THE CHEMICAL AND SENSORIAL EFFECTS OF PLANT-BASED FINING AGENTS ON WASHINGTON STATE RIESLING AND GEWÜRZTRAMINER WINES

By

LAURA ELLEN HILL

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of LAURA ELLEN HILL find it satisfactory and recommend that it be accepted.

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Carolyn F. Ross, Ph.D., Chair

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Charles G. Edwards, Ph.D.

_____

James Harbertson, Ph.D.

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Abstract

by Laura Ellen Hill
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Chair: Carolyn F. Ross

The objective of this study was to determine the chemical and sensory impact of plant-based fining agents on WA State Riesling and Gewürztraminer wines. Riesling and Gewürztraminer wines were made in from WA State (Paterson, WA) grapes. Following alcoholic fermentation, five fining agents were applied to the wines: bentonite (Gewürztraminer: 150 mg/100 mL; Riesling: 100 mg/100 mL), soy milk powder (Gewürztraminer: 2.16 mg/100 mL; Riesling: 3.24 mg/100 mL), Plantis Fine (Gewürztraminer: 15 mg/100 mL; Riesling: 25 mg/100 mL), Plantis AF (Gewürztraminer: 30 mg/100 mL) and Blankasit (30 ul/100 mL) and an unfined control. The resulting wines were evaluated for sensory attributes using a trained panel and for acceptability using a consumer panel. Solid-phase microextraction (SPME) was coupled with gas chromatography/mass spectrometry (GC/MS) to quantify selected the volatile compounds in both wines. For Gewürztraminer, the trained sensory panel found a difference in floral flavor, with the unfined control and Blankasit having the highest concentration and the remaining fining agents having lower concentrations ($p<0.05$). No

differences in acceptance were found between the Gewürztraminer wines ($p>0.05$). For Riesling, no significant differences in sensory attributes were found by the trained panelists. However, the consumer panel showed a significant difference in appearance acceptance of the wines, with the unfined control Riesling being less acceptable than the fined Riesling wines ($p<0.05$). In Gewürztraminer wine, the volatile compound concentrations that significantly differed between treatments included 3-methyl-1-butanol (malt, burnt aroma) and 1-hexanol (green aroma) which were both highest in the Blankasit-fined wine ($p<0.05$). Ethyl hexanoate (apple, fruit aroma) was highest in the soy milk powder-fined wine and ethyl dodecanoate (leaf aroma) was highest in the unfined wine. In Riesling, ethyl decanoate (grape aroma) and ethyl dodecanoate (leaf aroma) were significantly higher in the unfined wine compared to the fined wines. Many of the volatile compounds quantified were present at concentrations below odor threshold detection values, and therefore did not translate into sensorial differences in wine aroma or flavor. The fining agents applied in this study impacted the chemical properties of wines, specifically volatile composition, color parameters and protein stability; however these differences were not as apparent using sensory methods.

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CHAPTER 1

INTRODUCTION

The focus of this study was to determine the sensory and chemical effects of plant-based fining agents on Washington (WA) State Riesling and Gewürztraminer wines. Washington State is the second largest producer of premium wines, surpassed only by California. The wine industry in WA State is the source of many jobs, generating over $3 billion towards the state's economy each year (Washington Wine Commission 2009). Grapes are one of WA State's most abundant fruit crops, and white wine grapes comprise over 50% of the total crop (Washington Wine Commission 2009). The state industry has grown from approximately 19 wineries in 1981 to over 650 wineries today (Washington Wine Commission 2009). Due to the rapid growth of the WA wine industry, winemakers are constantly looking for ways to improve quality and production.

One of the most important indications of quality in white wine to consumers is appearance. Wine appearance is based on clarity and color, with white wine consumers expecting a perfectly clear, pale yellow table wine. Consumers desire a white wine clear of sediment or haze, as these characteristics can be indications of serious flaws in the wine. In order to ensure white wines remain clear and stable throughout their shelf-life, winemakers must remove sediment and unstable proteins from the wine prior to bottling.

The most common and cost effective practice to clarify and stabilize white wines is through the addition of fining agents during the winemaking process. Fining agents are settling aids that accelerate the flocculation and removal of wine sediment and protein. Fining agents can adsorb partially soluble molecules in wine and speed up their precipitation thereby preventing this phenomenon in the bottles and improving the quality

of wines for consumers. A wide array of fining agents are commercially available to the winemaking industry, including bentonite, isinglass, whey protein, egg whites, gelatin, casein, and wheat gluten. The selection of the appropriate fining agent is based on the winemakers' experience, availability and cost.

Although fining is important in order to ensure white wine stability, it also poses some potential problems. Some researchers and winemakers believe that fining agents can remove volatile aroma compounds from wine, negatively impacting the varietal character of a wine. Thus far, study results on this topic have been mixed. Some studies have found that fining agents do in fact remove aroma compounds impacting the sensory properties of wines, while other studies have reported that fining agents do not have a significant impact on wine sensory properties. Many studies have been performed examining fining agents, but only one study has been conducted specifically on WA State white wines (Sanborn 2008).

Due to newly proposed labeling regulations for the wine industry that would require winemakers to list fining agents on labels, there is a growing interest in the wine industry to find new more "label-friendly" fining agents that are not animal proteins or potential allergens. WA State's growing wine industry makes the demand for new fining agent alternatives an area of great interest, especially since there have not been extensive studies on fining agents in WA state wines and very few studies involving plant-based fining agents.

