and PVPP treatments may be substituted for SO_2 treatments in the prevention of Syval blanc wine browning.

Reducing protein

As previously mentioned, wine haze is primarily due to the presence of unstable proteins in solution. Protein stability is significantly influenced by wine pH. Wine proteins vary in charge; protein fractions can carry a net positive or net negative charge. When the positive and negative charges are equal (zero net charge), the protein is said to be neutral or at its isoelectric point (*pI*); at this point, the protein is least soluble in solution (Dawes et al., 1994; Boulton et al., 1996; Zoecklein et al., 1996; Gómez-Plaza et al., 2000; Harbertson, 2005). As the pH of the wine increases, the overall net charge becomes more negative; conversely, as the pH of the wine decreases, the overall charge

of the protein becomes more positive. When a protein is charged, it has an increased binding affinity for oppositely charged components in solution.

In white wine production, protein removal is vital, especially if other clarification methods are not applied. Specific fining agents target proteins through charge interactions, forcing the proteins to precipitate out of solution (Zoecklein et al., 1995; Boulton et al., 1996; Ribéreau-Gayon et al., 2006). The end result is a protein-stable wine less susceptible to wine haze and more appealing to the consumer.

Altering aroma and sensory properties

Fining can also alter wine aroma profiles by binding to free and bound aromatic compounds (Voilley et al., 1990; Moio et al., 2004; Armada and Falqué, 2007). For example, Moio et al. (2004) reported that fining with bentonite (80 g/hL), potassium caseinate (60 g/hL), silica gel (10 g/hL), gelatin (30 g/hL) and activated charcoal (20 g/hL) induced a loss of aroma compounds in Falanghina wine. Furthermore, a greater loss of glycosides suggested that direct interactions between glycosides and fining agents may exist, and that fining may negatively influence wine aging potential. In conclusion, the authors suggest that fining may result in decreased varietal character.

In a similar study, aroma and phenolic compounds in Parellada wine were reduced by fining with bentonite (0.5 g/L), potassium caseinate (0.4 g/L) a bentonite-gelatin mix (0.3-0.1 g/L), and microcrystalline cellulose (0.4 g/L) (Puig-Deu et al., 1996). Bentonite also significantly reduced the concentration of volatile and aromatic compounds, such as total flavonoids (6%), ethyl esters (46%), acetates (47%), alcohols (47%), and terpenes (42%). Potassium caseinate caused a 20% loss in flavonoids and a 22% loss in total acetates. The bentonite + gelatin treatment had a significant drop in

protein (32%) and total ethyl esters (46%), acetates (57%), alcohols (45%), and terpenes (37%). Microcrystalline cellulose resulted in losses of several volatiles, including flavonoids (23%), ethyl esters (19%), acetates (21%), total alcohols (18%), and terpenes (2%). Further, the protein fining agents had a greater effect on flavonoid concentrations than other polyphenols.

Few studies have used established sensory methods to evaluate the impact of fining on the sensory properties of wine. In fact, many authors include remarks concerning changes in organoleptic properties based on anecdotal tastings. However, Sims et al. (1995) used triangle tests to compare red and white wines fined with PVPP, casein, or gelatin. White wine treated with gelatin (0.6 g/L) was significantly different from an unfined control; no significant differences were observed between the control and wine treated with PVPP or casein. While neither gelatin or casein were significantly different from the control, PVPP (1.0 g/L) was perceived as different by the panel. Hence, consumers were able to differentiate between fined and unfined wines.

Fining trials

As a fining agent's performance can vary between wines, wineries conduct fining trials prior to fining to determine what type and concentration of fining agent to apply. Trials are conducted using similar techniques which will eventually be used in the cellar. Fining agent preparation, method of addition, method of assessing wine stability, and sensory impact are all components to consider when assessing the efficacy of wine fining during bench trials (Weiss and Bisson, 2002).

The type and concentration of fining agent used depends on the goals of the winemaker. To remove protein, fining agents targeting proteins should be considered. If

the winemaker seeks to reduce the tannin concentration, gelatin or egg white fining is practical. The selected fining agent should then be evaluated for performance. The production of lees, the reduction of haze, and the impact of the agent on the organoleptic properties of the wine should be considered and monitored throughout the fining trials. For example, if the objective of fining is to reduce turbidity, the production and height of sediment on the bottom of the fining vessel can indicate which agent concentration best eliminated haze-forming components from the wine (Bird, 2000). Instruments measuring turbidity or clarity can help determine the most effective fining agent and concentration required to minimize haze (Boulton et al., 1996). Alternatively, if the purpose of fining is to alter wine color, chromometric measurements could be used to determine the necessary fining agent concentration required to achieve the most favorable color.

Most importantly, fining trials help to avoid excessive fining. Over-fining may remove desired characteristics from the wine or impart unwanted sensory characteristics to the wine, which can be detrimental to quality. In general, an acceptable concentration of fining agent is lowered to a point at which it remains below a solubility condition or a taste threshold (Boulton et al., 1996). Determining the minimum concentration of fining agent necessary to clarify and stabilize a wine prior to cellar applications will greatly reduce the risk of over-fining.

Types of fining agents used in white wine production

Numerous fining agents are available to achieve wine clarity and stability (Table 1). Each agent has advantages and disadvantages with regards to wine flavor, cost, and loss of wine due to excessive lees production (Servili et al., 2000). Proteinaceous agents, including gelatin, casein, egg albumin, or isinglass, react with negatively charged

Table 1. Common fining agents and their source and application to winemaking.

Fining agent	Source	Purpose of application
Gelatin	Animal tissue	Removal of tannin and brown polymeric pigments
Isinglass	Fish bladder	Reduce phenolic compounds; add fruitiness to wine
Casein	Milk	Reduce wine haze and tannin content
Egg albumen	Egg whites	Reduce wine haze and tannin content
Bentonite	Clay, volcanic deposits	Protein removal
Tannin	Wood and grape seeds	Targets phenolic and protein compounds
Sparkalloid	Alginate	Clarification and settling aid
Polyvinylpolypyrrolidone	Synthetic polymer	Reduce polyphenols
Nylon	Synthetic polymer	Reduce polyphenols
Activated charcoal	Carbon	Decolorize and deodorize
Silica	Silica	Protein removal
Kieselsol	Silicon dioxide	Reduce phenolic compounds
Copper sulfate	Copper sulfate	Removal of H_2S
Potassium ferrocyanide*	Potassium ferrocyanide	Metal removal

* Application not permitted in the United States.

particles such as tannins. Alternatively, the non-protein materials like bentonite and tannins aid in the precipitation of proteins. A more thorough explanation of agents commonly applied by the wine industry follows, including each agent's mechanism of action and application to wine processing.

Earths

Bentonite

Bentonite is the most commonly used fining agent in the wine industry, particularly to achieve clarity and protein stability in white wine (Blade and Boulton, 1988; Zoecklein, 1988). Bentonite is a montmorillonite aluminum silicate clay weathered from volcanic material deposited in various regions of the world (Zoecklein et al., 1995; Boulton et al., 1996; Ribéreau-Gayon et al., 2006). The clay complex is arranged in plates which, upon hydration, expand to increase its surface area.

The treatment process of wine with bentonite includes three distinct physical reactions: dispersion of the agent, adsorption of the solutes, and settling of the complex (Blade and Boulton, 1988). Upon hydration, platelets swell and separate to increase the surface area available for cation exchange (Zoecklein, 1988; Boulton et al., 1996; Sarmento et al., 2000; Brady et al., 2002). This principally negatively charged molecule is then able to adsorb positively charged colloids suspended in solution. Once particles have adsorbed to the surfaces of bentonite, the molecule becomes heavy and settles due to the force of gravity.

While various types of bentonite exist, calcium and sodium bentonites are the predominant forms available in Europe and the United States, the latter form being more popular in the U.S. (Blade and Boulton, 1988; Zoecklein, 1988; Boulton et al., 1996;

Jackson, 2000). Between the two, sodium bentonite has been shown to have the highest adsorption capacity, suggesting this form to be more useful in removing suspended colloids, namely stable and unstable proteins (Blade and Boulton, 1988; Boulton et al., 1996; Sarmento et al., 2000). Sodium bentonite also reacts more quickly than the calcium form, reducing processing time. A drawback to sodium bentonite, however, is the excessive production of lees during fining. Calcium bentonite produces significantly more compact lees than sodium bentonite. For this reason, calcium bentonite is more commonly used in champagne production (*methodé champenoise*) to facilitate riddling, or the movement of yeast sediment to the neck of the bottle during secondary alcoholic fermentation (Pozo-Bayón et al., 2003; Vanrell et al., 2007).

Bentonite is predominantly used to remove proteins through charge-charge interactions. As negatively charged bentonite interacts with positively charged proteins, the complex precipitates from solution and is removed by racking or filtration (Figure 2). In few cases, positive reactive sites on bentonite have also been shown to bind with phenols and anthocyanins, reducing wine color and the potential for wine oxidation (Gómez-Plaza et al., 2000).

Bentonite additions are commonly made to finished wine prior to bottling but after several rackings. Bentonite can also be added to must pre-fermentation to reduce the need for wine clarification post-fermentation. However, pre-fermentation fining with bentonite considerably reduces the amount of yeast assimilable nitrogen in the must, which may potentially result in a stuck fermentation (Vos and Gray, 1979; Guitart et al., 1998; Weiss and Bisson, 2002).

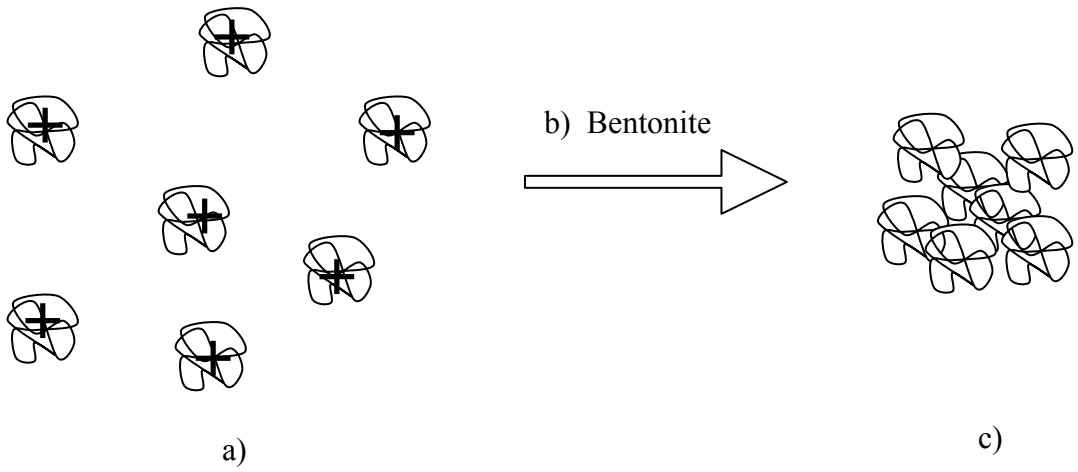


Figure 2. Electrostatic charges between a) positively-charged proteins and b) negatively charged bentonite result in c) a neutralized complex which will eventually clump together and precipitate from solution.

While bentonite is effective in wine clarification and stabilization, significant swelling upon hydration often causes bentonite to settle slowly. In these situations, diatomaceous earth must be added as a filtering aid to prevent clogging (Sarmento et al., 2000). Unfortunately, the resulting filter cake is a major source of winery waste. To limit winery waste, Sarmento et al. (2000) researched the protein adsorption capacity of silica gel, hydroxyapatite, and alumina as alternatives to sodium bentonite in wine clarification. However, the authors reported that no material tested had a higher adsorption capacity than sodium bentonite.

In addition to its swelling capacity, bentonite results in the production of excessive lees that must be separated from the wine. A slight loss of wine usually accompanies bentonite fining. Bakalilnsky and Boulton (1985) investigated the use of immobilized protease from *Aspergillus niger* at protein-stabilizing Chardonnay, Sauvignon blanc, Gewürztraminer, and Riesling. Only one Riesling replication was significantly stabilized (achieved a turbidity level < 20 NTU), demonstrating its inability to stabilize white wines against haze-inducing proteins.

Bentonite can alter wine aroma constituents. Armada and Falqué (2007) found that Albariño wines fined with bentonite had a lower concentration of terpenes and C_{13}-norisoprenoids (13%) and C_6 alcohols (33%). These volatile compounds are responsible for the varietal aroma of Albariño wines and a decrease in concentration could diminish overall quality.

Similarly, Voilley et al. (1990) reported a loss of aroma compounds in model wines fined with bentonite (10 g/L) or casein (10 g/L). A loss of ethyl hexanoate and isoamyl acetate was recorded for both fining agents. A greater loss in 1-hexanol was

observed from bentonite fining than casein fining (12.1% and 7.7%, respectively), whereas casein fining caused a greater loss of β-ionone (34.3% and 15.0%, respectively).

On the contrary, Pozo-Bayón et al. (2003) noted no effect on the volatile composition (primarily alcohols, esters, and fatty acids) in Spanish sparkling wine fined with bentonite (0.03 g/L). Bentonite is often added to the triage during sparkling wine production to encourage the flocculation and elimination of yeast. However, the single concentration of bentonite evaluated may have been too low to produce differences, especially considering concentrations of bentonite used in previous studies (10 g/L in Voilley et al., 1990; 0.5 g/L in Puig-Deu et al., 1996; 0.8 g/L in Moio et al., 2004).

In-line dosing for bentonite fining of wine is an alternative to batch-fining for protein removal in white wines. Adequate protein adsorption and turbidity levels were achieved in as little as three minutes at concentrations between 1000 – 1500 mg/L by Nordestgaard et al. (2007). In addition, no sensory differences were found between in-line dosed and batch-fined Chardonnay and Semillon-Chardonnay wines. Unfortunately, centrifugation was required to separate the bentonite from the wine and bentonite carryover in the wine was as high as 30%, suggesting that additional methods of clarification must be implemented to reduce carryover.

Proteins

Proteinaceous fining agents are usually of animal origin. Proteins have a high affinity for polyphenols, and interact with phenolic compounds by hydrogen bonding between the phenolic hydroxyl and the carbonyl oxygen of the peptide bond (Figure 3) (Zoecklein et al., 1995). Protein agents usually react with larger phenolic compounds as

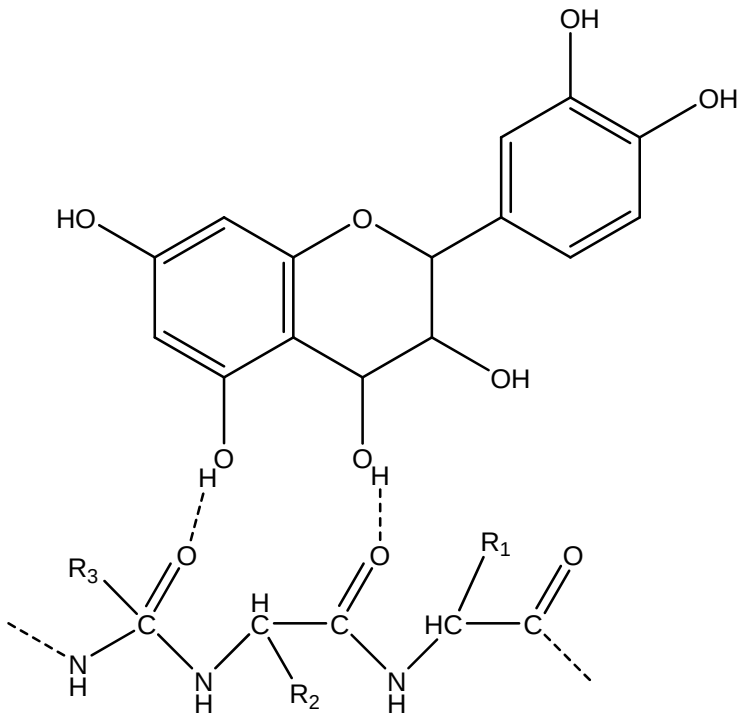


Figure 3. Hydrogen bonding between protein fining agent and phenol compound (Zoecklein et al., 1995).

they have more available hydroxyl groups (more binding sites) and form the strongest bonds. Isinglass, gelatin, caseins, and albumen are the most common proteinaceous agents used for fining.

Cosme et al. (2007) observed differences in the characteristics and functionalities of several classes of proteinaceous fining agents from different sources in a white wine blend: two potassium caseinates, two caseins, four egg albumins, four isinglasses, and seven gelatins from difference sources. Within each class, significant differences in total phenolic reduction were observed between specific products. The greatest decrease in total phenolics was observed using a gelatin. In addition, gelatins of low molecular weight removed more total phenolic compounds than gelatins of higher molecular weight. Furthermore, the author noted differences between gelatins concerning flavonoid and non-flavonoid removal. Similarly, flavonoid and non-flavanoid concentrations varied between isinglass of different sources. In general, caseins reduced a significantly larger percentage of non-flavonoid compounds (17.8%) than potassium caseinate (3.6%). In conclusion, the authors not only demonstrated variability between proteinaceous agents of different origins, but variability amongst sources as well.

Isinglass

Isinglass is a protein fining agent produced from sturgeon collagen (Boulton et al., 1996). The collagen fibers are available in sheets or powder forms. The triple helical structure of isinglass is critical for efficient clarification (Jackson, 2000). As the structure denatures at 29°C, it is vital to maintain low temperatures during preparation (Boulton et al., 1996). The agent acts based on charge interactions between the protein and

oppositely charged particles. Once electrostatically particles form a complex, the macrocolloids are no longer in solution and settle with gravity.