The objectives of this study were to examine the sensory and chemical impact of several commercially available fining agents on WA State Riesling and Gewürztraminer wines. Specifically, wines were made from the appropriate grapes and treated with either

plant-based fining agents (Plantis Fine, Plantis AF, soy milk powder, or Blankasit) or left as the unfined control. Sensory and chemical analyses were then performed on the wines to determine if the fining agents generated differences between wines treated with different fining agents.

A previous study was conducted to determine the impact of various fining agents on WA State Chardonnay and Gewürztraminer (Sanborn 2008). In this study, the various fining agents were applied to wines that had been cold settled or partially clarified and rough filtered. The study found few differences between the volatile profiles of the wines made using different fining agents, and fewer sensory differences between the wines. The lack of differences observed in this study was partially attributed to the use of wine that had been partially clarified, settled or filtered. To eliminate this variable and increase the possibility of observing significant differences between the fining agents, the current study proposed making wines at the Pullman Student Winery directly from the grapes. It was hypothesized that the application of different fining agents would result in wines that possessed chemical differences compared to the unfined control wine. These chemical differences would then translate into differences in the sensory profiles and consumer acceptance of the wines. Also, Riesling was used in place of Chardonnay in the present study because it has a more delicate, fruity sensory profile and it was hypothesized that it would be more impacted by fining. Riesling is also growing in popularity in the state and is the second most planted white varietal in WA State.

CHAPTER 2

LITERATURE REVIEW

The following literature review will present salient literature regarding the research project entitled "The Chemical and Sensory Effects of Plant-based Fining Agents on Washington State Riesling and Gewürztraminer Wines." By way of background, white wine quality will first be discussed. This will lead into a discussion of winemaking and the application of fining, a common practice in the winemaking industry employed to improve white wine quality, but with some possible negative impacts on the sensorial quality of wines. White wine agents, the chemical and sensorial effects of fining, and labeling issues will also be presented. Overall, this literature review will serve to provide background information as well as rationale for the research conducted on white wine fining at Washington State University.

I. Wine Quality

A. Wine Trends

In the United States, white wines are preferred by a majority of consumers, even with the growing popularity of red wines due to their greater health benefits (Washington Wine Commission 2009). The United States produces only 25-40% of the wine generated worldwide, but its rapidly growing industry has pushed it to the forefront of wine research (Jackson 2000).

Washington is the second largest premium wine producing region in the United States, behind California (Washington Wine Commission 2009). Grapes are Washington State's fourth largest fruit crop, with over 32, 000 acres of grapes planted (Washington Wine Commission 2009). In Washington, fifty-two percent of the grapes grown are

white varietals (Washington Wine Commission 2009), with cold tolerant grape cultivars, such as Riesling, Gewürztraminer, and Chardonnay being the most popular grapes to plant (Washington Wine Commission 2009). The wine industry contributes more than $3 billion to Washington state's economy annually and provides over 14, 000 jobs (Washington Wine Commission 2009), making it a vital component of the state's agricultural business. Washington's growing wine industry is constantly looking for ways to improve the quality of the wines they produce to compete with premium wine production in the United States and around the world.

B. Consumer Evaluation of Wine Quality

Wine quality is subject to individual consumer judgments, but can be characterized by many attributes including, color, aroma intensity, vitality (purity), complexity, subtlety, palate strength, length, balance and longevity (Meilgaard et al. 2007). Wine color is defined by hue (dominant color wavelength), strength or depth of color, purity or lack of tawny tones, and stability over time (Pérez-Caballero et al. 2003). Aroma intensity and vitality are the magnitude and quality of aromas in the wine, respectively (Zoecklein et al. 1999). Complexity is a term used to describe the level of harmony amongst all of a wine's sensory components (Zoecklein et al. 1999).

Sensory attributes of any food or beverage are generally perceived in the same order, regardless of the product with the first attribute perceived being appearance, followed by odor, consistency/texture, and then flavor and taste (Meilgaard et al. 2007). As the consumer's first point of contact with a wine is its appearance in the glass, the importance of appearance in acceptability is critical. Appearance is defined in wine by clarity, intensity and color (Bird 2005). Most consumers expect white table wines to be

brilliantly clear and have a pale yellow or straw color, and wines that deviate from these expectations can be interpreted as being of low quality (Lomolino and Curioni 2007). A study by Buteau et al. (1979) found that by appearance alone, panelists scored white wines lower perhaps because they associated darker wines with off-flavors and aromas. Consumers have also learned through experience to associate cloudiness in wines with negative sensory attributes caused by faulty wine treatments or microbial spoilage (Amerine and Roessler 1976). Even slight hazes are considered suspect to critical consumers (Siebert 1999).

C. Wine Industry Evaluation of Quality

In the wine industry, it is easier to identify quality wines through the absence of faults rather than the presence of positive attributes, and this is certainly true with respect to wine appearance (Jackson 2000). For instance, a brown hue in a white table wine can be an indication of oxidation or heat abuse, both conditions which impart undesirable flavors to the wine (Jackson 2000).

Haziness in a wine is always considered a serious fault thus winemakers invest extensive efforts towards producing clear wines with long shelf lives (Jackson 2000; Johnson and Robinson 2005). A wine is generally considered to be clear when it has a turbidity value that falls below 10 NTU (nephelometric turbidity units) (Bird 2005). Haze in white wines arises from different sources. Most hazes in wines are caused by resuspended sediment or unstable proteins precipitating out of the wine due to temperature abuse (Jackson 2000). Tartrate crystals can also cause haziness and appear as fine crystals or flake-like crystals if wines are not cold stabilized during winemaking. These tartrate crystals are also mistaken for glass fragments by uneducated consumers

(Jackson 2000). Casses are another type of wine haze that occur when metallic ions react with soluble proteins, but casse formations are not common (Catarino et al. 2008).

In addition to issues with chemical components in the wine, microbial spoilage can stem from bacterial contamination, resulting in haziness and many other quality-related issues. Microbial spoilage can cause more serious issues than haziness, namely unpleasant off-flavors and off-odors (Fugelsang and Edwards 2007). If unfavorable odors do not deter consumption of a contaminated wine, microbial spoilage can also cause gastrointestinal illnesses upon consumption (Boulton et al. 1996).

Clarity is an important indication of quality that can easily be controlled through haze prevention by a knowledgeable winemaker in the winery. Most winemakers check for wine clarity by subjecting a small wine sample to heat abuse (80ºC) and then visually checking the sample for any cloudiness after it has cooled to room temperature (Pocock and Rankine 1973). This simple heat stability test lets the winemaker know if the wine contains unstable proteins that should be removed.

II. White Wine Fining

A. White Winemaking

Every wine has different characteristics, even those of the same varietal or country of origin. Each wine is unique due to the numerous winemaking practices employed by winemakers around the world. Even though many unique winemaking techniques and traditions exist, most wineries follow the same basic process to make wine. The procedure is slightly different for the production of red and white wine as red wines are fermented in the presence of the grape skins, while white wines are fermented in the absence of skins. There is also more extensive stabilization and finishing

employed in white wines than in red wines due to the inherent instability and less forgiving nature of white wine appearance (Moio et al. 2004). Generally, white wines are fined to stabilize and clarify the final product, whereas red wines are rarely fined unless there is a serious fault present in the wine.

White winemaking starts with the harvest of the grapes (Figure 1). Once the grapes are harvested, they are crushed and destemmed, and then pressed to create a grape must. However, some winemakers skip the crushing stage and place white grapes directly in a bladder press for more gentle pressing. The must is settled overnight and racked off the settled sediment before it is inoculated with a selected yeast strain. The sediment is removed in order to avoid an overabundance of nutrients that undesirable microorganisms may use to grow before the selected yeast is able to start the alcoholic fermentation (Bird 2005). Most winemakers add a selected, purified yeast strain to the must so that the wines will have a more predictable fermentation and less variability in quality than an alcoholic fermentation that is allowed to occur spontaneously from native yeasts (Fugelsang and Edwards 2007).

Fermentation lasts between 7 to 14 days, provided that the fermentation does not become "stuck" due to a lack of nutrients, an unfavorable temperature, or the presence of competing microorganisms (Fugelsang and Edwards 2007). Different grape varietals have different fermentation rates at the same temperature due to variations in grape composition and different population levels of native yeasts on the grapes, but generally, higher environmental temperatures lead to faster fermentation rates (Ough 1964). Most white wine fermentations are carried out between 15 to 25ºC, which is slightly cooler than temperatures used for red wine fermentations (Bird 2005; Fugelsang and Edwards

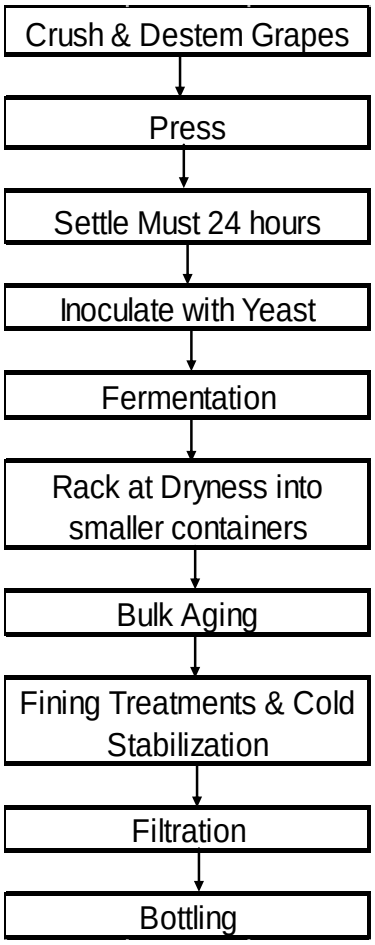

Figure 1. Process flow diagram of white winemaking procedure.

2007).

After fermentations have reached dryness, or a residual sugar of 2.5 g/L, the wines are racked off the sediment that has settled to the bottom of the vessels during fermentation. This sediment consists of dead yeast, grape proteins, tannins, and other large, unstable particles (Fukui and Yokotsuka 2003). The racking process is repeated as many times as necessary to clarify the wine before fining agents are applied to further clarify and stabilize the wine. The fining process is discussed in greater detail in the following sections. Following fining and cold stabilization, the wines are filtered and bottled, at which point the bottles are placed into storage until they are ready for sale.

For wine research purposes and in order to control as many experimental variables as possible, many wine researchers make wine on a small scale for experimentation, rather than purchasing wine made by a commercial winery. However, since many aspects of the winemaking process can impact the final quality of the wine produced, it is important to follow a process that mimics what is used in the winemaking industry. For experimental purposes, it is often impractical to use large, commercial size volumes of wine. It is easier to control all experimental variables and replicate treatments when the lots are kept as small as possible to carry out all experimental analyses (Weiss et al. 2001). Certain precautions must be taken to ensure that experimental findings are applicable to commercial-scale production. For instance, a small air leak in a large container may have minimal impact on the final wine, while a small air leak in a small container could be detrimental to the final wine quality (Boulton et al. 1996). Also, room temperature variations may be a greater challenge when using large containers, with sedimentation occurring much slower than in smaller lots (Boulton

et al. 1996). As long as researchers take proper measures to minimize variations in experimental wine production, their findings should be applicable to commercial-scale wine production.