Isinglass' principal purpose in winemaking is to modify wine phenols, to clarify, or to provide balance to a wine. It has also been said that isinglass enhances fruit character, though the mechanism is not fully understood (Zoecklein et al., 1995; Boulton et al., 1996; Hickman et al., 2000). One suggestion for this mechanism is that esters responsible for fruity aromas and flavors are enhanced when other volatile compounds, such as volatile phenols, are removed from wine. Isinglass adsorbs and precipitates phenols. When isinglass successfully removes volatile phenols that contribute to wine aroma, other concealed aromas become unmasked. Therefore, isinglass may indirectly enhance fruit aromas by removing masking aromas.

Isinglass may also enhance the brilliance and yellow color of white wines (Ribéreau-Gayon et al., 2006). Unlike other protein fining agents, isinglass is less active towards tannins and is less likely to affect astringency (Rankine, 1984; Zoecklein, 2002; Ribéreau-Gayon et al., 2006). The agent is also more effective at lower concentrations than gelatin or casein, reducing the risk of over-fining (Rankine, 1986; Boulton et al., 1996; Zoecklein, 2002). Unfortunately, isinglass can produce excessive lees, and it has been reported that excessive contact with wine may impart a fishy odor to the wine aroma (Zoecklein et al., 1995; Zoecklein, 2002).

Isinglass is usually added to a barreled wine. Like bentonite, isinglass can also be applied prior to fermentation (*i.e., methode champenoise*). Although isinglass preparation was tedious in the past, new, pre-hydrated products have facilitated its use (Zoecklein et al., 1995; Ribéreau-Gayon et al., 2006).

Gelatin

Gelatin is derived from collagen, the protein found in the skin and bones of animals. Unlike other protein-based fining agents, gelatin has relative high amounts of glycine, proline, and hydroxyproline (Rossi and Singleton, 1966; Calderon et al., 1968; Boulton et al., 1996). These amino acids assist in interactions between gelatin and wine tannins, and these higher molecular weight components eventually precipitate from solution (Rossi and Singleton, 1966; Calderon et al., 1968).

In wine production, gelatin is most commonly applied to remove tannins and brown polymeric pigments (Calderon et al., 1968; Boulton et al., 1996; Sarni-Manchado et al., 1999; Maury et al., 2001; Harbertson, 2005). Interactions between wine tannins and proteins also contribute to wine haze; therefore, protein removal with gelatin facilitates wine clarification (Calderon et al., 1968).

Wine color can be altered with the use of gelatin. Removing phenolic compounds with gelatin preserves red wine color during storage (Gómez-Plaza et al., 2000; Spagna et al., 2000). However, excessive additions can result in undesirable color loss in red wines (Jackson, 2000). In white wine production, the chemical oxidation of phenolic compounds (*i.e.,* atechins and proanthocyanidins) is the main form of browning, which affects the color, aroma, and taste of white wine (Singleton and Kramling, 1976; Spagna et al., 2000). Protein components in gelatin bind to wine polyphenols, forming complexes which precipitate out of solution and reduce browning potential.

Commercial gelatins vary widely in terms of amino acid composition and molecular weight, depending on their source (Boulton et al., 1996; Maury et al., 2001). Maury et al. (2001) characterized the fining abilities of a commercial gelatin (average

molecular weight 25,000 daltons) and gelatin fractions (16,000 to 190,000 daltons) in terms of tannin removal. Here, tannin precipitation with gelatin was selective for highly polymerized and galloylated tannins, and the 16,000 dalton gelatin precipitated more polymerized tannins than the 190,000 dalton gelatin. The author attributed the increased precipitating ability of the smaller molecular weight protein to its size, which allowed it to bind tannins with greater ease. This finding was in agreement with Sarni-Manchado et al. (1999), who noted that the largest molecular weight gelatin precipitated the least amount of proanthocyanidins. In addition, the gelatin fined wines were subjected to sensory evaluation by Maury et al. (2001) to determine if perceived astringency was affected by protein fining. In agreement, their results concluded that gelatin treated wines were significantly less astringent than unfined wine ($p < 0.05$).

Recent concerns have developed concerning the use of animal-based proteins, such as gelatin, as fining agents in wine production. For instance, bovine spongiform encephalopathy in humans has been linked to cattle, a source of gelatin (Jackson, 2000; Marchal et al., 2002a; Maury et al., 2003; Harbertson, 2005). In addition, consumer shifts towards "vegetarian" or "animal-free" products, as well as potential labeling laws requiring the declaration of all substances added to wine during production, have forced enologists to find alternatives to gelatin (Weber et al., 2007). Unfortunately, its effectiveness at tannin removal is so far unmatched.

Caseins

Caseins are a mixture of proteins precipitated from milk with high levels of glycine, proline, leucine, and lysine (Boulton et al., 1996; Zoecklein et al., 2000). As casein has a *pI* of 4.55, the protein is insoluble at pH 4-5 and is therefore prepared and

added to wine as an alkaline solution (O'Neal et al., 1950; Boulton et al., 1996). In solution, caseins form complexes with phenolic compounds via hydrogen bonding which, at lower wine pH, are precipitated out of solution. Casein is usually purchased as a powder, hydrated in alkaline medium, and added in doses of 10 to 20 g/hL (Ribéreau-Gayon et al., 2006).

Caseins are most commonly used to reduce haze and tannin concentrations, however they are less effective than gelatin or bentonite in terms of wine stabilization. For example, Cruess (1963) found that Muscatel and sherry wines fined with bentonite were more stable than those fined with casein.

Though the use of casein as a fining agent is not as popular as bentonite or gelatin, it has several applications. For example, Dambrouck et al. (2005) reported that casein or casein plus bentonite improved the foaming properties (foam height and foam stability) of Champagne wine compared to bentonite alone and a control. Here, bentonite caused a significant decrease in total protein content, grape invertase concentration, and foam quality, whereas casein only slightly reduced total protein content and grape invertase concentration when compared to the control. Such findings suggest that fining with casein may produce a higher quality Champagne product than using bentonite fining alone.

Albumen

Another protein commonly used in wine fining is albumen, an egg white mixture consisting primarily of ovalbumin. Egg white proteins are comprised of glucine, leucine, asparagine, valine, and serine. The *pI* lies between 4.55 to 4.90, similar to gelatin, casein, and isinglass (Boulton et al., 1996). An inexpensive and therefore widely practiced

method of albumen fining is with fresh egg whites. Egg whites are separated from the yolks, beaten, and added directly to the wine. Albumen is also available in solid form, which is rehydrated prior to its addition (Riberaeu-Gayon et al., 2006).

Albumen aids in wine clarification and tannin reduction, performing well in highly tannic red wines (Boulton et al., 1996). Several studies have been conducted evaluating albumen's ability to effectively clarify wines, as well as its impact on the quality of the finished wine (Meunier, 2003; Bonerz et al., 2004; Castillo-Sánchez et al., 2006). To date, no studies have found albumen to outperform other agents, and it has little effect on wine quality when compared to others.

Tannin

Enological tannin is a commercial product made from oak wood or grape pomace (Zoecklein et al., 1995; Jackson, 2000; Lee and Noble, 2003). It is water-soluble and partially soluble in ethanol. However, oak tannins may impart bitter, green, and astringent characters on a wine, whereas those extracted from grapes do not (Ribéreau-Gayon et al., 2006).

As a fining agent, tannin is primarily used to remove excess protein in white wines. Hydrogen bonds form between carboxyl or hydroxyl groups of the polyphenolic tannins and carbonyl groups of the protein peptide bonds, causing the proteins to precipitate (Calderon et al., 1968; Jackson, 2000). Tannin applications are less popular than bentonite as the adsorptive capacity of tannin is much less (Ribéreau-Gayon et al., 2006). However, regions such as Champagne commonly use tannins for clarification and stabilization.

Tannin-gelatin mixtures are often used to simultaneously target phenolic and protein compounds. Tannins can be added post-gelatin fining to remove excess gelatin, or to tannin-deficient wines to increase perceived astringency (Zoecklein et al., 1995; Ribéreau-Gayon et al., 2006).

Tannins can be used to stabilize wine color during fermentation as well as assist in color development during ageing, though regulations in certain wine regions prohibit the addition of tannin to increase color intensity (Zoecklein et al., 1995).

Synthetics

PVPP

Polyvinylpolypyrrolidone, or PVPP, is a commercially available vinyl polymer produced by polymerizing vinylpyrrolidone in the presence of an alkali (Figure 4). It is most commonly sold as Polyclar AT, an insoluble white powder (Doner et al., 1993; Ribéreau-Gayon et al., 2006).

PVPP is frequently found in food and cosmetic applications, and is commonly used by winemakers to reduce astringency and retard browning (Ough, 1960; Caputi et al., 1969; Gómez-Plaza et al., 2000). PVPP binds with monomeric and polyphenols through hydrogen bonds between the carboxyl groups and hydroxyl groups of phenolics (Gómez-Plaza et al., 2000; Jackson, 2000). In return, undesirable bitterness and astringency is diminished from the wine.

PVPP is also efficient at removing formed brown complexes and correcting discoloration (Jackson, 2000; Ribéreau-Gayon et al., 2006). Ough (1960) reported that PVPP removed more color from Cabernet Sauvignon than gelatin. In addition, the PVPP

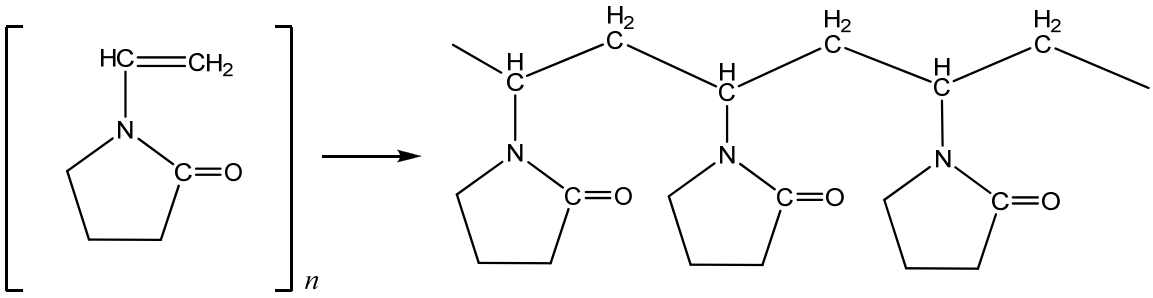


Figure 4. Polymerization of vinylpyrrolidone into polyvinylpolypyrrolidone (PVPP) (Riberaeu-Gayon et al., 2006).

wines produced more color haze than the gelatin fined wines. While PVPP (25 – 50 mg/L) had better filtration properties and precipitated more tannin than gelatin, sensory analysis revealed that gelatin-treated wine was preferred over PVPP-treated wine. Furthermore, PVPP-treated wine had less aroma and flavor than the gelatin fined wines.

Likewise, Castillo-Sanches et al. (2006) showed that red Vinhão wine fined with PVPP (1 g/L) had a greater color loss than 0.2 g/L gelatin, 0.2 g/L egg albumin, and 0.6 g/L casein after 20 months storage. The PVPP also caused a greater loss in anthocyanin content in the wines exposed to rotation by rotary vat.

In agreement, Sims et al. (1995) noted that post-fermentation additions of PVPP resulted in reduced total and polymeric phenols and significantly lighter color red wine. Additionally, the author also observed that pre- and post-fermentation additions of PVPP reduced total and flavonoid phenols, lightened color, and improved resistance to browning in white muscadine wine. Nevertheless, Gómez-Plaza et al. (2000) monitored the phenolic composition of Monastrell red wine fined with either PVPP or bentonite at bottling during a 12 month storage period and found that PVPP-fined wine showed higher anthocyanin content, wine color, and total color pigments than either the bentonite treatment on an unfined wine after 12 months.

Nylon

Nylon is a synthetic fiber consisting of repeating polymer units linked by peptide bonds. It was the first successful synthetic polymer made from coal, air, and water. It is legal in most countries for wine fining.

As with PVPP, nylon has carbonyl functional groups on its surface that adsorb to phenols and similar compounds. Fuller and Berg (1965) reported that nylon successfully

reduced the color of white table wines and slowed browning, more so than casein but less effectively than carbon, with little influence on wine quality. However, nylon is significantly less efficient than PVPP and as a consequence, is not commonly used commercially (Boulton et al., 1996).

Activated carbon

Activated carbon is purified charcoal with a high internal porosity and an increased adsorptive surface area (Jackson, 2000). It is a nonspecific adsorptive agent with an affinity for benzenes and their derivatives (Zoecklein et al., 1995).

Activated carbon is used in extreme situations to decolorize or deodorize a wine. It remoes color pigments and phenolics (Boulton et al., 1996; Lopez et al., 2001). Unfortunately, activated carbon can also induce the oxidation of ethanol, leading to increased concentrations of acetaldehyde which can impart pungent or suffocating aromas to the wine, and thus its use by wineries is limited (Zoecklein et al., 1995; Clarke and Bakker, 2004). Furthermore, Cruess (1963) found that wines fined with carbons imparted a "carbon taste" to the wine. While it has been reported that activated carbon can aid in microbial stability by adsorbing vitamins that may be used by spoilage organisms, little evidence exists.

Lopez et al. (2001) studied the effect of activated charcoal in combination with casein, potassium caseinate, albumin, and gelatin on the sensory properties of fino sherry. Here, the aromatic profile was not altered by the fining agents employed. The application of activated charcoal, however, resulted in a decrease of the polyphenolic content of the sherry. Nevertheless, these sherries had similar browning tendencies to unfined wines.

Silicon dioxide

Silica

Silicon dioxide is an oxide of silicon found in natural products such as sand or quartz. Its initial fining application was as a substitute for tannic acid in gelatin fining (Zoecklein et al., 1995). The agent is used most commonly in white and rosé wine production to expedite settling (Boulton et al, 1996).

Silica is a negatively charged particle used to neutralize, flocculate, and precipitate positively charged proteins (Ribéreau-Gayon et al., 2006). Negatively-charged silica electrostatically binds with positively-charged proteins, which eventually settle from solution. However, Sarmento et al. (2000), reported that bentonite had a higher adsorption capacity and protein-binding affinity than silica gel and would be better suited for stabilizing protein in wine and must.

Suspensions of silica are often combined with gelatin treatments to reduce tannin hazes and to prevent potential overfining with gelatin. When combined with other agents, silica accelerates clarification, reduces the amount of residual protein fining agent, and facilitates filtration (Ribéreau-Gayon et al., 2006). In addition, silica has little to no impact on the organoleptic properties of wine and carries little risk of overfining (Ribéreau-Gayon et al., 2006).

Kieselsol

Kieselsol is an aqueous suspension of silicon dioxide commonly used to remove bitter phenolic compounds from white wine. Kieselsol also has the ability to remove positively or negatively charged particles as it is available in both positively or negatively

charged forms (Jackson, 2000). To date, no research has been published regarding the impact of Kieselsol on the chemical or sensory properties of wine.

Polysaccharides

Agar/Gum arabic

Agar is an unbranched polysaccharide derived from cell membranes of algae. It is gelatinous by nature and is used as a stabilizer in food products (Fennema, 1996). Gum arabic, a natural gum from the acacia tree (*Acacia Senegal* and *Acacia seyal*), is a mixture of saccharides and glycoproteins (Fennema, 1996). Like agar, it is used as a food stabilizer.

Both agar and gum arabic partially neutralize surface charges on dispersed colloids, preventing the colloids from repelling one another and allowing them to coagulate and precipitate or to dissolve (Zoecklein et al., 1995). Presently, neither agar or gum arabic are commonly used for fining in the United States. They have been replaced by other alginate-based materials such as Sparkalloid or Klearmor.

Sparkalloid/Klearmor

Sparkalloid and Klearmor are positively-charged, alginate-based materials which aid in the settling of finely suspended matter (Boulton et al., 1996). They are sold as a powder which forms a viscous colloidal solution when hydrated. Both agents are used to enhance clarity and filterability. Like agar and gum arabic, Sparkalloid and Klearmor neutralize surface charges of naturally occurring protective colloids, allowing them to dissolve into solution or coagulate and precipitate out of solution (Boulton et al., 1996). They produce relatively compact lees and have not been found to affect color, odor, or flavor (Zoecklein et al., 1995).

Metal removal

Blue fining, or *Moslinger,* is the application of potassium ferrocyanide to wine to remove transition metal cations (Zoecklein et al., 1995; Boulton et al., 1996). Potassium ferrocyanide is not permitted for use in the United States, while colloidal forms, such as Cufex, are legal. Chelating agents and commercial resins have also been applied to reduce elevated metal concentrations from wine (Boulton et al., 1996).

Other fining applications

Fining encompasses functionalities other than wine clarification and stabilization. Reductions in Ochratoxin A (a potential human carcinogen found in food and wine) (Castellari et al., 2001; Fernandes et al., 2007) and certain pesticides (Cabras et al., 1995; Soleas and Goldberg, 2000) have been reported as a consequence of fining.

Ochratoxin A (OTA) is a carcinogenic mycotoxin produced by fungi such as *Aspergillus* and *Penicillium* (Fernandes et al., 2007). Although OTA is broken down during grape processing, levels higher than that allowed by the European Union (2 μk/Kg) have been detected in wine (Fernandes et al., 2007).

In response to growing concerns by the EU regarding OTA levels in wine, Fernandes et al. (2007) evaluated the efficacy of several fining agents at reducing OTA concentrations. Here, the authors reported that the most efficient removal of OTA was achieved by egg albumen and gelatin fining in red wines. White wine fined with PVPP and casein did not significantly differ in OTA from the control wine. These findings differed slightly from those reported by Castellari et at. (2001), who reported that potassium caseinate (150 g/hL) and activated carbon (10 g/hL) were more successful than silica gel, gelatin, and albumen at removing sufficient levels of OTA, and did so without

affecting total polyphenolic levels. Potassium caseinate removed 82% of OTA, while carbon was the most efficient OTA adsorbant.