B. Wine Turbidity & Stability

As described previously, clarity is one of the leading consumer quality requirements for white wine. A wine's appearance is the consumer's first impression of the wine and a hazy or turbid white wine is unattractive to consumers. Wine turbidity arises from the presence of particles suspended in the wine that impede light rays and diffuse some light in various directions, making the wine seem cloudy or even opaque. This phenomenon is known as the Tyndall effect (Ribéreau-Gayon et al. 2000). Turbidity can be measured by turbidimeters, which measure the light diffused in a given direction, with measurements expressed as NTU (nephelometric turbidity units) (Ribéreau-Gayon et al. 2000). However, most wineries do not have turbidimeters and simply judge turbidity by appearance.

Wine turbidity results from the presence of compounds in the wine. Common wine turbidity sources include dust, grape tissue, yeast, bacteria, or grape colloids. Other sources include particles formed from proteins, pectins, gums, metallocolloids, and polyphenol degradation products. (Mesquita et al. 2001) Over time, these particles often settle out of solution on their own and are removed by racking, but this process can be expedited by fining, centrifugation, or filtration. Fining is generally used in combination with racking and filtration to produce a clear, stable product of high quality. Centrifugation uses rotation at a high speed to expedite settling (Jackson 2000). The centrifuge compacts the sediment into a pellet so that the wine can easily be racked off of

the pellet. Centrifugation follows the same principle as spontaneous settling, but requires minutes, rather than days or weeks (Jackson 2000). Centrifugation is extremely effective at removing wine sediment but requires the use of expensive equipment that many small wineries do not have the resources to purchase. Filtration is extremely effective for removing large particles from wine such as grape tissue and yeasts, but cannot remove the colloids that cause protein hazes without the aid of fining agents because these proteins are too small (Hsu et al. 1987).

Stability is another important attribute of commercial wines. Winemakers must be able to stabilize wines before bottling to ensure wine quality under reasonable storage conditions or risk suffering tremendous financial losses. Some wine polysaccharides and polyphenols can contribute to haze formations and hinder tartrate crystal precipitation (Vernhet et al. 1996). As wine polysaccharides arise from the grape, each grape varietal has a different polysaccharide composition. Thus in order to increase wine stability, it is beneficial to remove these polysaccharides and polyphenols from the wine. All white wines should be checked for protein stability through heat stability testing prior to bottling (Pocock and Rankine 1973). Compared to red wines, white wines are more likely to develop protein hazes as they do not have a high polyphenol concentration to bind and precipitate labile proteins before bottling (Fukui and Yokotsuka 2003; Cabello-Pasini et al. 2005). It is essential for winemakers to be confident that the wine's clarity will not change at any point after bottling so that they will not suffer inventory and subsequent financial losses.

C. Fining Background

Fining is defined in the wine industry as "the deliberate addition of an adsorptive compound that is followed by the settling or precipitation of partially soluble components from the wine" (Boulton et al. 1996). All materials used for the purpose of fining are called fining agents, regardless of their mechanism of removal or targeted compound (Boulton et al. 1996).

Fining is an effective practice for removing particles and increasing wine stability. Fining agents can remove unstable compounds that are soluble in wine as well as complexing factors that can form between proteins and tartaric acid in white wines (Siebert and Lynn 1997; Hsu and Heatherbell 1987b). White wines are more often fined than red wines because they are more susceptible to browning and turbidity due to their pale color and transparent appearance, both undesirable characteristics to consumers.

D. Fining Agent Mechanisms

Winemakers use fining agents to enhance clarity, color, aroma, flavor and stability in wines. (Sims et al. 1995) Due to the complex nature of wine, many factors impact the effectiveness of the fining agents including the actual agent, the method of the agent's preparation, the method of addition, the addition rate, the wine pH, metal content of the wine and fining agent, wine temperature, wine age, and previous wine treatments. (Weiss et al. 2001)

All fining agents behave differently in wines since they come in many forms, including proteins, earths, synthetic molecules, pectins, gums, metallocolloids, and by-products of polyphenols. Different fining agents have unique requirements and capabilities. Some agents are better than others at removing specific molecules or faults from wines. For example, one fining agent is ideal for color correction in wines

(activated carbon), while another is better suited for tannin reduction, or increasing clarity and stability (bentonite). Most commonly used fining agents have been adequately researched in order to understand their functionality and ideal application, but there are new fining agents being developed which still need to be studied to learn their best uses within the winemaking process. The fining agent itself is the most important determinant of fining agent effectiveness, and as its properties cannot be easily changed, a winemaker must thoroughly understand its characteristics to use it successfully.

Most fining agents cannot be added directly to wines as they are commercially available. They must be prepared in a particular manner before they are added in the proper concentration to the wines. As fining agent reactions are all surface reactions it is important to hydrate the agents prior to addition and mix the wine well after the fining agent is added (Weiss et al. 2001). All fining agents should be prepared and added to the wine according the manufacturer's recommendations in order to perform properly in the wine, provided these recommendations are also within federal guidelines and regulations.

Since wine pH influences the charge density of molecules in the wine, protein stability is primarily dependent on wine pH. Wines with a lower pH require a lower concentration of fining agent than a corresponding wine with a higher pH. This concentration difference is due to the difference between the isoelectric point (pI) of the proteins and the wine pH. The pI of a protein is the pH of the solution at which the protein carries no net charge, and is therefore insoluble in solution, meaning it is highly unstable. A greater difference between pH and pI yields greater reaction potential with fining agents of the opposite charge (Dawes et al. 1994; Ough 1992). Compared to a wine

with a higher pH, a wine with a low pH will have more strongly charged proteins that will interact more readily with fining agents.

Although it is undesirable, metals may be introduced into the wines via fining agents or from harvesting and processing equipment. Since metals have highly charged ions associated with them, they can interfere with fining efficiency. Winemakers attempt to limit metal introductions as high metal concentration in either the wine or the fining agent slurry can negatively affect fining agents' activity and flocculation in the wine. To minimize the potential of metal introduction, many winemakers use deionized water to hydrate fining agents.

Another parameter to impact wine fining potential is the wine temperature. The temperature affects many fining agents' reaction times thus it is critical to carry out fining trials under the same conditions as the bulk fining treatment. Also, protein fining agents tend to be more effective at lower temperatures because the slower reaction time allows more contact time between the wine and the fining agent (Yokotsuka and Singleton 1995). Fining agents that work via hydrogen bonding will be unaffected by temperature (Yokotsuka and Singleton 1995).

As wine ages, more and more compounds will settle out of the wine by gravity. As a consequence, older wines require less fining agent to remove the remaining unstable colloids (Fukui and Yokotsuka 2003). Prior wine treatments will have an effect similar to aging on subsequent fining treatments in that there will be fewer unstable compounds still present in the wine. This is similar to the action of spontaneous settling during aging and its removal of unstable compounds from solution (Hsu et al. 1987). Winemakers

may use multiple fining treatments to achieve a brilliantly clear wine, with each subsequent treatment requiring a lower dosage of fining agent.

Three mechanisms exist through which fining agents work to remove particles from wines, electrostatic interaction, bond formation, or absorption/adsorption. Different classes of fining agents work via different mechanisms. In electrostatic interaction, fining agents induce particles of opposite charge to coalesce with the agent, forming larger particles that settle from the solution due to their increased density (Hsu and Heatherbell 1987b; Dawes et al. 1994). The second mechanism, bond formation, usually involves hydrogen bond formation between particles in the wine and the fining agent. This mechanism also forms a larger, denser particle that settles out of solution. Finally, in absorption/adsorption, wine particles adhere to the surface of the fining agent and settle out of solution. Many fining agents employ some sort of electrostatic interaction as they react in the wine so they are able to form strong bonds with wine particles having a positive or negative charge, creating a molecule that is large enough to precipitate out of solution (Siebert and Lynn 1997). The strong interaction between fining agents and wine molecules makes fining the best known way to stabilize and clarify wines (Boulton et al. 1996).

E. Fining Trials

As grape and wine composition impact fining agent performance, fining trials must be conducted on wines from each vintage. In order to accurately determine the effective concentration of fining agent needed in the wine, these trials must be performed under the same conditions of the bulk fining treatments (Weiss et al. 2001). In these initial small scale fining trials, smaller volumes of the wine are used to determine the

proper fining dosage prior to applying the doses to the bulk treatments (Ough 1992). Over-fining can lead to poor clarification while under-fining will not properly stabilize the wine.

In fining trials, several concentrations of the fining agent are added to small volumes of wine (Figure 2). Five to six concentrations at regular intervals can be determined from the previous year's results or five to six concentrations within the manufacturer's recommended dosage range can be used. Following addition, the wines are monitored daily, visually or with a turbidimeter until the wines appear clear or measure less than 10 NTU. The wines at each concentration are then measured for turbidity to determine the lowest dosage level that produced a clear wine (Bird 2005). If the winery does not have a turbidimeter, they can also measure the height of the lees formed in each volume to determine the proper dosage level. Provided that all conditions other than the wine volume are the same as those that will exist in the bulk wine, the dose determined from the fining trial should yield similar results to the small scale trial but likely more time will be required for the larger volume.

At the optimum fining agent concentration, the amount of colloid added should be equal to the amount of colloid removed from the wine (Bird 2005). It is important to determine the smallest amount of fining agent that needs to be added to the wine to be effective and to avoid wasting fining agent or losing large volumes of wine. In addition, the shortest amount of contact time for clarification should be employed so that no excess fining agent is left in the wine and there is less opportunity for flavor transfer. Excess fining agent left in the wine could lead to sediment in bottles, impart off-odors or flavors,

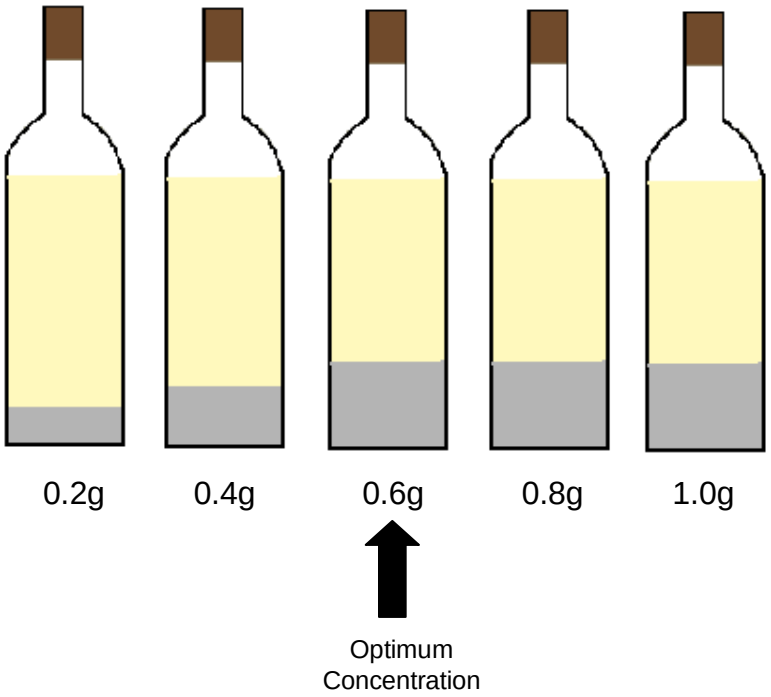

Figure 2. A fining trial experiment showing five increasing concentrations of fining agent to a wine. Successive concentrations of fining agent are added to the same volume of wine to determine the level of fining agent that causes no further effect as measured by the level of sediment in the bottles or wine turbidity (Bird 2005). A control of zero fining agent is also used for the trial. In the above fining trial, the optimum concentration of fining agent was 0.6 g.

and could potentially cause allergic reactions in susceptible individuals (Weber et al. 2007; Rolland et al. 2006).