The presence of pesticides in must has been shown to not only slow the rate of alcoholic fermentation, but to have detrimental effects on the aroma quality of red and white wines. Soleas and Goldberg (2000) reported that bentonite showed little effect at diminishing pesticide concentrations, while a post-fermentation treatment with 0.25 g/L kieselsol was successful at reducing levels. In another study, Cabras et al. (1995) found that activated charcoal (20 g/hL) allowed almost complete elimination of insecticide residues in an Italian red wine, whereas the agents bentonite (100 g/hL), potassium caseinate (100 g/hL), gelatin (20 g/hL), PVPP (80 g/hL), and silicon dioxide (50 g/hL) showed no or moderate influence on removing insecticide from the wine.

Recently, bovine spongiform disease has forced winemakers to seek alternatives to animal-derived fining agents. Marchal et al. (2002a; 2002b) investigated the use of wheat gluten as a clarifying agent in white musts and wines. When Chardonnay must was treated with gluten (20 or 40 g/hL), a 70% decrease in turbidity was observed (Marchal et al., 2002a). With 20 g/hL, gluten imparted a similar turbidity as musts fined with a tannin-gelatin mixture (5 g/hL). At a higher level of 40 g/hL, gluten generally clarified the wine to a higher degree than 60 g/hL bentonite. However, depending on the specific gluten product, gluten was not as effective as 1 g/hL isinglass or 10 g/hL casein. Additionally, gluten treatments produced less lees than the bentonite treatments.

Alternative fining agents

Alternatives to current fining agents are often sought after by researchers. Bonerz et al. (2006) evaluated the use of Cfine®, a material extracted from the skins of deep-sea

fish, as a potential fining agent in Pinot Noir production. At a concentration of 1.4 mg/L, Cfine® resulted in wines of brighter color, better clarity, and less bitterness and astringency than wines fined with gelatin, isinglass, casein, egg albumen, or PVPP. However, the long-term stability of wines fined with Cfine® was not evaluated.

In another study, Bonilla et al. (2001) found baker's yeast to be successful at reducing browning in Spanish white wines. The results agreed with those of Razmkhab et al. (2002) who observed a similar affect in sherry.

Chitin, a polysaccharide derived from the outer skeleton of insects and crustaceans, has been useful to fine wine (Vincenzi et al., 2005). At low concentrations, Chitin stabilized wine without significantly modifying the protein content. In support, Chitin reduced wine haze by 80%. According to the authors, proteins contribute significantly to the organoletpic properties of white wine; protein removal could impact sensorial qualities, and the use of bentonite severely diminishes a wine's aroma profile. However, the study did not include a sensory panel or a chemical evaluation of aroma compounds present in either treatment to support their claim.

Polysaccharides (*i.e.*, alginates) successfully remove proteins from wine. Cabello-Pasini et al. (2005) found that polysaccharides extracted from seaweed efficiently reduced protein. The authors noted that tannins were not adsorbed by the polysaccharide and suggested its use as a fining agent capable of precipitating protein without affecting tannin content.

A zirconia-packed column was compared to bentonite in its ability to remove protein from Spanish white wine (Salazar et al., 2006). Though not as effective as bentonite, zirconia did successfully reduce the protein content to a stable level (< 2

ΔNTU). In addition, no sensory differences were detected by an untrained panel between the zirconia and bentonite treatments. However, the cost and feasibility of applying the packed-column method in a winery setting were not addressed.

Labeling laws

The European Parliament (EP) recently adopted Directive 2003/89/EC, declaring that specific substances used in the production of food products must be declared on product labels for they present a risk to allergic individuals (Weber et al., 2007). The regulation also includes imports. The list includes products derived from egg, milk, and fish.

To comply with labeling laws, winemakers must produce wines free of such components. In response to the EP's directive, Weber et al. (2007) investigated finished wine for residual levels of fining agents. Here, no residual levels of potassium caseinate (6 and 30 g/hL), isinglass (50 and 250 ml/hL), or fish gelatin (10 and 50 g/hL) were detected. However, the author noted significant residual levels of dried egg white (4 and 20 g/hL) and lysozyme (25 and 50 g/hL). In a similar study, Rolland et al. (2006) reported that albumen, isinglass, and non-grape-derived tannins left negligible residual levels in commercial Australian wines.

The Codex Alimentarius was created by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to regulate general standards such as food labeling and additives in food production (www.codexalimentarius.net, 2008). Certain individuals have an immunological reaction to the ingestion of gluten and must therefore avoid gluten-containing products. In accordance with the Codex Alimentarius, food products must contain < 10 mg/L gluten

to be labeled as gluten free. Marchal et al. (2002a) found that red wines treated with 6, 12, and 18 g/hL wheat gluten had < 0.25 or 2.5 g/hL residual deaminated gluten proteins.

As wine label restrictions begin to severely impact how wine is produced in the United States, types and concentrations of fining agents may be targeted. Therefore, it is necessary to continue investigations into the efficacy and impact of fining on wine quality and to seek out new fining agents that satisfy labeling mandates.

CHAPTER II

THE IMPACT OF FINING ON THE CHEMICAL AND SENSORY PROPERTIES OF WASHINGTON STATE CHARDONNAY AND GEWÜRZTRAMINER WINES

MATERIALS AND METHODS

Wine

Unfined Gewürztraminer (2.9% w/v residual sugar; 0.8 g/L titratable acidity; pH 3.07; 11.4% v/v ethanol) and Chardonnay (0.1% residual sugar; 0.6g/L titratable acidity; pH 3.51; 14.4%/volume ethanol) wines were obtained from Chateau Ste. Michelle winery (Paterson, WA) at the end of alcoholic fermentation. The Gewürztraminer wine had been rough filtered, or passed through a filter of large pore size, and the Chardonnay wine had been cold stabilized. The wines were transported in sterilized, food-grade, 30-gallon plastic drums to Washington State University (Pullman, WA) for fining studies. Initial turbidity measurements were made using a turbidmeter (Orbeco-Helliage, Farmingdale, NY) and expressed in nephelometric turbidity units (NTUs).

Both wines were held at approximately 8°C until utilized. Free SO_2 levels were adjusted to between 20 and 30 mg/L with potassium metabisulfite (JT Baker, Inc., Phillisburg, NJ) and headspaces were flushed with N_2 gas.

Fining trials

Trials were performed to determine appropriate concentrations for each fining agent. The treatments applied were an unfined control, bentonite (500, 750, 1000, 1250, and 1500 mg/L), isinglass (15, 60, 75, 90, and 105 mg/L), Sparkalloid (300, 360, 420,

480, and 540 mg/L), activated charcoal (100, 250, 350, 450, 500 mg/L), whole milk (50, 250, 500, 750, 1000 mg/L), or wheat gluten (50, 100, 200, 300, and 400 mg/L).

Fining agent preparation

Sodium bentonite (Crosby and Baker, Scott Labs, Walla Walla, WA), Sparkalloid (Cellar Pro, Steinbart Wholesale, CA), and isinglass (Ichtyocolle, Scott Labs, Walla Walla, WA) were prepared according to manufacturer recommendations. Bentonite was hydrated 24 hrs prior to wine addition. Sparkalloid powder (31.6 g) was added to 1000 ml boiling water, boiled for 10 minutes, and was added to the wine. Isinglass powder (10 g) was added to 50 ml deionized water and heated for five minutes until dissolved two hours prior to wine addition. A slurry of GemPro HiQ wheat gluten (Manildra Group, USA, MO) was prepared by adding 50 g gluten to 1000 ml.

Proportions of the bentonite, isinglass, Sparkalloid, whole milk (Ferdinand's Creamery, Pullman, WA), activated charcoal (hydrocarbon trap fill, Varian, USA) and wheat gluten were pipetted into 50 ml of wine. Wines were then mixed and allowed to settle at 13°C. After seven days, turbidity levels were measured at room temperature.

Samples were subsequently subjected to a heat stability test (Pocock and Rankine, 1973). Samples were heated at 80˚C for six hours, cooled to 4˚C for twelve hours, and assessed for turbidity at 22˚C.

Wine Fining

Wines were racked off into 5-gallon glass carboys after adjusting free SO_2 levels to 20 - 30 ppm using potassium metabisulfite. The prepared fining agents were added to the wines at 1000 mg/L bentonite, 60 mg/L isinglass, 360 mg/L Sparkalloid, 450 mg/L activated carbon, 500 mg/L whole milk, or 400 mg/L wheat gluten. The fining

agents were stirred into the wines and allowed to settle at approximately 12.8°C (Figure 5).

After seven days, turbidity levels were measured prior to being filtered through a 0.45μm membrane filter (Vitipore Plus 0.45 μm, GusmerEnterprises, Fresno, CA), bottled, and closed with natural corks (Tri-State, Moscow, ID). The closures were visually examined for flaws and defective corks were discarded. Chardonnay treatments were bottled in 750-ml glass Burgundy bottles and Gewürztraminer treatments were bottled in 750-ml Hock bottles (Saxco Pacific Coast, Tacoma, WA). Wines were stored at approximately 4°C until chemical and sensory analysis.

Chemical analysis

All volatile standards, as well as potassium hydrogen phthalate, tartaric acid, and bovine serum albumin, were purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (NaOH) and sodium chloride (NaCl) were purchased from JT Baker (Phillisburg, NJ). Coomassie blue reagent was purchased from BioRad Laboratories, Inc. (Hercules, CA).

For all chemical analyses, three bottles of each treatment were evaluated in duplicate per varietal. Sulfur levels were monitored using the Aeroation-Oxidation Method for SO_2 Analysis (Buechsenstein and Ough, 1978). Alcohol, pH, titratable acidity, and volatile acidity were analyzed in accordance to AOAC methods (2000). Alcohol content was measured using an ebulliometer (Presque Isle Wine Cellars, North East, PA); pH was measured using an Accumet AB15 Plus pH meter (Fisher Scientific, USA); and titratable acidity (TA) was analyzed using a TitroLine Easy autotitrator

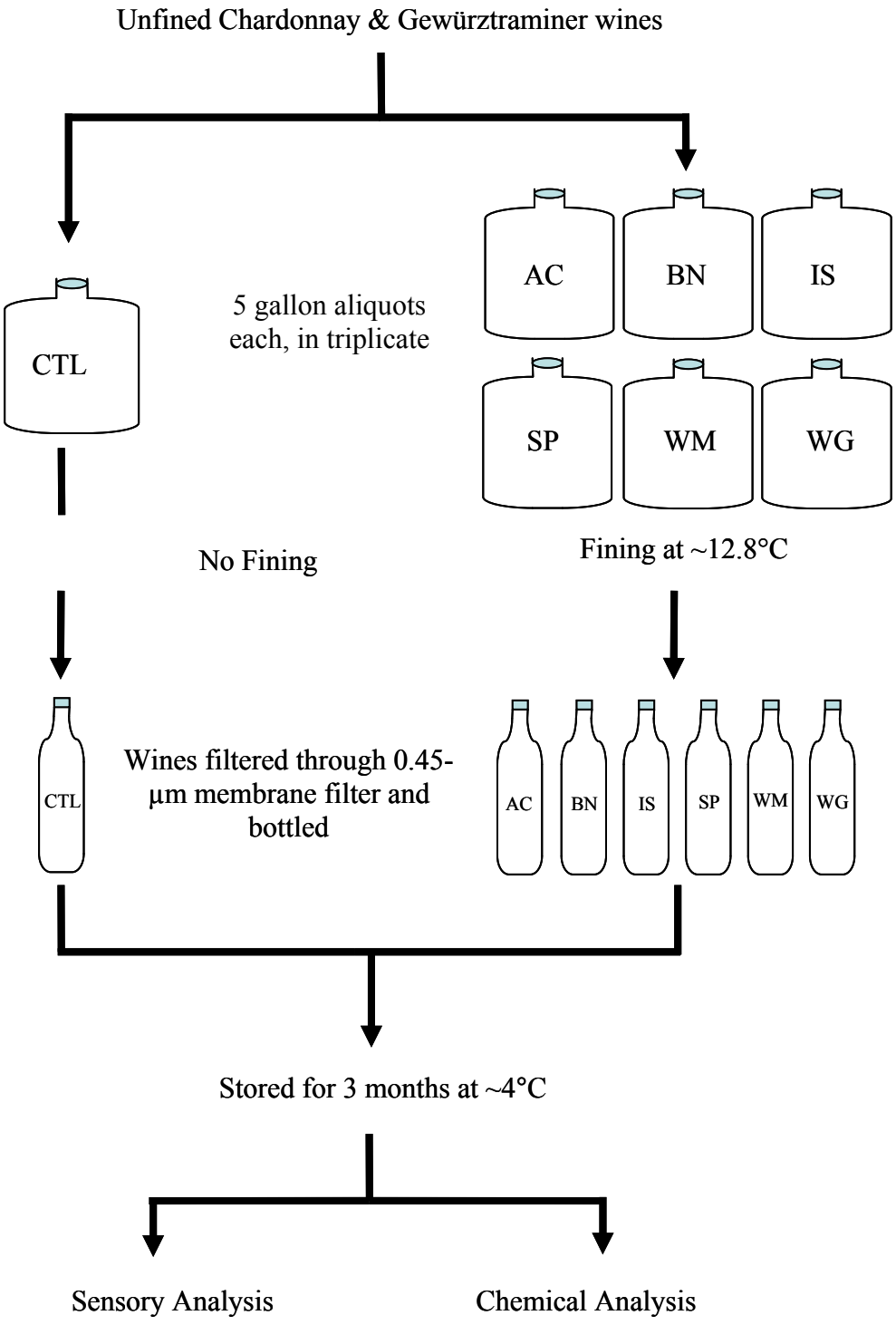


Figure 5. Fining of Chardonnay and Gewürztraminer wines. AC: Activated Carbon: AC; Bentonite: BN; Isinglass: IS; Sparkalloid: SP; Whole Milk: WM; Wheat Gluten: WG; Control: CTL.

(Schott Instruments, Deutschland, Germany).

Protein contents were measured using a modified Bradford assay employing Coomassie Brilliant Blue (Bradford, 1976; Murphey et al., 1989) with Genesys 10uv spectrophotometer (Thermo Electron Corp., Madison, WI).

Finally, color was analyzed according to Pérez-Caballero et al. (2003) using a Genesys 10uv spectrophotometer (Thermo Electron Corp., Madison, WI).

Volatile Analysis

Solid-phase microextraction was used to extract various volatile compounds from the wines. Prior to use, a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was pre-conditioned at 250°C for 30 min. The optimized SPME parameters were as follows: 2 ml of sample was placed into a 4-ml amber glass vial with 0.65 g NaCl (6M) and a magnetic stir bar (Steffen and Pawliszyn, 1996; Whiton and Zoecklein, 2000; Howard et al., 2005; Bohlscheid et al., 2006). The vial was securely capped with a Teflon-coated silicon septum and allowed to equilibrate for five min while being stirred magnetically at ambient temperature (~22°C). After equilibration, the sample headspace was extracted for 45 min at ambient temperature while being magnetically stirred and introduced into the injection port of the gas chromatograph.

GC/MS analyses were carried out using a Hewlett-Packard 5890 gas chromatograph coupled to an HP-5973 mass selective detector. The GC was equipped with a 0.75-mm i.d. deactivated injection liner (Supelco, Bellefonte, PA). Chromatographic separations were achieved using a 60-m length, 0.32-mm i.d., 0.25-µm film thickness, DB-1 column (J&W Scientific). The injector temperature was maintained at 200°C. The injection was made in splitless mode at 200°C for 5 minutes.

Helium was used as the carrier gas with a constant flow rate of 1 ml/min. The oven temperature settings were as follows: 33°C for 5 min; 5°C/min ramp to 50°C; 2°C/min ramp to 225°C; hold at 225°C for 13 min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV. The temperature of the detector was maintained at 230°C. Data were collected in SCAN mode from the mass range 35 to 550 m/z. Identification of volatiles was confirmed using retention time of spectra match of standard compounds. Secondary confirmation was conducted using NIST mass spectra library.

The internal standards, 1-pentanol and 1-dodecanol, were selected based on their response recoveries and retention time when compared to target volatile compounds. For each calibration standard and sample, 4-µl of 1-pentanol (5,000 mg/L in 50% v/v ethanol) and 1-µl of 1-dodecanol (1,000 mg/L in 50% v/v ethanol) were added five minutes prior to extraction, giving concentrations of 10 mg/L 1-pentanol and 0.5 mg/L 1-dodecanol in solution.

Sensory Analysis

Forced Choice Duo-Trio Test

Bottled wines were stored for four months at 4°C prior to difference testing by an untrained panel. A forced choice duo-trio test (constant reference) was used to determine whether or not fined wines differed from the unfined (control) wines. Each varietal was evaluated over a two day period, with three treatments per varietal (and a control) evaluated each day, for a total of four evaluation days. Thirty panelists participated on each evaluation day and received a non-monetary incentive for their participation. Panels were conducted in individual sensory booths in the Sensory Facility of the Food Science

and Human Nutrition Building at Washington State University, Pullman, WA. Booths were equipped with yellow colored lights to mask visual differences in the wines. Demographic data were collected from each panelist. Panelists were recruited through email, internet announcements, and bulletins posted throughout the Food Science and Human Nutrition Building at Washington State University, Pullman, WA.

A test-sensitivity analyzer was used to determine the α-risk, β-risk, and power of the test (Meilgaard, Civille, and Carr, 1999). The inputs were as follows: 30 panelists were required (n); 20 number of correct guesses were needed for a difference to be significant (x); there was a 0.5 probability of a correct guess by a panelist (ρ_o) using the duo-trio test; and a proportion distinguisher (ρ_d) of 0.3, indicating that no more than 30% of the population could detect a difference at the calculated β-risk. Based on the inputs, it was calculated that there was a 0.65 probability of a correct response by a panelist, an α-risk was 0.05, a β-risk was 0.5, and that the power of the test (1-β) was 0.5.

Each panelist was presented with water, crackers, a napkin, and a cuspidor. Panelists were presented with three samples per flight, for a total of three flights. Per flight, the control sample (labeled Reference) was presented along with two samples (one control sample, one treatment sample) individually marked with a three-digit code. Twenty-five milliliters of each sample was served at 10°C in clear ISO/INAO wine glasses and covered with plastic petri dishes. Wines were maintained at 10°C in a water bath (VWR1155 Refrigerated Constant Temperature Circulator, Polyscience, Niles, IL). Panelists were asked to examine, sniff, and/or taste each sample and then determine which of the two coded samples differed from the Reference sample. Evaluations were

recorded on laptop computers and analyzed using Compusense five Release 4.6 software (Ontario, Canada).

Trained Panel

Twelve Washington State University students and staff (11 females and 1 male, ages 23-70) were selected to participate on the trained panel. Panelists were recruited through email and online Washington State University announcements. Panelist selectively was based on availability. Panelists received non-monetary gifts at the end of each training and evaluation session as incentives. All training and evaluation sessions were conducted in the Sensory Facility of the Food Science and Human Nutrition Building at Washington State University, Pullman, WA.

Panelists met for a total of nine one-hour training sessions. Demographic information was collected from each panelist. During the first training sessions, panelists were introduced to various basic taste and aroma standards prepared in a commercial base wine (Franzia Refreshing White Wine, Ripon, CA). These standards were selected to represent taste and aroma attributes found in Chardonnay and Gewürztraminer wines by a small focus group of experienced wine tasters prior to the trained panel. Panelists evaluated a 30-ml wine sample (Chardonnay control from fining experiment) for taste, aroma, and flavor; the same was done with a sample of the Gewürztraminer control. Panelists evaluated each taste standard in-mouth, noting locations in the mouth in which the taste sensations were perceived. Panelists were familiarized with 15-cm line scales (with high and low anchors) and asked to score taste intensities using the scales. Aroma standards were individually evaluated by sniffing each standard and describing, as a group, the odor they perceived.

For the second session, taste and aroma standard concentrations were reduced by one half which and were revisited by panelists at the beginning of the session. Panelists evaluated a 30-ml sample (experimental Chardonnay control) for aroma, taste, and flavor. Evaluations were discussed by the group and terms were combined, added, and/or eliminated (Table 2). The process was repeated with the Gewürztraminer control.

In the third and fourth training sessions, taste and aroma standards were revisited. Two commercial Chardonnay (2005 Columbia Winery Chardonnay, 2004 Arbor Crest Chardonnay) and two Gewürztraminer wines (2005 Columbia-Crest Gewürztraminer, 2005 Columbia Winery Gewürztraminer) were individually evaluated for taste, aroma, and flavor and the discussed as a group. During the fifth session, the panelists practiced evaluating samples of commercial and experimental wines in sensory booths and became familiarized with sensory evaluation software (Compusense five Release 4.6, Ontario, Canada). Evaluation mean intensities and standard deviations for each wine attribute were analyzed using Microsoft Excel (Microsoft Office, 2004).

During the sixth training session, panelists received feedback regarding their performance in relation to the group (based on attribute mean intensities). Individual outlying scores were considered unreliable and of low validity, and panelists were encouraged to revisit standards representing attributes which they had the most difficulties. The wines evaluated in the booth were then revisited and discussed as a group. During training, it was decided that during final evaluations, panelists would receive a control sample (unfined treatment) with each treatment as a reference. The panel individually evaluated the control treatment (for each varietal) and through discussion, the group decided on appropriate intensities of each attribute for each control

Table 2. Taste, aroma, and flavor-by-mouth descriptors and standards developed by a trained panel (n = 12) for the evaluation of Chardonnay and Gewürztraminer treatments.

Term	Standard (in 200 ml base wine)
Taste	
Sweet	Sucrose (JT Baker, Phillisburg, NJ) - 4 g
Sour	Citric acid (JT Baker, Phillisburg, NJ) - 0.4 g
Bitter	Quinine sulfate (Sigma, St. Louis, MO) - 2 mg
Aroma/Flavor	
Fruity/Lychee/Citrus	
Apricot	Canned apricots in syrup (Safeway Select) - 10 ml
Peach	Canned peaches in syrup (Safeway Select) - 10 ml
Lychee	Canned lychee in syrup (Walong Markering Inc, Buena Park, CA) - 200 ul
Citrus	Citrus extract (1 drop each orange and lemon extracts in 100 ml base wine (McCormick Inc., Hunt Valley, MD)) - 50 ul
Floral/Honey	
Floral	Floral essence (1 drop floral essence (Wine Awakenings, Ontario, Canada) in 150 ml base wine) - 3 ml
Honey	Raw Honey (Silverbow, Moses Lake, WA) - 1 g
Herbaceous/Veggie	Canned asparagus (Safeway Select) - 1 ml
Cooked vegetable	Canned green beans (Safeway Select) - 1 ml
Herbaceous	1 in^2 green pepper in 10% ethanol - 1 ml
Spicy	
Nutmeg	0.1 g nutmeg (Spice Islands, San Francisco, CA) in 250 ml base wine - 15 ml
All-spice	0.1 g all-spice (Spice Islands, San Francisco, CA) in 250 ml base wine - 15 ml
Chemical/Hot	
Ethanol	95% Ethanol - 20 ml

sample. These intensities were revisited during the following two training session to ensure proper in placement on the line scale and were used as a reference for the remainder of training and sample evaluations.

In the eighth training session, the panelists evaluated three Chardonnay and Gewürztraminer wines in the booths and were provided with control sample as reference. Results were analyzed as in training session five and feedback was provided during the final training session. This final session was also used to discuss any further concerns or comments of the panel.

Bottled wines were held for seven months at 4°C prior to evaluations. After training, panelists participated in four days of evaluations (two days per varietal) in individual testing booths equipped with white lighting. A complete balanced block experimental design was used and each panelist was presented with each treatment twice. Taste and aroma standards used during training were available to the panelists prior to evaluations. Wine bottles were held at approximately 18°C in a water bath (VWR1155 Refrigerated Constant Temperature Circulator, Polyscience, Niles, IL) and were poured immediately prior to serving. Twenty-five ml sample aliquots were presented in ISO/INAO clear wine glasses and covered with plastic petri dishes. Samples were labeled with three-digit codes were served in random order. Panelists were asked to evaluate each sample for attribute intensity using a 15-cm line scale. Accompanying each sample was a reference sample (unfined control treatment) and its attribute intensities, determined during panel training.

Statistical Analysis

Chemical data were analyzed using two-way analysis of variance (ANOVA) and compared using Fishers's least significant difference (LSD) (XLSTAT, Addinsoft, Paris, France). Compusense five Release 4.6 (Ontario, Canada) was used to collect all sensory data and analyze data from the forced choice duo-trio test. A two-way fixed-effects ANOVA (including wine and panelist) and Fisher's LSD was used to analyze trained panel data for significant differences (XLSTAT, Addinsoft). Panelist x treatment interactions were included in the ANOVA model as they were found to be significantly different between treatments.

RESULTS AND DISCUSSION

Chemical analysis

Turbidity

The initial turbidities of unfined Chardonnay and Gewürztraminer wines were 211 and 27.8 NTU, respectively. All fining concentrations achieved acceptable turbidity levels of < 10 NTU (Weiss and Bisson, 2002) in both varietals (Table 3). Concentrations that achieved the lowest turbidity level in one of the two varietals were selected for the fining experiment. Concentrations were kept identical between varietals to allow for comparison between the Chardonnay and Gewürztraminer wines.

Turbidity levels were again assessed after fining and prior to filtration (Table 4). In the Chardonnay, the only treatment to exceed 10 NTU was the control (CTL) (10.9 NTU). Wine fined with whole milk (WM) (6.4 NTU), wheat gluten (WG) (6.2 NTU), Sparkalloid (SP) (5.7 NTU), bentonite (BN) (5.0 NTU), and isinglass (IS) (3.0 NTU)

Table 3. Mean turbidity levels (NTU) by treatment (mg/L) from fining trial in Chardonnay and Gewürztraminer wines measured by an Orbeco-Helliage turbidmeter. Treatment means with different letters within columns differ at $p \leq 0.05$ using Fisher's LSD. AC: activated charcoal; BN: bentonite; IS: isinglass; WM: whole milk; SP: Sparkalloid; WG: wheat gluten; CTL: control.

Treatment (mg/L)		Chardonnay	Gewürztraminer
AC	100	6.6a	1.1a
	250	6.7a	1.2a
	350	6.8a	1.2a
	450	6.1b	1.4a
	500	6.6a	1.4a
BN	500	2.7a	1.4a
	750	0.7d	1.1b
	1000	1.2c	1.0b
	1250	1.7b	1.4a
	1500	1.3bc	1.1b
IS	15	6.7a	1.8a
	60	5.4c	1.6a
	75	6.6a	1.6a
	90	5.0c	1.6a
	105	6.1b	1.9a
WM	50	2.8a	7.5a
	250	3.8a	7.5a
	500	2.8b	6.8ab
	750	2.3bc	6.9ab
	1000	1.1c	6.6b
SP	300	8.5a	2.6d
	360	7.6b	3.5d
	420	6.9bc	5.6c
	480	6.7cd	8.0b
	540	6.1d	9.9a
WG	50	7.6c	2.6c
	100	9.6b	2.9b
	200	13.3a	3.6a
	300	5.0d	3.2b
	400	3.8d	2.9d

Table 4. Mean turbidity values (NTU) of Chardonnay and Gewürztraminer treatments prior to bottling as measured by an Orbeco-Helliage turbidmeter. The time passed between the initiation of fining and turbidity readings is indicated by time (days). Wines were bottled within 24 hours of turbidity readings listed. Means with different letters within columns differ at $p \leq 0.05$ using Fisher's LSD. AC: activated charcoal; BN: bentonite; IS: isinglass; WM: whole milk; SP: Sparkalloid; WG: wheat gluten; CTL: control.

Wine	Treatment	Time (d)	Turbidity (NTU)
Chardonnay			
	AC	7	9.4a
	BN	7	5.0bc
	IS	7	3.0c
	WM	7	6.4b
	SP	7	5.7b
	WG	7	6.2b
	CTL	7	10.9a
Gewürztraminer			
	AC	7	17.1b
	BN	7	8.1c
	IS	7	2.5d
	WM	7	24.5a
	SP	7	1.2d
	WG	7	22.5a
	CTL	7	23.0a
	AC*	21	19.1
	WM*	21	18.2
	WG*	21	18.1

*Treatments with turbidity levels >10 NTU after 7 days; 1 carboy per was treatment allowed to settle an additional 14 days, at which time turbidity levels were again measured.

were significantly lower in turbidity. Marchal et al. (2002) found that fining with wheat gluten (40 g/hL) resulted in a more clarified Chardonnay more than bentonite, though neither clarified as well as isinglass or casein. In the present study, wheat gluten and bentonite did not significantly differ in turbidity.

In the Gewürztraminer, only BN (8.1 NTU), IS (2.5 NTU), and SP (1.2 NTU) achieved turbidity levels < 10 NTU after seven days fining (Table 4). The activated charcoal (AC), WM, and WG treatments were above 10 NTU and were therefore fined an additional 14 days. However, turbidity levels remained above 10 NTU after 21 days. Further chemical and sensory results were based on AC, WM, or WG fined for 7 days.

It is not fully understood why turbidities achieved from fining did not agree with the bench trials for the AC, WM, and WG treatments. Marchal et al. (2002) found that turbidity levels decreased with increasing additions of wheat gluten (10 to 40 g/hL). In the present study, greater concentrations of wheat gluten (> 400 mg/L) may have been required to achieve clarification. In addition, a smaller volume of wine was used in the fining trials, which may have allowed for better mixing of the fining agent when compared to the larger volume of wine used in the fining experiment.

Turbidity measurements of Chardonnay were taken after three months storage in the bottle (Table 5). The WG treatment (2.4 NTU) was significantly higher than the other treatments, which did not significantly differ from one another.

After 3 months storage (Table 5), bentonite treated Gewürztraminer had the lowest turbidity level (0.4 NTU). WM had the greatest turbidity (2.8 NTU) and was significantly higher than the control, suggesting that it induced haze.

Table 5. Turbidity (NTU), total protein (mg/L), titratable acidity (g/100 ml), volatile acidity (g/100 ml), and color measurements of Chardonnay and Gewürztraminer wines taken 3 months after storage in bottle. Means with different letters within rows differ at $p \leq 0.05$ using Fisher's LSD. AC: activated charcoal; BN: bentonite; IS: isinglass; WM: whole milk; SP: Sparkalloid; WG: wheat gluten; CTL: control.

Treatment	Chemical parameter				
	Turbidity (NTU)	Total protein (mg/L)	Titrable acidity (g/100 ml)	Volatile acidity (g/100 ml)	Color ('L')
Chardonnay					
AC	0.6^{b}	9.36^{d}	0.571	0.046	99.95^{a}
BN	0.6^{b}	8.41^{e}	0.577	0.051	99.96^{a}
IS	0.7^{b}	10.15^{a}	0.558	0.048	99.96^{a}
WM	0.8^{b}	10.23^{a}	0.556	0.046	99.87^{c}
SP	0.7^{b}	9.53^{c}	0.541	0.046	99.95^{a}
WG	2.4^{a}	10.23^{a}	0.542	0.047	99.93^{b}
CTL	0.5^{b}	9.98^{b}	0.560	0.052	99.81^{d}
Gewürztraminer					
AC	1.9^{ab}	76.88^{a}	0.770^{de}	0.037^{b}	99.99
BN	0.4^{d}	17.92^{e}	0.804^{b}	0.044^{ab}	100.00
IS	0.9^{cd}	71.50^{abc}	0.840^{a}	0.040^{ab}	99.99
WM	2.8^{a}	63.07^{d}	0.757^{ef}	0.045^{ab}	99.99
SP	2.5^{ab}	70.40^{bc}	0.787^{c}	0.042^{ab}	99.98
WG	2.1^{ab}	68.12^{cd}	0.771^{d}	0.041^{ab}	99.99
CTL	1.6^{bc}	76.63^{ab}	0.756^{f}	0.049^{a}	100.00

For either varietal, all treatments had turbidity levels < 10 NTU after storage; however, more differences were observed between Gewürztraminer treatments than Chardonnay. The Chardonnay control dropped from 211 to 10.9 NTU (at bottling), suggesting that the majority of haze-forming material naturally settled from the wine. Similarities in turbidity observed between AC, BN, IS, WM, SP, and CTL treatments after bottle storage were most likely attributed to natural settling rather than fining. Wheat gluten, however, caused an increase in haze in the Chardonnay. On the contrary, the Gewürztraminer wine had more haze at bottling (23.0 NTU), which resulted in greater differences in fining agent performance. Additionally, filtration was more critical than fining in removing turbidity in AC, WM, and WG fined Gewürztraminer.

Protein

Chardonnay fined with BN (8.41 mg/L) had the lowest concentration of protein (Table 5). In addition, SP (9.53 mg/L) and AC (9.36 mg/L) treatments significantly reduced protein when compared to the control (9.98 mg/L). The IS, WM, and WG treatments were greater than the control (10.15, 10.23, and 10.23 mg/L, respectively). These three agents are protein-based and may have contributed the increase in protein content.

In the Gewürztraminer, the AC (76.88 mg/L), IS (71.50 mg/L), and CTL (76.63 mg/L) treatments were highest in protein (Table 5). BN had the lowest protein concentration (17.92 mg/L) (Table 5), a 57.71 mg/L reduction. This finding agrees with Dambrouck et al. (2005), who reported that BN (10 to 50 g/hL) was most successful at removing protein from champagne. Both WM (63.07 mg/L) and WG (68.12 mg/L) treatments were low in protein, but neither were as low as BN. While the ability of

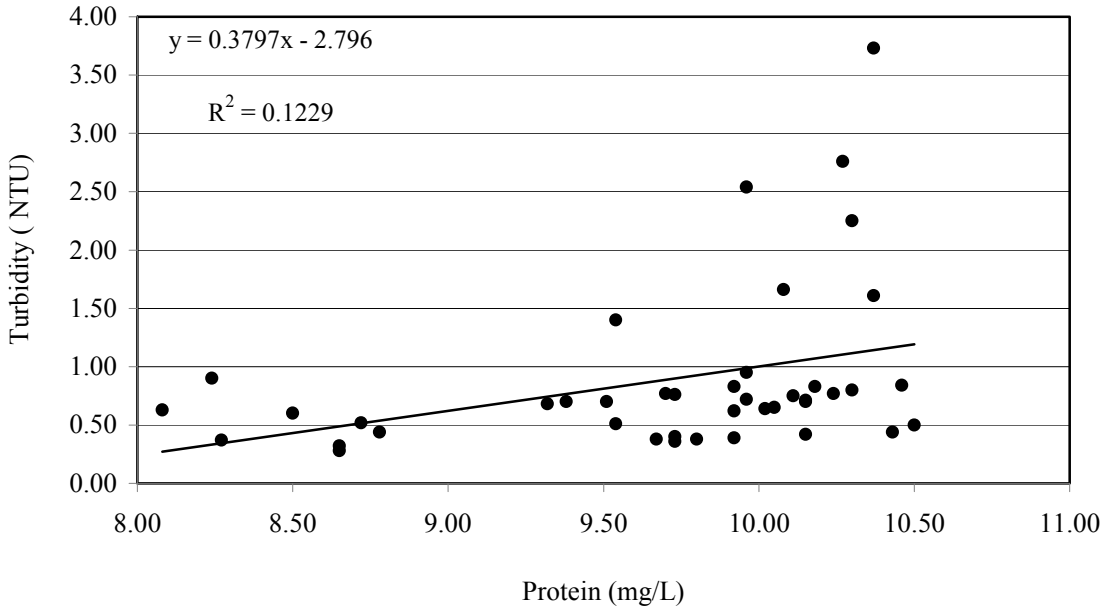
isinglass to reduce protein levels in Gewürztraminer wine has not been extensively studied, it appeared to be ineffective, as did activated charcoal. Additionally, protein levels in AC-treated wine were not unexpected as carbon does not target proteins.