III. White Wine Fining Agents

A. Bentonite

Bentonite, the most common fining agent used in the wine industry, is classified as an earth. It is an aluminum-silica clay mined all over the world (Ough 1992) but in the United States it is primarily mined in Wyoming. It is available in calcium, magnesium, and sodium bentonite forms, but sodium bentonite is the most commonly used form in wine fining due to its superior ability to bind proteins. Sodium bentonite tends to have more exposed surface area for protein binding because its platelets separate better in wine than calcium bentonite (Amerine and Joslyn 1970).

Bentonite works through adsorptive interactions between the negatively charged bentonite platelet surfaces and positively charged proteins in the wine. Since the bentonite platelet edges are positively charged, there can also be a small amount of binding with negatively charged phenolics (Catarino et al. 2008; Hsu and Heatherbell 1987b). Bentonite fining is also known to prevent casse formation in bottled wines by halting copper formation in wines with problematic metal levels through an unknown mechanism (Catarino et al. 2008). Because it is an aluminum-silica clay, bentonite fining also tends to increase aluminum levels in wines making it important to use the minimum dosage necessary to produce clarification (Catarino et al. 2008).

One negative aspect of using bentonite for wine fining is that it tends to form voluminous and loosely packed lees that can range from 3-10% of the total wine volume (Tattersall et al. 2001). Also, its handling and disposal are becoming a greater concern, costing the US

wine industry approximately $300-500 million annually (Hoj et al. 2000). The loose compaction and large volume of lees may lead to significant wine volume losses. Fining during cold stabilization may enhance lees compaction as the formation of potassium bitartrate crystal will help compact the lees. This method is particularly effective when the wine pH is below the pKI of tartaric acid (3.65) as free hydrogen ions are released into solution upon potassium bitartrate crystal formation, lowering the wine pH (Yokotsuka and Singleton 1995; Dawes et al. 1994). A lower pH favors increased positive charges on proteins, enhancing the bentonite activity (Dawes et al. 1994).

Bentonite must be hydrated for up to 24 hours before it is added to the wine and is usually added as a 5% solution in water or wine. This preparation is crucial so that the clay has time to swell and increase its surface area for reaction. Once added to the wine, the bentonite needs to be well mixed in order to maximize interactions with the wine. After mixing, the bentonite is settled before the wine is racked off the lees. Bentonite is primarily used in white wines and juices to remove proteins and enzymes that can catalyze browning reactions of polyphenol oxidases in juice (Main and Morris 1991).

B. Gelatin

Gelatin is made from collagen, the primary structural protein in skin and bones. It has a positive charge in juice or wine (pI of 4.7) and reacts with negatively charged particles via hydrogen bonding (Yokotsuka and Singleton 1987). Gelatin preferentially binds with larger molecules that have more potential hydrogen binding sites (Singleton 1967).

Gelatins vary greatly in quality and are rated based on purity and bloom, defined as the gelatin's ability to absorb water. The practice of counterfining, the application of a

second fining agent to aid in the removal of the first fining agent, is often used in conjunction with gelatin (Boulton et al. 1996). To aid in removal of the gelatin from the wine, a silica dioxide fining agent is often used as a counterfining agent. Even with counterfining, using a high bloom gelatin in conjunction with silica dioxide can lead to unreacted gelatin remaining in solution (Hahn and Possman 1977). The number of potential bonding sites determines gelatin effectiveness, so the size of the gelatin molecule is extremely important. Lower molecular weight gelatin reduces the rate of precipitation but enhances clarification and lees compaction (Yokotsuka and Singleton 1995).

Gelatin has the ability to strip wine character by removing aroma compounds that contribute to varietal character, so only high purity gelatins that are free of undesirable flavors and odors should be used for fining purposes (Sims et al. 1995).

C. Isinglass

Isinglass is made from sturgeon collagen and is available as a prehydrolyzed form or as a fibrous form known as flocked isinglass (Cosme et al. 2007). Isinglass adsorbs and precipitates polymeric phenols and tannins, allowing it to reduce color and astringency in wines (Cosme et al. 2009). Unlike several other protein fining agents, it does not require extensive counterfining but it can form a bulky, glue-like precipitate that is difficult to remove (Ough 1992). Isinglass yields compact lees (less than 2% of the treated volume), but due to the low density of the flakes formed, they can form hydrophobic interactions with wood tannins in barrels or casks (Cosme et al. 2009). The prehydrolyzed form needs to be hydrated in cool water for 20-30 minutes before wine

addition, and the flocked form needs to be hydrated for 24 hours in pH adjusted water (Rankine 1984).

Isinglass is typically used on white wines to bring out fruit character without dramatically altering tannin levels since it is less reactive towards condensed tannins than other protein fining agents (Rankine 1984). Isinglass degrades over time, resulting in an unpleasant fishy odor that can easily be transferred to the wine. To prevent this, isinglass should not be stored for long periods of time (Rankine 1984).

D. Casein

Casein is the primary protein in milk (Cosme et al. 2007; Ough 1992) and occurs as a positively charged molecule in solution. It is available in a purified form that is soluble in alkali solution or as sodium or potassium caseinate, which is soluble in water (Cosme et al. 2008). Both forms require hydration before they are added to wine. Casein flocculates at wine pH and the precipitate adsorbs and removes suspended material via electrostatic interaction as it settles in wine (Cosme et al. 2007). Casein is not as effective as carbon at removing oxidized color and flavor but it does not catalyze oxidative deterioration, which has been associated with using carbon in wines (Singleton and Draper 1962). Like gelatin, it is often counterfined with tannin or silicon dioxide.

It is commonly used in white or sherry wines to remove oxidized character and color. It is also used to prevent pinking in susceptible wines such as Chardonnay and Pinot blanc.

E. Albumin

Egg albumin is one of the few fining agents commonly used in red wines. It is usually added as fresh or frozen egg whites that have been gently whipped. As a general

rule, one gram of albumin precipitates two grams of tannin (Peynaud 1984). Albumin removes tannin by forming hydrogen bonds with the hydroxyl groups on tannin molecules (Cosme et al. 2007).

Egg whites are rarely used in white wine fining because of their need for extensive counterfining (Zoecklein et al. 1999). Red wines do not require counterfining because of the high level of tannins naturally present in the wine, allowing for fining agent compaction and removal (Siebert et al. 1996). Egg whites are thought to remove less fruit character than gelatin, so they are often added to red wines to reduce astringency before bottling (Cosme et al. 2009).

F. Activated Carbon

Activated carbons are adsorbent fining agents that are used to modify the sensory characteristics of juices, wines, and spirits (Singleton and Draper 1962). The carbon activation process creates pores within the carbon particles, which gives the carbon high internal porosity and surface area. It has nonspecific physical adsorption, but tends to favor electrostatic interactions with weakly polar molecules, especially ones containing benzene rings or their derivatives (Singleton and Draper 1962). Smaller phenolics are effectively removed by activated carbon.

G. PVPP

Polyvinylpolypyrolidone (PVPP) is a synthetic, high molecular weight fining agent that has an affinity for low molecular weight phenolics and performs similarly to protein fining agents. It forms hydrogen bonds between carbonyl groups on the polyamide (PVPP) hydrogens and the hydrogen groups on phenolic compounds (Siebert and Lynn 1997). PVPP is available in various particle sizes so that it can selectively bind

certain phenols. It mainly binds and removes small phenolics such as catechins and anthocyanins, browning precursors in white wines, because it contacts only a few reactive groups on phenolic compounds (Sims et al. 1995). PVPP may be used in combination with activated carbon to remove browning in white wines. However, some winemakers have found that PVPP can strip wine complexity by removing delicate aromas formed during aging (Sims et al. 1995). It is prepared as a 5-10% (w/v) slurry in wine or must and mixed for at least one hour before addition to the wine to be treated. The Bureau of Alcohol, Tobacco, Firearms and Explosives (www.atf.gov/pub) regulates that PVPP must be removed from wine by filtration prior to bottling.

H. Tannins

Tannins used for fining are usually extracted from nutgalls or grape seeds. They carry no net charge in wine solution, and are used as a counterfining measure with gelatin as they form hydrogen or hydrophobic bonds with proteins (Hagerman et al. 1998). Tannins are also added to red wines low in grape tannins to increase astringency (Zoecklein et al. 1999). Tannic acid solutions are generally prepared as a 1% (wt/vol) solution in 70% ethanol. BATF regulates this usage of tannic acid, stating that total tannin content may not be increased by more than 150 mg/L (www.atf.gov/pub).

I. Silica sol

Silica sols are aqueous suspensions of silica dioxide, and there are several generic names for these products, including silica gel and Kieselsol. Silica sols were initially used in Germany as a substitute for tannic acid in gelatin fining (Zoecklein et al. 1999). Silica sol electrostatically binds with positively charged proteins to initiate flocculation and settling (Armada and Falqué 2007). This reaction depends on particle size, shape,

and surface type, as well as particle size distribution and charge density within the silica sol suspension. The nature of the silica particle dictates what size and how many molecules can bind with it. The particle size also determines the relative charge strength of the silica particle.

Silica sols are frequently used in combination with protein fining agents for clarification. Silica sols are now favored for counterfining over tannins because they create a smaller lees volume, have faster precipitation, result in greater clarity, and are thought to remove less of the wine's varietal character (Armada and Falqué 2007; Ribéreau-Gayon et al. 1999). Silica sols tend to have a limited shelf-life (usually less than two years) and should not be frozen. Their use is regulated by BATF, and they must be completely removed by filtration before bottling (www.atf.gov/pub).

J. Alginates

Alginates are the most commonly used polysaccharides for fining, and are frequently derived from the algae cell walls. They are negatively charged and are usually bound to an inert carrier, such as diatomaceous earth (Cabello-Pasini et al. 2005). Their mechanism of action for fining purposes is through electrostatic interaction (Cabello-Pasini et al. 2005). Alginate reactivity and clarification capability is best in wines with a pH of 3.5 or less (Dawes et al. 1994). Counterfining alginates with gelatin or bentonite may speed up the clarification process.

Sparkalloid and Klearmor are products consisting of negatively-charged alginates on diatomaceous earth carrier in solution that are commonly used in the US wine industry. They are primarily used to improve clarity and filterability. They have little adsorptive capability and work by electrostatic interaction. Both Sparkalloid and

Klearmor must be hydrated in hot water at 60 g/L before addition to wine or juice while the solution is still hot (Zoecklein et al. 1999). Alginates produce very compact lees, even though the dosage levels are similar to those of bentonite.