Figure 6a compares turbidities after 3 months storage to protein concentration. In Chardonnay, the highest turbidity level was in the WG treatment (2.4 NTU), which had the highest protein concentration (10.23 mg/L). Little linearcorrelation existed between turbidity and protein concentration for the remaining treatments, suggesting that protein levels were too low to trigger differences in turbidity.

In the Gewürztraminer, the lowest turbidity levels were observed in samples of lowest protein concentration (Figure 6b). In general, turbidity levels increased with increasing protein concentration, although the highest turbidity levels were not observed in samples of highest protein concentration. Hsu and Heatherbell (1987) found that protein fractions which contribute to wine protein instability are of lower molecular weight and lower *pI*. It is thought that the agents in the present study did not efficiently remove haze-forming protein fractions from the wine. Alternatively, Dawes et al. (1994) reported that proteins interact with various wine components (*i.e.,* phenolics), which may reduce their binding affinity with fining agents.

The unfined Chardonnay and Gewürztraminer wines differed in protein (9.9 and 76.6 mg/L in the control treatments, respectively). Additionally, certain agents performed differently between varietals. For example, isinglass was capable of reducing protein in Chardonnay but not in Gewürztraminer. The majority of wine proteins originate in the grape (Mesquita et al., 2001); therefore, wine varietals vary in protein concentration. Furthermore, proteinaceous fining agents (*i.e.,* wheat gluten, fish glue,

a)



b)

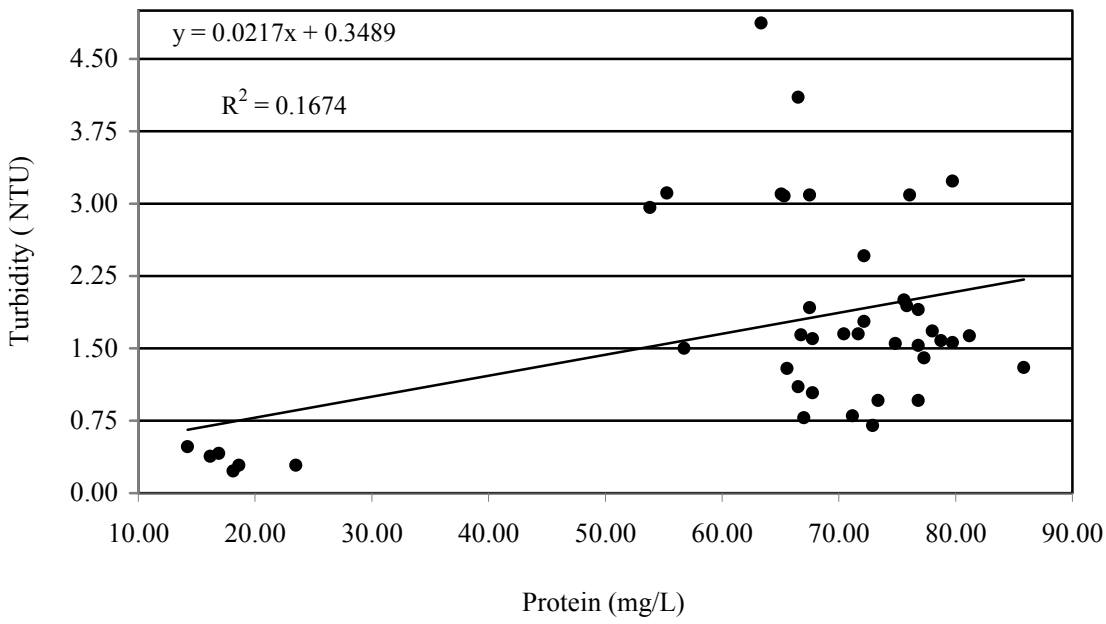


Figure 6. Scatter plot of protein concentration (mg/L) vs. mean turbidity (NTU) in a) Chardonnay and b) Gewürztraminer treated wines after 3 months storage in bottle following application of fining treatments.

and casein) vary greatly in proteic composition. Factors such as *pI* and molecular weight differ depending on the source of the agent and may influence how well a particular agent performs in wine clarification (Marchal et al., 2002a).

Conversely, both whole milk and wheat gluten significantly reduced protein in both varietals, though neither was as effective as bentonite. However, considering that neither agent significantly reduced turbidity, protein monitoring during bench trials may be a better indicator of fining performance than turbidity.

pH/Ethanol/Volatile acidity/Titratable acidity

In the Chardonnay, no significant differences in ethanol concentration, pH, TA, or VA were noted. In the Gewürztraminer, however, significant differences were observed between titratable and volatile acidities (Table 5). The IS treatment (0.840 g/L) was significantly higher in titratable acidity than any other treatment. The WM (0.757 mg/L) and CTL (0.756 mg/L) were similar in titratable acidity and were the lowest of all treatments. For both varietals, all treatments had titratable acidity levels typical to white wines (Boulton et al., 1996), suggesting that the fining agents studied did not affect titratable acidity.

Volatile acidity is a measurement of volatile acids in wine, mainly acetic acid, has a vinegar or sour aroma and flavor. Both the Chardonnay and Gewürztraminer treatments had acceptable concentrations of volatile acidity (Table 5), considering the aroma threshold for volatile acidity is around 0.07% (0.07 g/100 ml) (Amerine and Roessler, 1976) and the legal limit is 0.12% (1.2 g/L) (Boulton et al., 1996). For either wine variety, the control treatment had the highest level of volatile acidity, indicating that fining reduced the concentration of volatile acids in both Chardonnay and

Gewürztraminer. Significant differences were observed in Gewürztraminer between the CTL (0.049 g/100 ml), which had the highest percentage of volatile acidity, and AC (0.037 g/100 ml) treatment. Activated charcoal has an extremely high surface area and thus a high adsorption capacity for certain compounds, such as aromatic compounds (Moio et al., 2004; Ugarte et al., 2005). Therefore, activated carbon was capable of adsorbing volatile acids from Gewürztraminer, reducing volatile acidity.

Color

The 'L' component of the tristimulus color method indicates the lightness or darkness of a sample, with a value of 100 being white and a value of 0 being black (Main and Morris, 1991). The 'a' component is indicative of the degree of green and red in the sample. A positive 'a' value indicates red in the sample while a negative 'a' value indicates the sample is greener in color. The 'b' component represents the intensity of yellow and blue, with a positive 'b' value being more yellow and a negative 'b' value being more blue.

The 'a' and 'b' values did not significantly differ between treatments in the Chardonnay wine. However, the 'L' value of AC (99.95), BN (99.96), IS (99.96), and SP (99.95) were all significantly higher than the other three treatments (Table 5). The lowest mean 'L' value was the control (99.81). These results agree with those found by Lopéz et al. (2001), who found that sherry wine fined with activated charcoal reduced color intensity, and with Cosme et al. (2008), who observed that fining with casein, potassium caseinate, isinglass, egg albumin, and gelatin reduced the color intensity of white wine. Protein fining is often used to remove by adsorptive precipitation compounds that lead to changes in color *i.e.,* phenolic compounds.

The WG and WM treatments did not reduce the 'L' value in Chardonnay when compared to the other fining agents. Whole milk is known to improve the color of white wines; the fat content reduces the protein's adsorption capacity, preventing it from adsorbing polyphenols and other molecules responsible for color (Ribéreau-Gayon et al., 2006). All fining treatments produced wines significantly whiter than the control, indicating that fining had an effect on the color of Chardonnay independent of fining agent type.

There were no significant differences in color measurements between Gewürztraminer treatments (Table 5), indicating the fining did not affect the color of the Gewürztraminer wine after three months storage. Lopez et al. (2001) found differences in Sherry wine fined with different combinations of fining agents after one year in the bottle.

Volatile analysis

Individual peak areas were compared to internal standards to generate compound-to-internal standard ratios, which were used to construct standard curves (Table 6). The gas chromatogram was split in half according to retention times, and compounds in the upfield portion of the chromatogram (eluting in the first half of the chromatogram) were compared to 1-pentanol while compounds downfield (eluting in the second half of the chromatogram) were compared to 1-dodecanol.

Fourteen different volatile compounds were monitored in Chardonnay (Table 7). Several other compounds were detected in Chardonnay but were not quantified due to weak chromatographic signals. The analytes were selected based on preliminary volatile analysis of Chardonnay as well as those shown to be present in Chardonnay wine

Table 6. Range and linearity used for the quantification of aroma compounds. All calibration standards were prepared in 50% ethanol and extracted using solid phase microextraction. Standards were introduced to gas chromatograph using a 0.75-mm i.d. deactivated injection liner and compounds were separated by a DB-1 column. R.T. refers to the retention time (min) of the compound. R^2 represents the linearity of the calibration curve. Calibration curve ranges (mg/L) include the lowest and highest concentration for each compound.

	R.T. (min)	Volatile Compound	R^2	Calibration curve range (mg/L)	Regression equation[a]
A	4.685	Ethyl acetate	0.997	0.05 - 2.5	$y = 0.1656x + 0.0049$
B	4.970	2-methyl-1-propanol	0.992	0.5 - 10.0	$y = 0.0187\ x - 0.0108$
C	8.180	3-methyl-1-butanol	0.999	0.5 - 10.0	$y = 0.0587\ x + 0.0278$
D	8.294	2-methyl-1-butanol	0.999	0.5 - 10.0	$y = 0.5602\ x + 0.019$
E	11.274	Ethyl butanoate	0.996	0.05 - 2.5	$y = 2.7609\ x + 0.3338$
F	14.518	1-hexanol	0.999	0.5 - 10.0	$y = 0.491\ x - 0.482$
G	15.842	3-methyl-1-butanol acetate	0.997	0.05 - 2.5	$y = 7.1497\ x + 1.5818$
H	16.138	2-methyl-1-butanol acetate	0.997	0.05 - 2.5	$y = 4.8784\ x + 1.0498$
I	16.229	Ethyl hexanoate	0.990	0.05 - 2.5	$y = 17.96\ x + 4.5676$
J	22.516	Benzeneethanol	0.995	0.5 - 10.0	$y = 0.001\ x - 0.0003$
K	26.541	Linalool	0.966	0.001 - 1.0	$y = 5.5453\ x + 0.0159$
L	26.598	Ethyl octanoate	0.979	0.05 - 2.5	$y = 4.551\ x + 0.8888$
M	30.172	2-phenylethyl acetate	0.995	0.05 - 2.5	$y = 1.222\ x + 0.0156$
N	31.010	Nerol	0.978	0.01 - 1.0	$y = 0.96\ x$
O	31.501	L-α-terpinol	0.964	0.05 - 1.0	$y = 0.2213\ x + 0.0008$
P	32.222	Ethyl decanoate	0.948	0.05 - 2.5	$y = 4.1877x + 0.634$
Q	36.162	Ethyl dodecanoate	0.953	0.001 - 2.5	$y = 3.5872\ x$

[a] x denotes the concentration of volatile compound (mg/L) and y denotes the ratio of peak area of the volatile compound to its respective internal standard; compounds ethyl acetate through ethyl hexanoate were compared to 1-pentanol, compounds benzeneethanol through ethyl dodecanoate were compared to 1-dodecanol.

Table 7. Mean concentrations (mg/L) of volatile compounds in bottled Chardonnay wines 3 months after storage following application of fining treatment. Means with different letters within rows differ at $p \leq 0.05$ using Fisher's LSD
AC: activated charcoal; BN: bentonite; IS: isinglass; WM: whole milk; SP: Sparkalloid; WG: wheat gluten; CTL: control.

	Treatment						
Volatile Compound	AC	BN	IS	WM	SP	WG	CTL
Alcohols							
2-methyl-1-propanol	25.4	25.1	29.2	27.9	27.3	27.8	23.6
3-methyl-1-butanol	251	248	257	252	237	254	244
2-methyl-1-butanol	3.91	3.71	4.08	3.91	4.00	3.96	3.95
1-hexanol	0.753	0.811	0.796	0.808	0.872	0.730	0.741
Benzeneethanol	482	406	389	403	319	368	320
Ethyl esters							
Ethyl butanoate	0.348	0.378	0.382	0.385	0.292	0.355	0.376
Ethyl hexanoate	1.19	1.30	1.23	1.29	0.990	1.12	1.35
Ethyl octanoate	3.42	2.96	3.02	3.27	2.32	3.11	3.09
Ethyl decanoate	0.810	0.724	0.726	0.785	0.570	0.742	0.804
Ethyl dodecanoate	0.021^{bc}	0.017^{c}	0.018^{c}	0.026^{ab}	0.026^{ab}	0.023^{bc}	0.031^{a}
Acetate esters							
Ethyl acetate	39.6	42.5	41.6	43.1	35.6	40.4	38.5
3-methyl-1-butanol acetate	2.94	3.36	3.26	3.21	2.72	2.96	3.28
2-methyl-1-butanol acetate	< 0.05	< 0.05	0.05	< 0.05	< 0.05	< 0.05	0.05
2-phenylethyl acetate	0.178	0.157	0.154	0.167	0.123	0.156	0.156

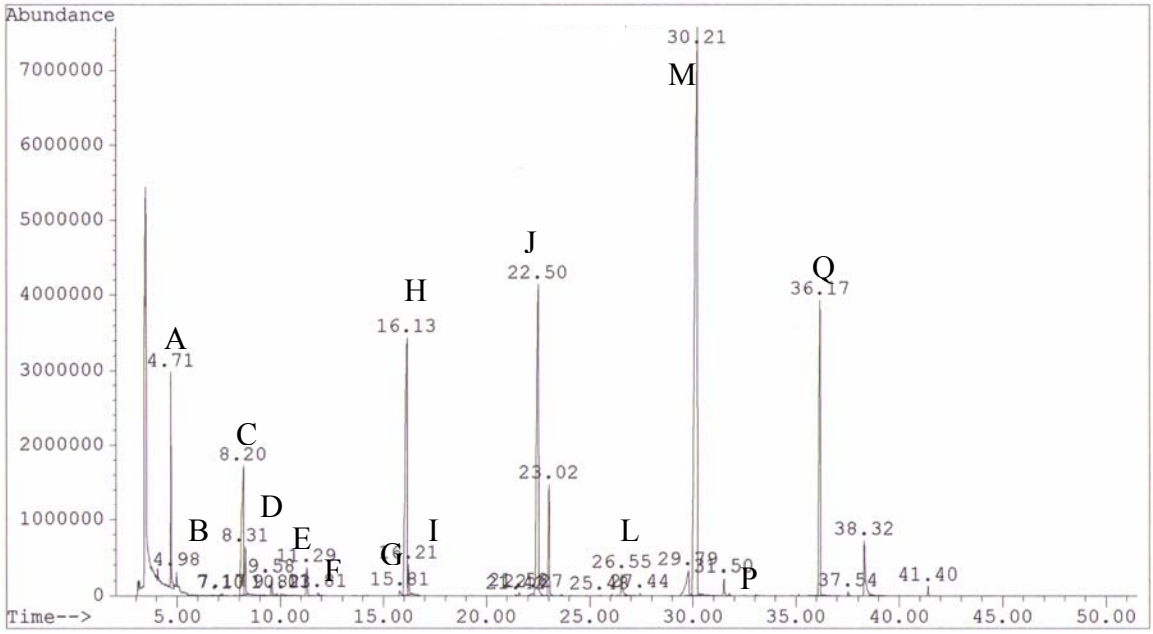
(Whiton and Zoecklein, 2000; Wondra and Berovič, 2001; Lee and Noble, 2003; Howard et al., 2005). A gas chromatogram of a Chardonnay control sample is displayed in Figure 7a.

In the Chardonnay, ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, and benzeneethanol had the greatest concentrations (Table 7). Lee and Noble (2003) showed ethyl acetate, 2-methyl-1-propanol, and benzeneethanol to be characteristic volatile compounds in California Chardonnay. Certain "oak" compounds characteristic to Chardonnay, such as oak lactones, eugenol, 4-vinyl guaiacol, and vanillin (Lee and Noble, 2003) were not found in the Chardonnay as the wines were not aged in oak.

The only compound to significantly differ between treatments was ethyl dodecanoate, which highest in the control (0.031 mg/L) and lowest in Chardonnay treated with BN (0.017 mg/L). The use of bentonite has been shown to have repercussions on the concentration of varietal aroma compounds in wines when used prior to alcoholic fermentation (Voilley et al., 1990; Puig-deu et al., 1996). Stable linkages between bentonite and aroma compounds of must and wine (hexanol, ethyl hexanoate, and isoamyl acetate) result in a loss in compound concentration as they settle from solution with bentonite (Voilley et al., 1990; Moio et al., 2004). Ethyl dodecanoate is a high molecular weight ethyl ester, formed during alcoholic fermentation, and is responsible for a leafy or soapy odor detected in wines (Acree and Arn, 2004; Bisson, 2005; Clary et al., 2006). For the most part, the volatile profile of the Chardonnay wine appeared to be unaffected by the fining agents applied.

Nineteen volatile compounds were monitored in the Gewürztraminer wine (Table 8), which were selected based on their significance to the varietal character of

a)



b)

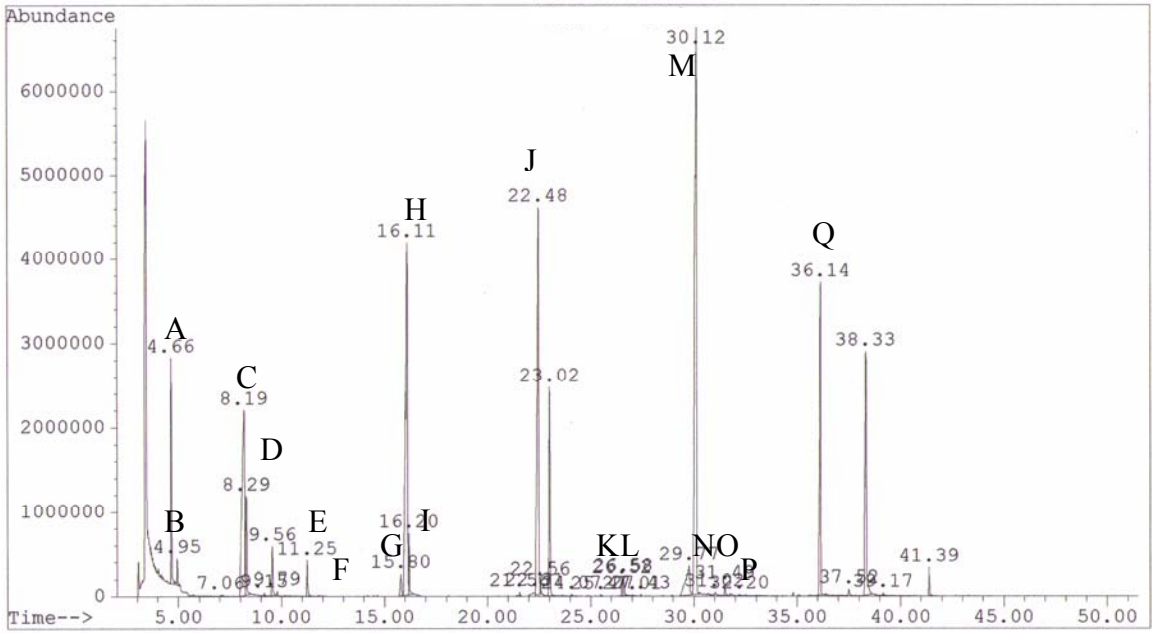


Figure 7. Gas chromatogram of a) Chardonnay and b) Gewürztraminer control treatments after 3 months storage in bottle following application of fining treatments. Chromatograms were obtained using SPME with a PDMS/DVB fiber on a DB-1 column. Letters reflect compounds described in Table 6.

Table 8. Mean concentrations (mg/L) of volatile compounds in bottled Gewürztraminer wines 3 months after storage following application of fining treatment. Means with different letters within rows differ at $p \leq 0.05$ using Fisher's LSD. AC: activated charcoal; BN: bentonite; IS: isinglass; WM: whole milk; SP: Sparkalloid; WG: wheat gluten; CTL: control.

	Treatment						
Volatile Compound	AC	BN	IS	WM	SP	WG	CTL
Alcohols							
2-methyl-1-propanol	32.9^{a}	30.7^{a}	21.1^{b}	23.3^{b}	20.9^{b}	22.6^{b}	27.7^{ab}
3-methyl-1-butanol	202^{a}	191^{b}	182^{bc}	184^{bc}	182^{c}	183^{bc}	190^{bc}
2-methyl-1-butanol	5.18^{a}	4.77^{bc}	4.60^{bcd}	4.58^{cd}	4.41^{d}	4.22^{d}	4.84^{b}
1-hexanol	1.81^{a}	1.72^{a}	1.76^{a}	1.71^{a}	1.73^{a}	1.54^{b}	1.71^{a}
Benzeneethanol	74.7^{ab}	36.2^{d}	85.2^{ab}	65.1^{bc}	51.5^{cd}	45.3^{d}	64.5^{c}
Ethyl esters							
Ethyl butanoate	0.232^{a}	0.221^{a}	0.238^{a}	0.225^{a}	0.226^{a}	0.183^{b}	0.243^{a}
Ethyl hexanoate	0.870	0.854	0.701	0.839	0.876	0.750	0.819
Ethyl octanoate	0.940	0.571	1.11	0.981	1.038	0.910	0.955
Ethyl decanoate	0.177^{bc}	0.041^{d}	0.158^{bc}	0.151^{bc}	0.282^{a}	0.145^{c}	0.195^{b}
Ethyl dodecanoate	0.025^{c}	0.008^{e}	0.006^{e}	0.017^{d}	0.031^{ab}	0.026^{bc}	0.028^{ab}
Acetate esters							
Ethyl acetate	27.4^{a}	25.1ab^{c}	24.0b^{cd}	22.8^{cd}	22.1^{d}	23.4^{cd}	26.3^{ab}
3-methyl-1-butanol acetate	2.55^{ab}	2.54^{ab}	2.78^{a}	2.48^{b}	2.55^{ab}	2.10^{c}	2.50^{b}
2-methyl-1-butanol acetate	0.111^{a}	0.102^{a}	0.120^{a}	0.087^{a}	0.105^{a}	0.043^{b}	0.110^{a}
2-phenylethyl acetate	0.028^{b}	0.010^{d}	0.026^{b}	0.027^{b}	0.036^{a}	0.018^{c}	0.025^{b}
Terpenes							
Linalool	0.014^{ab}	0.008^{c}	0.014^{ab}	0.014^{ab}	0.014^{ab}	0.013^{ab}	0.012^{b}
Nerol	0.014^{a}	0.009^{c}	0.011^{ab}	0.010^{bc}	0.010^{ab}	0.011^{ab}	0.007^{c}
L-α-terpinol	0.015^{a}	0.009^{b}	0.017^{a}	0.015^{ab}	0.018^{a}	0.015^{a}	0.013^{ab}

Gewürztraminer and their commonality in wine (Reynolds et al., 1989; Flores et al., 1991; Reynolds et al., 1996; Guth, 1997a; Girard et al., 2002). Among these compounds are linalool, nerol, and L-α-terpinol, terpenes responsible for the floral notes common to Gewürztraminer wines (Girard et al., 2002). A sample chromatogram of the Gewürztraminer control can be found in Figure 7b.

In Gewürztraminer, 2-methyl-1-propanol, 3-methyl-1-butanol, and benzeneethanol were among the compounds found of highest concentration. These fusel oils contribute desirably to a wine's bouquet at low concentrations, and their concentration increases with extended aging (Clarke and Bakker, 2004). Consequently, excessive levels (> 300 mg/L) can add negative characteristics to a wine *i.e.,* pungent odors.

Several differences were observed in the volatile composition of Gewürztraminer wine (Table 8). For example, all higher alcohols evaluated differed significantly between treatments. Overall, WG reduced the alcohol concentration more so than the other fining agents evaluated. For 2-methyl-1-propanol, the CTL (27.7 mg/L), AC (32.9 mg/L), and BN (30.7 mg/L) treatments had the highest concentration, whereas the IS, SP, and WG treatments had the lowest (21.1, 20.9, and 22.5 mg/L, respectively). Benzeneethanol is known to enhance the roasted, toasty aroma in wine (Peinado et al., 2004). The compound was greatly affected by the use of SP (51.5 mg/L), WG (45.3 mg/L), and most significantly BN (36.2 mg/L). The IS treatment had the highest concentration (85.2 mg/L). 1-hexanol was of lowest concentration in the WG treatment (1.54 mg/L).

For both 3-methyl-1-butanol and 2-methyl-1-butanol, the AC treatment (202 mg/L and 5.18 mg/L, respectively) was significantly higher than any other treatment.

Lopez et al. (2001) found no significant differences in the aroma profile between unfined sherry wine and those fined with activated charcoal. The difference between the former finding and that of the present study could be explained by the differences in concentration of activated charcoal applied. Lopez et al. (2001) used a concentration of 180 mg/L whereas 450 mg/L of activated concentration were used in this experiment. The adsorption capacity of activated charcoal depends on its dosage, and a high dosage could affect the components of the aroma (Lopez et al., 2001).

Esters are secondary aromas derived from alcoholic fermentation. Lower aliphatic ethyl esters (short chain) add fruity characteristics to wine whereas higher, longer-chained ethyl esters contribute soapy, oily, or waxy notes (Clarke and Bakker, 2004). Significant variations in ester concentrations were observed between treatments. Ethyl acetate, which contributes to the sweet, fruity aroma of Gewürztraminer wines (Guth, 1997a; 1997b), was significantly higher in the AC treatment (27.4 mg/L) when compared to the IS (24.0 mg/L), SP (22.1 mg/L), WM (22.8 mg/L), and WG treatments (23.4 mg/L). The compound was lowest in the SP treatment (22.1 mg/L). 3-methyl-1-butanol acetate (2.10 mg/L) and 2-methyl-1-butanol acetate (0.043 mg/L) were significantly decreased by wheat gluten. 2-phenylethyl acetate, which adds floral, fruity, and honey notes to wine, was drastically reduced by bentonite (0.010 mg/L). The highest concentration was noted in the SP treatment compound was enhanced by SP (0.036 mg/L), which was higher than the control (0.025 mg/L). The BN treatment had the lowest amount of ethyl decanoate (0.041 mg/L), which contributes fruity and grape aromas to wine. Along with the IS treatment (0.006 mg/L), BN produced the lowest

values of ethyl dodecanoate (0.008 mg/L). Both compounds were highest in the SP treatment, though ethyl dodecanoate was different from the control.

Overall, wheat gluten reduced the concentration of the lower aliphatic esters (*i.e.,* ethyl butanoate or ethyl hexanoate). The higher aliphatic ethyl esters (ethyl decanoate or ethyl dodecanoate) were most affected by bentonite, which resulted in the lowest concentrations of these compounds.

Slight differences in the three terpene compounds investigated were observed. One compound characteristic to Gewürztraminer is linalool (Amerine and Roessler, 1976). The lowest concentration of this compound was observed in the BN treated wine (0.008 mg/L). This finding agrees with Armada and Falqué (2007), who reported that bentonite (60 g/hL) significantly reduced the total concentration of of monoterpenes and C_{13}-norisoprenoids (13%), specifically linalool, geraniol, β-pinene, and limonene, in Albariño wine. Moio et al. (2004) observed that Falanghina wines fined with bentonite (80 g/hL) suffered significant losses in linalool and geraniol.

Several factors affecting the interaction between fining agents and free or bound volatile compounds have been suggested (Voilley et al., 1990; Moio et al., 2004; Armada and Falqué, 2007), including the chemical nature of the volatile compound (*i.e.,* polarity, functional groups, structure) or physical properties of the fining agent (*i.e.,* pI). For example, fining agents can directly adsorb aroma compounds. Strong linkages between bentonite and ethyl esters have been shown to reduce the concentration of these aroma compounds during bentonite fining (Voilley et al., 1990).

Additionally, volatile concentrations could indirectly decrease in concentration as a result of interactions between volatile compounds and macromolecules in the wine, the

latter eventually being adsorbed by the fining agent. Consequently, part or all of the volatile compound is eliminated along with the fining agent. For instance, Voilley et al. (1990) found interactions between aldehydes and amino acids in an aqueous medium. Should bentonite be added to the medium, charge-charge interactions between the negatively charged bentonite and positively charged proteins would form and precipitate out of suspension. As a result, aldehydes (*i.e.,* hexanal and hexenals, responsible for grassy flavors at low concentrations) are removed from the wine (Clarke and Bakker, 2004). Moreover, yeast have been shown to retain ethyl esters in their cell walls (Voilley et al., 1990). Settling aids, such as Sparkalloid, are used to facilitate the settling of yeast and other macromolecules from wine. Along with the yeast, ethyl esters are removed from the wine, indirectly affecting the wine's aroma and flavor.

Furthermore, fining agents derived from animals may contain a certain amount of fat *i.e.,*whole milk. On one hand, volatile compounds may be absorbed and removed from the wine with the fining agent. On the other hand, the fat content reduces the protein's adsorption capacity, preventing it from adsorbing compounds that may contribute to aroma and flavor (Ribéreau-Gayon et al., 2006).

Sensory analysis

Forced Choice Duo-Trio Test

Demographic data collected from the untrained panel is presented in the Appendix. Twenty of the 30 panelists were needed to distinguish between treatments to achieve significance ($p \leq 0.05$) (Meilgaard et al., 1999).

The untrained panel was unable to tell a significant difference fined and unfined Chardonnay ($p \leq 0.05$). However, at least 50% of panelists were able to distinguish between the control and BN, WM, or WG fined wines, hinting at significance (Table 9).

Similar results were found in Gewürztraminer wine (Table 9). While no significant differences were observed ($p \leq 0.05$), a large percentage of panelists were able to distinguish between the control and the IS, WM, and SP fined Gewürztraminer.

Whole milk was the only fining agent to bring about substantial differences (detected by at least 50% of the panel) for both varietals; the other agents affected only a single varietal. Therefore, it is suggested that wine varieties react differently to processing methods such as fining, as was demonstrated in the volatile analysis.

One explanation for the lack of differences between treatments in either varietal may be the temperature at which the wine was served during the panel. Ross and Weller (2008) found that white wines served at 18ºC had higher aroma intensities than those served at 10ºC and 12ºC, suggesting that white wines should be served warmer than chilled to achieve the wine's maximum aroma profile.

In addition, the power of the test was 0.5, which was low. Statistical power is the probability that a Type II error will not be made, or that the test will reject a false null hypothesis (Meilgaard et al., 1999). As the power increases, the chance of a Type II error occurring will decrease (1-β). The β-risk was 0.5, which was large. By decreasing the β-risk, the probability of missing a difference that truly exists will decrease, and the statistical power increases. Here, it is likely that differences were missed because the power was too low.

Table 9. Number of untrained panelists able to distinguish between fining treatment and an unfined Chardonnay and Gewürztraminer wines. A total of 30 panelists evaluated each treatment. Twenty of the 30 panelists were needed to distinguish between treatments to achieve significance ($p \leq 0.05$). AC: activated charcoal; BN: bentonite; IS: isinglass; WM: whole milk; SP: Sparkalloid; WG: wheat gluten; CTL: control.

Treatment	Number of correct responses by panelists	% panelists able to distinguish between treated and untreated wines	*Significance
Chardonnay			
AC	10	33.3	NS
BN	19	63.3	NS
IS	11	36.7	NS
WM	19	63.3	NS
SP	11	36.7	NS
WG	19	63.3	NS
Gewürztraminer			
AC	14	46.7	NS
BN	14	46.7	NS
IS	18	60.0	NS
WM	15	50.0	NS
SP	17	56.7	NS
WG	14	46.7	NS

*NS not significant at $p \leq 0.05$.

Trained Panel

Of the 13 attributes evaluated by a trained panel, only spicy aroma and floral/honey flavor significantly differed between Chardonnay treatments (Table 10). Isinglass significantly lowered both spicy aroma (1.5) and the floral/honey flavor (3.0), the lowest mean intensities for either attribute. WG produced the highest concentration of spicy aroma (2.3), whereas the highest concentration of the floral/honey flavor was observed in the WM treatment (4.4). The results for the floral/honey flavor should be interpreted cautiously as a significant panelist effect was observed (Table 11), indicating that individual panelists varied in how they rated that particular attribute. In fact, significant panelist effects were observed for many of the attributes in either wine variety. This could be attributed to differences in panelist sensitivity to certain attributes or to insufficient training with standards for particular attributes *i.e.*, those that had a panelist effect. It could also be a result of variability in use of the scale between panelists or individual anatomical and physical differences (Næs, 1991; Noble et al., 1991). These factors are difficult to eliminate, even through training. The short period of training could also be a factor, considering the panelists only received nine hours of training on the attributes evaluated. Interestingly, these findings are similar to Meunier (2003), who reported no noticeable differences in the appearance, aroma, and flavor of Chardonnay wine fined with various concentrations collagen, skim milk, or isinglass.

An odor threshold is the concentration in which an odor-active compound is perceived by an individual. Several factors contribute to the odor threshold of a compound. Aroma volatile compounds are more volatile at higher temperatures and therefore have a greater aroma impact with increased temperature. The presence of wine

Table 10. Mean intensity ratings for Chardonnay and Gewürztraminer treatments as determined by a trained panel (n = 12) using a 15-cm unstructured line scale. Replicate evaluations were made over two evaluation days. Means with different letters within rows differ at $p \leq 0.05$ using Fisher's LSD. AC: activated charcoal; BN: bentonite; IS: isinglass; WM: whole milk; SP: Sparkalloid; WG: wheat gluten; CTL: control.

	Treatment						
Attribute	AC	BN	IS	WM	SP	WG	CTL
Chardonnay							
Aroma							
Fruit/lychee/citrus	7.0	7.1	6.7	6.9	6.9	7.5	6.7
Floral/honey	5.0	5.3	4.7	4.6	5.4	5.0	4.7
Herbaceous/veggie	3.3	3.8	3.5	3.8	3.6	4.3	3.8
Spicy	1.9^{ab}	1.7^{ab}	1.5^{b}	2.0^{ab}	1.8^{ab}	2.3^{a}	1.7^{ab}
Chemical/hot	10.3	9.7	10.6	10.2	9.8	9.7	10.2
Taste							
Sweet	2.4	1.9	1.4	2.0	1.6	1.6	1.4
Sour	6.8	6.4	6.9	6.8	7.3	7.2	6.9
Bitter	10.6	10.8	10.1	10.2	10.9	10.1	10.6
Flavor							
Fruit/lychee/citrus	4.6	4.8	4.3	4.9	4.4	4.7	4.6
Floral/honey	4.2^{ab}	3.8^{ab}	3.0^{b}	4.4^{a}	3.7^{ab}	3.8^{ab}	4.1^{ab}
Herbaceous/veggie	4.1	4.4	4.1	4.7	4.5	4.6	4.6
Spicy	1.4	1.3	1.1	1.4	1.3	1.5	1.4
Chemical/hot	11.8	11.6	11.6	11.8	11.8	11.3	11.9
Gewürztraminer							
Aroma							
Fruit/lychee/citrus	10.7	11.0	11.4	11.1	10.9	10.6	11.3
Floral/honey	9.3	9.6	8.8	9.5	9.1	8.8	9.3
Herbaceous/veggie	5.3	5.0	5.8	4.8	4.3	4.9	4.4
Spicy	6.4	6.4	5.5	6.3	6.7	6.0	6.3
Chemical/hot	3.7	3.6	3.9	3.4	3.4	3.4	3.2
Taste							
Sweet	11.1	11.8	11.5	11.2	10.5	11.1	11.4
Sour	8.9	8.9	9.7	8.9	9.0	9.2	8.5
Bitter	3.1	2.8	3.3	2.9	3.4	3.2	3.1
Flavor							
Fruit/lychee/citrus	11.0	10.8	11.1	10.6	10.5	11.1	10.1
Floral/honey	7.3	7.4	7.7	7.6	7.7	7.2	7.7
Herbaceous/veggie	3.5	3.3	4.1	3.6	3.7	4.1	3.7
Spicy	6.0	5.8	5.7	5.0	5.8	5.5	6.0
Chemical/hot	4.8	4.5	4.8	4.2	4.6	4.8	4.9

Table 11. Calculated F-values and significant interactions of the trained panel for Chardonnay and Gewürztraminer wines.

	Interaction			
Attribute	Rep	Pan	Trt	Pan*Trt
Chardonnay				
Aroma				
Fruit/lychee/citrus	0.007	3.73**	0.536	1.12
Floral/honey	0.048	3.47**	0.560	1.04
Herbaceous/veggie	1.35	1.31	0.835	0.919
Spicy	0.018	1.64	2.26*	1.28
Chemical/hot	5.61*	1.63	0.779	1.25
Taste				
Sweet	2.50	10.3**	1.48	1.25
Sour	0.269	6.27**	0.434	0.739
Bitter	3.50	6.52**	0.765	1.73
Flavor				
Fruit/lychee/citrus	0.400	0.400**	0.455	0.735
Floral/honey	0.001	7.04**	2.31*	0.880
Herbaceous/veggie	1.82	4.34**	0.718	0.969
Spicy	0.006	4.98**	1.10	0.873
Chemical/hot	0.537	6.41**	0.378	0.920
Gewürztraminer				
Aroma				
Fruit/lychee/citrus	0.380	2.48*	0.524	1.35
Floral/honey	0.491	7.62**	0.770	0.877
Herbaceous/veggie	0.742	1.65	2.02	1.29
Spicy	0.011	3.55**	0.904	0.880
Chemical/hot	0.103	4.88**	0.350	0.773
Taste				
Sweet	0.049	1.26	1.30	0.676
Sour	0.866	8.09**	0.618	0.558
Bitter	1.06	0.919	0.450	0.786
Flavor				
Fruit/lychee/citrus	1.55	3.93**	0.665	1.02
Floral/honey	5.25*	6.61**	0.754	0.861
Herbaceous/veggie	0.304	4.78**	0.906	1.11
Spicy	5.43*	5.01**	1.76	1.42
Chemical/hot	0.120	3.24**	0.900	1.62*

Rep: Replicate; Pan: Panelist: Trt: Treatment

* Significance at $p \leq 0.05$.

** Significance at $p \leq 0.01$.

constituents, including ethanol and sugar, also determine the volatility of aroma compounds (Clarke and Bakker, 2004). The concentration of the compounds, along with the concentration of other volatile compounds in the same medium, will also impact the threshold of aroma compounds. Additionally, sensorial impressions of certain volatile compounds will change depending on the concentration of that compound in the air.

The odor thresholds of several important wine volatile compounds have been published (Guth, 1997a; 1997b; Peinado et al., 2004; Zea et al., 2001). While the odor-activity of volatile compounds was not investigated in this study, published values were used to draw comparisons between the volatile and sensory data.

For example, no significant differences were observed between the lower aliphatic ethyl esters (*i.e.,* ethyl butanoate, ethyl hexanoate, or ethyl octanoate) in Chardonnay, although several esters exceeded their odor threshold values and theoretically produced aromas capable of being detected by the panelists (Table 12). Esters generally contribute to the fruity aroma of wine. Therefore, the volatile results concur with the sensory results, as no differences were found in fruity aroma.

Surprisingly, no sensory differences were found in the Gewürztraminer wine (Table 10), considering the number of differences observed in the volatile composition. In addition, only three attributes did not have a significant panelist effect, suggesting that panelist variability may have prevented treatment differences from being observed *i.e.,* use of scale, differences in sensitivity to certain attributes, anatomical differences.

The degree of difference between compound concentration could be another explanation for the lack of sensory differences found between Gewürztraminer treatments. For example, ethyl acetate is responsible for sweet and fruity notes

Table 12. Odor thresholds (OT) concentrations (mg/L) in wine and aroma descriptors of volatile compounds found in Chardonnay and Gewürztraminer wines.

Volatile compounds	OT (mg/L)	Aroma descriptor
Alcohols		
2-methyl-1-propanol	40[¥]	acid, fruit, floral[*]
3-methyl-1-butanol	30[¥]	whiskey, pungent[§]
2-methyl-1-butanol	--	wine-like, fusel[§]
1-Hexanol	1.1[f]	herbaceous, woody, alcohol[§]
Benzeneethanol	900[f]	toasty, roasted[f]
Ethyl esters		
Ethyl butanoate	0.4[*]	strawberry, apple, banana, rum[f]
Ethyl hexanoate	0.08[f]	fruit, pineapple, green-apple, banana[§τ]
Ethyl octanoate	0.58[*]	fruit, floral, pear, sweet[f]
Ethyl decanoate	0.5[*]	brandy, oil, grape[§]
Ethyl dodecanoate	--	mango, leafy, soapy[§]
Acetate esters		
Ethyl acetate	12.0[*]	pineapple, balsamic, sweet[f]
3-methyl-1-butanol acetate	1.5[f]	banana, fruit, sweet[§]
2-methyl-1-butanol acetate	1.5[f]	fruit, banana, candy[§]
2-phenylethyl acetate	1.8[f]	rose, apple, sweet, honey[f]
Terpenes		
Linalool	0.015[f]	floral, citrus, sweet[§]
Nerol	0.5[*]	fresh, sweet, rose[f]
L-α-terpineol	1.0[*]	lily, sweet, cake, mint[ψ]

[¥] Guth (1997b)
[*] Zae et al. (2001)
[ψ] Lee and Noble (2003)
[§] Acree and Arn (2004)
[f] Peinado et al. (2004)
[τ] Sarrazin et al. (2007)

found in wine, such as pineapple (Acree and Arn, 2004). The concentration of ethyl acetate found in all Gewürztraminer treatments was about twice the concentration of the odor threshold, indicating that the compound quite possibly contributed to the fruity characteristic found by the panelist. However, differences between treatments did not differ by more than 5 mg/L, which may have been too insignificant to be detected by panelists. Additionally, 3-methyl-1-butanol acetate, known to contribute banana, fruity, and sweet characteristics to wine (Acree and Arn, 2004; Clary et al., 2006), has an odor threshold of roughly 1.5 mg/L (Peinado et al., 2004), which is lower than that found in the present study. Guth (1997a) found 3-methyl-1-butanol acetate to be one of the highest odor active compounds in Gewürztraminer. Nevertheless, the panel did not detect differences in fruity aroma/flavor and sweet taste, suggesting that chemical differences were not significant enough to induce perceivable differences.

Flores et al. (1991) noted that Gewürztraminer fined with bentonite (30 g/hL) was significantly lower in cooked vegetative aroma and higher in chemical aroma than an unfined control when evaluated by a trained panel. In fact, other than chemical aroma, all attributes were scored higher in the control than the fined wine. Panel training was similar between studies, as were the number of panelists. Conversely, significant panelist-by-replication interactions were observed in both studies, making it difficult to generalize the sensory implications of bentonite fining on Gewürztraminer wine.

Isinglass is said to enhance fruity aromas in wines. On one hand, this was demonstrated in the Gewürztraminer, which had the highest fruit aroma and flavor intensities in the isinglass treatment. On the other hand, the opposite was observed in the Chardonnay, where isinglass had the lowest fruit aroma and flavor intensities. These data

suggest that isinglass will behave differently depending on the wine variety, and that winemakers should consider fining trials prior to fining to establish the sensory impact of isinglass on the fruity notes of specific wines.

With regards to terpenes, nerol and L-α-terpinol concentrations were substantially lower than their odor thresholds (0.5 and 1 mg/L, respectively) (Acree and Arn, 2004) and their contribution to the floral aroma and/or flavor was most likely minimal. However, the odor threshold for linalool is approximately 0.015 mg/L (Peinado et al., 2004), similar to the concentrations found in the Gewürztraminer, and may have been responsible for the high floral intensities observed by the panel. Surprisingly, the panel was unable to distinguish between fining treatments, especially considering the low concentration of linalool in the BN treatment. This indicates that the chemical differences between treatments were not significant enough to generate sensory differences.

CONCLUSIONS

The chemical and sensory differences found between fining treatments in the Chardonnay and Gewürztraminer wines support the hypothesis of this study, and the null hypothesis was rejected. Activated charcoal, wheat gluten, and whole milk clarified Chardonnay but failed to diminish wine turbidity (< 10 NTU) in Gewürztraminer wine, whereas bentonite, isinglass, and Sparkalloid achieved wine clarity in both varietals. Bentonite was most effective at reducing protein in both varietals. Neither wheat gluten nor whole milk were as effective in reducing turbidity as bentonite. Isinglass, bentonite, Sparkalloid, and activated charcoal all produced whiter Chardonnay wines when

compared to an unfined control; conversely, Gewürztraminer color was unaffected by any fining agent.

Fining had more impact on the volatile profile of Gewürztraminer wine than Chardonnay, and few differences in the sensory properties were observed in either wine. Bentonite significantly reduced linalool levels in Gewürztraminer, and while sensory differences were not significant, winemakers should take precautions when bentonite fining Gewürztraminer. Differences in spicy aroma and floral/honey flavor of Chardonnay fined with wheat gluten and isinglass, respectively, should be interpreted with caution as they were accompanied by significant panelist interactions. Based on the differences observed between varietals, bench trials are strongly encouraged when determining the type and concentration of fining agent. Alone with turbidity, protein content and sensory impact should be assessed throughout fining trials to ensure proper agent doses when fining.

SUGGESTIONS FOR FUTURE STUDIES

First, particular fining concentrations applied in this present study did not clarify Gewürztraminer. It was thought that the volume of wine used in bench trials was not large enough to mimic the fining experiment, or allowed for better fining agent distribution when compared to fining experiment. Larger-scale fining trials may produce more reliable results, and may have a greater impact on the sensory properties. In addition, the wines should be revisited after a longer period of storage to determine if chemical and/or sensory changes occur over time. Gas Chromatography/Olfactometry may provide interesting results with regards to the odor activity of the wines as well, and may help explain the lack of differences found between Gewürztraminer treatments by the trained panel.

Secondly, it would be of interest to investigate the impact of different fining agents of similar functionality on the sensory properties of wine. For example, both gelatin and isinglass are used to reduce phenolic levels in red wine. However, their effectiveness can differ, depending on the wine medium and phenolic compounds present. Therefore, coupling a fining agent's efficacy at phenolic removal with its overall sensorial effect (*i.e.*, astringency and color) could aid winemakers in selecting the most suitable agent from a particular class of fining agents to achieve their winemaking goals. Additionally, the implications of fining on both the chemical and sensory properties at extremely high doses would demonstrate the risk of loss in quality from over-fining.

Moreover, this study demonstrated that varietals react differently to fining. The volatile composition of Riesling, known for its fruity and floral bouquet, may respond

similarly to fining as did Gewürztraminer. Riesling's popularity to the Washington State wine industry makes it a likely candidate for future processing research, such as fining.

The application of fining to other areas of enology should be explored. The ability of fining agents to reduce residual pesticide concentrations in wine is of high interest. Examining the potential for certain materials to remove or reduce unwanted sensory properties could help the wine industry battle issues such as spoilage by acetic acid bacteria or *Brettanomyces*. Reductions in excessive aromas, such as herbaceous and grassy, could help winemakers correct sensory problems that originated in the vineyard but are difficult to alter during vinification.

Lastly, new fining agents should be sought after. As processing and labeling regulations tighten by the EU, and possibly in the US, new products must be available to wineries to facilitate processing. Plant-derived agents should be explored with regards to wine clarification and stabilization, and eventually sensory impact.

LITERATURE CITED

Achaerandio, I., V. Pachova, C. Güell, and F. López. Protein adsorption by bentonite in a white wine model solution: effect of protein molecular weight and ethanol concentration. Am. J. Enol. Vitic. 52(2): 122-126 (2001).

Acree, T., and H. Arn. Flavornet and human odor space. www.flavornet.org. 2004.

Amerine, M., and E. Roessler. Wines: Their Sensory Evaluation. W.H. Freeman and Company, San Francisco (1976).

Ancín C., B. Ayestarán, M. Corroza, J. Garrido, and A. González. Influence of prefermentation clarification on the higher alcohol contents of wines. Food Chem. 55(3):241-249 (1996).

Armada, L., and E. Falqué. Repercussion of the clarification treatments agents before the alcoholic fermentation on volatile composition of white wines. Eur. Food Res. Technol. 225:553-558 (2007).

Bakalinsky, A., and R. Boulton. The study of immobilized acid protease for the treatment of wine proteins. Am. J. Enol. Vitic. 36(1):23-29 (1985).

Bayly, F., and H.W. Berg. Grape and wine proteins of white wine varietals. Am. J. Enol. Vitic. 18(1):18-32 (1967).

Bird, D. Understanding Wine Technology: The Science of Wine Explained. DBQA Publishing, Great Britain. 2000.

Bisson, L. Metabolism of *Dekkera/Brettanomyces* isolated from wine. Am. Vine. Found. Project #295 (2005).

Blade, W., and R. Boulton. Adsorption of protein by bentonite in a model wine solution. Am. J. Enol. Vitic. 39:193-199 (1988).

Bohlscheid, J.C., X.D. Wang, D.S. Mattinson, and C.G. Edwards. Comparison of HS-SPME and XAD-2 methods to extract volatile compounds produced by *Saccharomyces* during wine fermentations. J. Food Qual. 29:1-15 (2006).

Bonerz, D.P.M., D.G. Bloomfiels, S.I. Dykes, G.L. Creasy, and M.S. Pour Nikfardjam. A new gentle fining agent for Pinot Noir. Aust. N.Z. Grape. Wine. 54(3/4):86-95 (2004).

Bonilla, F., M. Mayen, J. Merida, and M. Medina. Yeasts used as fining treatment to correct browning in white wines. J. Agric. Food Chem. 49:1928-1933 (2001).

Boulton, R., Singleton, V., Bisson, L., and R. Kunkee. Principles and Practices of Winemaking. CBS Publishers and Distributors, New Delhi. 1996.

Bradford, M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the priciple of protein-dye binding. Anal. Biochem. 72:248-254 (1976).

Brady, N., and R. Weil. The Nature and Properties of Soils. 13th ed. Prentice Hall, Upper Saddle River, N.J. (2002).

Buechsenstein, J.W., and C.S. Ough. SO_2 determination by aeration-oxidation: a comparison with Ripper. Am. J. Enol. Vitic. 29(3):161-164 (1978).

Cabello-Pasini, A., N. Victoria-Cota, V. Macias-Carranza, E. Hernandez-Garibay, and R. Muñiz-Salazar. Clarification of wines using polysaccharides extracted from seaweeds. Am. J. Enol. Vitic. 56(1):52-59 (2005).

Cabras, P., V. Garau, F. Pirisi, M. Cubeddu, F. Cabitza, and F. Spanedda. Fate of some insecticides from vine to wine. J. Agric. Food Chem. 43:2613-2615 (1995).

Calderon P., J. Van Buren, and W. Robinson. Factors influencing the formation of precipitates and hazes by gelatin and condensed hydrolyzable tannins. J. Agric. Food Chem. 16(3):479-482 (1968).

Caputi, A., T. Brown, and M. Ueda. Residual polyvinylpyrrolidone in wine. Am. J. Enol. Vitic. 20:152-154 (1969).

Castellari, M., A. Versari, A. Fabiani, G. Parpinello, and S. Galassi. Removal of ochratoxin A in red wines by means of adsorption treatments with commercial fining agents. J. Agric. Food Chem. 49:3917-3921 (2001).

Castillo-Sánchez, J.J., J.C. Mejuto, J. Garrido, and S. García-Falcón. Influence of wine-making protocol and fining agents on the evolution of the anthocyanin content, colour, and general organoleptic quality of Vinhão wines. Food Chem. 97:130-136 (2006).

Clarke, R.J., and J. Bakker. Wine flavour chemistry. Blackwell Publishing, Oxford, UK. 2004.

Clary, C., A. Gamache, M. Cliff, J. Fellman, and C. Edwards. Flavor and aroma attributes of Riesling wines produced by freeze concentration and microwave vacuum dehydration. J. Food Proc. Pres. 30:393-406 (2006).

Cosme, F., J.M. Ricardo-da-Silva, and O. Laureano. Protein fining agents: characterization and red wine fining assays. Ital. J. Food Sci. 1(19):39-56 (2007).

Cosme, F., J.M. Ricardo-da-Silva, and O. Laureano. Interactions between protein fining agents and proanthocyanidins in white wine. Food Chem. 106:536-544 (2008).

Cruess W. 1963. A study of several problems of clarification of wine with casein. Am. J. Enol. Vitic. 14(3):137-138 (1963).

Dambrouck, T., R. Marchal, C. Cilindre, M. Parmentier, and P. Jeandet. Determination of grape invertase content (using PTA-ELISA) following various fining treatments versus changes in the total protein content of wine. Relationships with wine foamability. J. Agric. Food Chem. 53(22):8782-8789 (2005).

Dawes, H., S. Boyes, J. Keene, and D. Heatherbell. Protein instability of wines: influence of protein isoelectric point. Am. J. Enol. Vitic. 45(3):319-326 (1994).

Doner, L., G. Bécard, and P. Irwin. Binding of flavonoids by polyvinylpyrrolidone. J. Agric. Food Chem. 41:753-757 (1993).

Dupin, I., B. McKinnon, C. Ryan, M. Boulay, A. Markides, G. Jones, J. Williams, and E. Waters. *Saccharomyces cerevisiae* mannoproteins that protect wine from protein haze: their release during fermentation and lees contact and a proposal for their mechanism of action. J. Agric. Food Chem. 48:3098-3105 (2000).

Fernandes, A., N. Ratola, A. Cerdeira, A. Alves, and A. Venâacio. Changes in Ochratoxin A concentration during winemaking. Am. J. Enol. Vitic. 58(1):92-96 (2007).

Fennema, O. Food Chemistry, 3rd ed. Marcel Dekker, Inc. New York. 1996.

Flores, J.H., D.A. Heatherbell, L.A. Henderson, and M.R. McDaniel. Ultrafiltration of wine: effect of ultrafiltration on the aroma and flavor characteristics of White Riesling and Gewürztraminer wines. Am. J. Enol. Vitic. 42(2):91-96 (1991).

Fuller, W.L., and H.W. Berg. Treatment of white wine with Nylon 66. Am. J. Enol. Vitic. 16:212-218 (1965).

Girard, B., L. Fukumoto, G. Mazza, P. Delaquis, and B. Ewert. Volatile terpene constituents in maturing Gewürztraminer grapes from British Columbia. Am. J. Enol. Vitic. 53(2):99-109 (2002).

Girotti, S., F. Fini, L. Bolelli, L. Savini, E. Sartini, and G. Arfelli. Chemiluminescent determination of total antioxidant capacity during winemaking. Lumine. 21:233-238 (2006).

Gómez, M., R. Brandt, N. Jakubowski, and J. Andersson. Changes of the metal composition in German white wines through the winemaking process. A study of 63 elements by inductively coupled plasma-mass spectrometry. J. Agric. Food Chem. 52:2953-2961 (2004).

Gómez-Plaza, E., R. Gil-Muñoz, J.M. López-Roca, M. L. de la Hera-Orts, and A. Martínez-Cultíllas. Effect of the addition of bentonite and polyvinylpyrrolidone on the colour and long-term stability of red wines. J. Wine Res. 11(3):223-231 (2000).

Guitart, A., P. Herhández Orte, and J. Cacho. Effect of different clarification treatments on the amino acid content of Chardonnay musts and wines. Am. J. Enol Vitic. 49(4):389-396 (1998).

Guth, H. Identification of character impact odorants of different white wine varieties. J. Agric. Food Chem. 45:3022-3026 (1997a).

Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. J. Agric. Food Chem. 45:3027-3032 (1997b).

Harbertson J. Clarification: fining to reduce astringency. WSU Wine and Grape Res Ext News. 15(3):8-10 (2005).

Hickman, D., T. Sims, C. Miles, A. Bailey, M. Mari, and M. Koopmans. Isinglass/collagen: denaturation and functionality. J. Biotech. 79:245-257 (2000).

Howard, K., J. Mike, and R. Riesen. Validation of a solid-phase microextraction method for headspace analysis of wine aroma components. Am. J. Enol. Vitic. 56(1):37-45 (2005).

Hsu, J., and D. Heatherball. Heat-unstable proteins in wine. I. Characterization and removal of bentonite fining and heat treatment. Am. J. Enol. Vitic. 38(1):11-16 (1987).

Jackson, R. Wine Science. 2nd ed. Academic Press, San Diego (2000).

Karagiannis, S., and P. Lanaridis. Insoluble grape material present in must affects the overall fermentation aroma of dry white wines made from three grape cultivars cultivated in Greece. Sens. Nutri. Qual. Food. 67(1):369-374 (2002).

Kotseridis, Y., and R. Baumes. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. J. Agric. Food Chem. 48:400-406 (2000)a.

Kotseridis, Y., A. Razungles, A. Bertrand, and R. Baumes. Differentiation of the aromas of Merlot and Cabernet Sauvignon wines using sensory and instrumental analysis. J. Agric. Food Chem. 48:5383-5388 (2000)b.

Lee, S.J., and A. Noble. Characterization of odor-active compounds in Californian Chardonnay wines using GC-olfactometry and GC-mass spectrometry. J. Agric. Food Chem. 51:8036-8044 (2003).

López, S., R. Castro, E. García, J. Pazo, and C. Barroso. The use of activated charcoal in combination with other fining agents and its influence on the organoleptic properties of sherry wine. Eur. Food Res. Technol. 212:671-675 (2001).

Main, G.L., and J.R. Morris. Color of Riesling and Vidal wines as affected by bentonite, Cufex®, and sulfur dioxide juice treatments. Am. J. Enol. Vitic. 42(4):354-357 (1991).

Main, G.L., and J.R. Morris. Color of Seyval blanc juice and wine as affected by juice fining and bentonite fining during fermentation. Am. J. Enol. Vitic. 45(4):417-422 (1994).

Marais, J., and A.C. Houtman. Quantitative gas chromatographic determination of specific esters and higher alcohols in wine using freon extraction. Am. J. Enol. Vitic. 30(3):250-252 (1979).

Marchal, R., L. Marchal-Delahaut, A. Lallement, and P. Jeandet. Wheat gluten used as a clarifying agent of red wines. J. Agric. Food Chem. 50:177-184 (2002a).

Marchal, R., L. Marchal-Delahaut, F. Michels, M. Parmentier, A. Lallement, and P. Jeandet. Use of wheat gluten as clarifying agent of musts and white wines. Am. J. Enol. Vitic. 53(4):308-314 (2002b).

Maury, C., P. Sarni-Manchado, L. Sandrine, V. Cheynier, and M. Moutounet. Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. Am. J. Enol. Vitic. 52(2):140-145 (2001).

Maury, C., P. Sarni-Manchado, L. Sandrine, V. Cheynier, and M. Moutounet. Influence of fining with plant proteins on proanthocyanidin composition of red wines. Am. J. Enol. Vitic. 54(2):105-111 (2003).

Meilgaard, M., G.V. Civille, and B.T. Carr. Sensory Evaluation Techniques. CRC Press, Boca Raton (1999).

Mesquita, P., M. Piçarra-Pereira, S. Monteiro, V. Loureiro, A. Teixeira, and R. Ferreira. Effect of wine composition on protein stability. Am. J. Enol. Vitic. 54(2): 324-330 (2001).

Meunier, M. Collagen as a fining agent for whites and red wines. Aust. N.Z. Grape. Wine. 9:120-122 (2003).

Miller G., Amon J., Gilson R., and R. Simpson. Loss of wine aroma attributable to protein stabilization with bentonite or ultrafiltration tangentielle. Aust grapegrower winemaker. 256:49-50 (1985).

Moio, L., M. Ugliano, A. Gamtui, A. Genovese, and P. Piombino. Influence of clarification treatment on concentrations of selected free varietal aroma compounds and glycoconjugates in Falanghina must and wine. Am. J. Enol. Vitic. 55(1):7-12 (2004).

Morris, J.R., and G.L. Main. Fining agents for wine. Proc. 14th NM Conf. (1995).

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

Murphey, J., J. Powers, and S. Spayd. Estimation of soluble protein conetrations of white wines using Coomassie brilliant blue G-250. Am. J. Enol. Vitic. 40(3):189-193 (1989).

Naish, M., M. Clifford, and G. Birch. Effect of gelatin (a model for salivary PRP) on the sensory astringency of 5-*o*-caffeoylquinic acid and tannic acid. Ann. N.Y. Acad. Sci. 855:823-827 (1998).

Nordestgaard, S., Y. Chuan, B. O'Neill, E. Waters, L. Deans, P. Policki, and C. Colby. In-line dosing of white wine for bentonite fining with centrifugal clarification. Am. J. Enol. Vitic. 58(2): 283-285 (2007).

Ough, C.S. Gelatin and polyvinylpyrrolidone compared for fining red wines. Am. J. Enol. Vitic. 11:170-173 (1960).

O'Neal, R., L. Meis, and W.V. Cruess. Observations on the fining of wines with casein. Am. J. Enol.Vitic. 1:69-72 (1950).

Panagiotakopoulou, V., and J.R. Morris. Chemical additives to reduce browning in white wines. Am. J. Enol. Vitic. 42(3):255-260 (1991).

Peinado, R.A., J. Moreno, M. Medina, and J.C. Mauricio. Changes in volatile compounds and aromatic series in sherry wine with high gluconic acid levels subjected to aging by submerged flor yeast cultures. Biotechnol. Lett. 26:757-762 (2004).

Pérez-Caballero, V., F. Ayala, J. Echávarri, and A. Negueruela. Proposal for a new standard OIV method for determination of chromatic characteristics of wines. Am. J. Enol. Vitic. 54(1):59-62 (2003).

Pocock, K.F., and B.C. Rankine. Heat test for detecting protein instability in wine. Aust. Wine Brew. Spirit Rev. 91(5):42-43.

Pozo-Bayón, M.A., E. Pueyo, P.J. Martín-Álvarez, and M.C. Polo. Polydimethylsiloxane solid-phase microextraction-gas chromatography method for the analysis of volatile compounds in wines. Its application to the characterization of varietal wines. J. Chrom. A. 922:267-275 (2001).

Pozo-Bayón, M.A., E. Pueyo, P.J. Martín-Álvarez, A.J. Martínez-Rodríguez, and M.C. Polo. Influence of yeast strain, bentonite addition, and aging time on volatile compounds of sparkling wines. Am. J. Enol. Vitic. 54(4):273-278 (2003).

Puig-Deu, M., E. López-Tamames, S. Buxaderas, and M.C. Torre-Boronat. Influence of must racking and fining procedures on the composition of white wine. Vitis. 35(3):141-145 (1996).
Rankine, B.C. Use of isinglass to fine wines. Aust. NA. Grape. Wine. 249:16 (1984).

Razmkhab, S., A. Lopez-Toledano, J. Ortega, M. Mayen, J. Merida, and M. Medina. Adsorption of phenolic compounds and browning products in white wines by yeasts and their cell walls. J. Agric. Food Chem. 50:7432-7437 (2002).

Reynolds, A.G., and D.A. Wardle. Influence of fruit microclimate on monoterpene levels of Gewürztraminer. Am. J. Enol. Vitic. 40(3):149-154 (1989).

Reynolds, A.G., D.A. Wardle, and M. Dever. Vine performance, fruit composition, and wine sensory attributes of Gewürztraminer in response to vineyard location and canopy manipulation. Am. J. Enol. Vitic. 47(1):77-92 (1996).

Ribéreau-Gayon, P., Glories, Y., Maujean, A., and D. Dubourdieu. Handbook of Enology. II: The Chemistry of Wine Stabilization and Treatments, 2nd ed. John Wiley and Sons, Ltd, Bordeaux, France (2006).

Rolland, J., E. Apostolou, K. Deckert, M. de Leon, J. Douglass, I. Glaspole, M. Bailey, C. Stockely, and R. O'Hehir. Potential food allergens in wine: double-blind, placebo-controlled trial and basophil activation analysis. Nutrition. 22:882-888 (2006).

Ross, C.F**.,** and K. Weller. Effect of serving temperature on sensory attributes of red and white wine. J. Sens. Stud. (In press., 2008).

Rossi Jr., J., and V. Singleton. Flavor effects and adsorptive properties of purified fractions of grape-seed phenols. Am. J. Enol. Vitic. 17:240-246 (1966).

Salazar, F., I. Achaerandio, M. Labbé, C. Güell, and F. López. Comparative study of protein stabilization in white wine using zirconia and bentonite: physiochemical and wine sensory analysis. J. Agric. Food Chem. 54:9955-9958 (2006).

Sarmento, M., J. Oliveira, and R. Boutlon. Selection of low swelling materials for protein adsorption from white wines. Int. J. Food Sci. Tech. 35:41-47 (2000).

Sarni-Manchado, P., A. Deleris, S. Avallone, V. Cheynier, M. Moutounet. Analysis and characterization of wine condensed tannins precipitated by proteins used as fining agents in enology. Am. J. Enol. Vitic. 50(1):81-86 (1999).

Sarrazin, E., Dubourdieu, D., and P. Darriet. Characterization of key-aroma compounds of botrytized wines, influence of grape botrytization. Food Chem. 103(2):536-545 (2007).

Saywell, L.G. Clarification of wine. Ind. Eng. Chem. 26(9):981-982 (1934).

Servili, M., G. de Stefano, P. Piacquadio, and V. Sciancalepore. A novel method for removing phenols from grape must. Am. J. Enol. Vitic. 51(4):357-361 (2000).

Sims, C., J. Eastridge, and R. Bates. Changes in phenols, color, and sensory characteristics of Muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. Am. J. Enol. Vitic. 46(2):155-158 (1995).

Singleton V and Kramling T. 1976. Browning of white wines and an accelerated test for browning capacity. Am J Enol Vitic. 27(4):157-160 (1976).

Soleas, G., and D. Goldberg. Potential role of clarifying agents in the removal of pesticide residues during wine production and their effects upon wine quality. J. Wine Res. 11(1):19-34 (2000).

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

Spagna, G., R. Barbagallo, and P. Pifferi. Fining treatments of white wines by means of polymeric adjuvants for their stabilization against browning. J. Agric. Food Chem. 48:4619-4627 (2000).

Steffen, A., and J. Pawliszyn. Analysis of flavor volatiles using headspace solid-phase microextraction. J. Agric. Food Chem. 44:2187-2193 (1996).

Ugarte, P., E. Agosin, E. Bordeu, and J.I. Villalobos. Reduction of 4-ethylphenol and 4-ethylguaiacol concentration in red wines using reverse osmosis and adsorption. Am. J. Enol. Vitic. 56:30-36 (2005).

Vanrell, G., R. Canals, M. Esteruelas, F. Fort, J. Canals, and F. Zamora. Influence of the use of bentonite as a riddling agent on foam quality and protein fraction of sparkling wine (Cava). Food Chem. 104:18-155 (2007).

Vincenzi, S., M. Polesani, and A. Curioni. Removal of specific protein components by chitin enhances protein stability in a white wine. Am. J. Enol. Vitic. 56(3):246-254 (2005).

Voilley, A., C. Lamer, P. Dubois, and M. Feuillat. Influence of macromolecules and treatments on the behavior of aroma compounds in a model wine. J. Agric. Food Chem. 38:258-251 (1990).

Vos P, and R. Gray. The origin and control of hydrogen sulfide during fermentation of grape must. Am. J. Enol. Vitic. 30:187-197 (1979).

Weber, P., H. Steinhart, and A. Paschke. Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA. J. Agric. Food Chem. 55:3127-3133 (2007).

Weiss, K., and L. Bisson. Effect of bentonite treatment of grape juice on yeast fermentation. Am. J. Enol. Vitic. 53:28-36 (2002).
Whiton, R.S., and B.W. Zoecklein. Optimization of headspace solid-phase microextraction for analysis of wine aroma compounds. Am. J. Enol. Vitic. 51(4):379-382 (2000).

Wondra, M., and M. Berovič. Analyses of aroma components of Chardonnay wine fermented by different yeast strains. Food Technol. Biotechnol. 39(2):141-148 (2001).
www.codexalimentarius.net (2008).

Zea, L., L. Moyano, J. Moreno, B. Cortes, and M. Medina. Descrimination of the aroma fraction of Sherry wines obtained by oxidative and biological ageing. Food Chem. 75:79-84 (2001).

Zhang, Z., and J. Pawliszyn. Headspace solid-phase microextraction. Anal. Chem. 65:1843-1852 (1993),

Zoecklein, B. Bentonite fining of juice and wine. Virginia Cooperative Extension Publication No. 463-014 (1988).

Zoecklein, B., Fugelsang, K., Gump, B., and F. Nury. Wine analysis and production. CBS Publishers and Distributors, New Delhi. 1995.

Zoecklein, B. Enology notes #11: Seasonal variation influencing protein stability. http://www.fst.vt.edu/extenstion/enology/EN/contentextenologynotes11.html accessed 1/18/2006. 2000.

Zoecklein, B. Enology notes #43: Fining agents, isinglass and structural balance, and summary of fining agent use.
http://www.fst.vt.edu/extension/enology/EN/contentextenologynotes43.html. 2002.

APPENDIX

Demographic data obtained from Forced Choice Duo-Trio Test. Three treatments of each varietal were evaluated per day in comparison to the control (unfined) treatment of the same varietal. A total of 30 panelists participated each day.

Parameter	Evaluation Day			
	Chardonnay Day 1	Chardonnay Day 2	Gewürztraminer Day 1	Gewürztraminer Day 2
Age (years)				
21-30	14	16	10	16
31-40	2	5	8	6
41-50	6	4	5	3
51-60	8	5	6	4
61-70	0	0	1	1
70+	0	0	0	0
Gender				
Female	21	19	16	18
Male	9	11	14	12
Wine Consumption (days/month)				
1-5	13	17	18	21
6-10	7	5	4	2
11-20	7	6	5	4
21-30	2	1	2	1
31-40	0	0	0	1
40+	1	1	1	1