A study performed by Cabello-Pasini et al. (2005) compared bentonite to agar, carrageen, and alginic acid for their ability to precipitate proteins in Chenin blanc wine. Results showed that the three polysaccharides extracted from seaweeds showed comparable effectiveness as bentonite towards removal of proteins in wine.

K. Gum Arabic

Gum Arabic primarily consists of arabinose and is harvested from acacia trees. Because it is a stable colloid, it has been shown to stabilize other colloids and interrupt bitartrate crystal growth, delaying their precipitation in wine by binding to unstable molecules (Dawes et al. 1994; Vernhet et al. 1996; Gibson 2003). However, this delay is short-lived and not as effective as cold-stabilization for preventing crystal formation in bottles. Currently, gum arabic is not used extensively in the US wine industry. Researchers have found that gum arabic can also be used in red wines to stabilize wine pigments and prevent turbidity at an addition rate of 5-20 g/hl (Ribéreau-Gayon et al. 2000).

L. Yeast

Yeast fining is accomplished by adding fresh yeast, at a rate up to 10%, to wine and then filtering or centrifuging the wine to remove the yeast (Zoecklein et al. 1999). Yeast fining is traditionally carried out using the *Saccharomyces cerevisiae* wine yeast. Yeast cell walls consist of mannoproteins and play a role in the complexation of polyphenols and metals in wine and must (Lomolino and Curioni 2007). Thus, during

fermentation, yeasts may reduce metal concentration and when added post-fermentation, these yeast may prevent metal casse formation (Langhans and Schlotter 1987).

While yeast fining is not currently one of the more common fining agents used, it has found some applications. Yeast can adsorb phenolic compounds and reduce browning and oxidative character in wine (Lopez-Toledano et al. 2007; Razmkhab et al. 2002). Thus far yeast fining has been found to be less efficient at removing browning than other fining agents but has less impact on sensory attributes of the wine (Razmkhab et al. 2002). For this reason, some researchers (Razmkhab et al. 2002) propose substituting yeast fining for charcoal or PVPP fining. Yeast fining has also been used to remove herbaceous and off-odors from white wines (Razmkhab et al. 2002).

M. Plant proteins

An issue of growing importance and popularity in the wine industry is developing more environmentally-friendly winemaking practices. In addition, the market for vegetarian or vegan wines is growing along with the concern of using fining agents that are potential allergens (European Union 2007; Rolland et al. 2006; Weber et al. 2007). Both the European Union and Australia have imposed new labeling laws that require winemakers to list potential allergens used as fining agents (European Union 2003). This has generated an interest in substituting animal-based protein fining agents with equally effective plant protein alternatives. Thus far, the plant proteins that have been studied as fining agents include pea protein, vegetable protein, soy protein, and lupin protein. Plant proteins perform similarly to animal proteins in wine, and adsorb negatively charged molecules in the wine (Fischerleitner et al. 2003).

There have been few studies on plant based fining agents, but a growing number of plant and vegetable protein fining agents are available on the market. Winery suppliers are starting to advertise allergen-free and vegetable-based fining agents due to demand for these types of products in the wine industry.

A study by Fischerleitner et al. (2003) compared the effectiveness of vegetable and animal proteins as fining agents. For the plant protein fining agents, this study used soya protein, lupin protein, and vegetable protein and compared them to gelatin, casein, albumin, isinglass, and whey protein. These fining agents were applied to both red and white wines, with chemical and sensory analysis used to compare the treated wines. Results showed that the fining agents had little effect on white wine color but some fining agents, particularly albumin, left residue in the white wines. Overall, this study found that the application of the plant protein fining agents resulted in comparable precipitation of proteins and phenolics to the animal protein fining agents.

Another study was conducted by Maury et al. (2003) investigating the fining ability of white lupin, wheat gluten, hydrolyzed gluten compared to gelatin in red wine. Results showed that the hydrolyzed glutens and white lupins were the most selective for proanthocyanidins. As evaluated by changes in turbidity, hydrolyzed gluten and white lupin performed similarly to the gelatin in fining of the wines. All of the fining agents decreased turbidity more than spontaneous settling and selectively precipitated condensed tannins, but no agent was able to precipitate simple phenolics from the wines. No sensory testing was performed in this study.

IV. Fining Effects on Chemical and Sensory Properties of Wine

Due to the importance of wine quality, it is a primary concern to winemakers that fining agents do not strip wines of varietal character. Many studies have been conducted applying different fining agents to various wines with varied results. The impact of fining agents, particularly the new plant-based agents, on the chemical and sensory properties of wine needs to be further explored.

Moio et al. (2004) examined the effects of must clarification procedures, including spontaneous settling, settling with the addition of pectic enzyme, filtration, fining with bentonite, silica gel, casein, and carbon, and fining followed by filtration on varietal aroma in Falanghina wines. All clarification treatments were found to decrease levels of glycosylated aroma precursors for linalool, geraniol, benzyl alcohol, 2-phenylethyl alcohol, and eugenol, leading to lower concentrations of these free aroma compounds in the final wines. Clarification treatments that used fining agents caused the largest decreases in these compounds. This study also compared the effects of using clarification treatments before and after alcoholic fermentation, and concluded that pre-fermentation clarifications led to improved sensory characteristics in white wines. This finding was attributed to the removal of the grape solids prior to fermentation. The removal of grape solids at this stage was thought to promote ester production and limit fusel alcohol production during alcoholic fermentation, improving the overall aromatic quality of the wine (Moio et al. 2004).

Armada and Falqué (2007) also studied the effects of fining with bentonite and silica gel on the aroma composition of white wines. This study found that compared to the unfined control, bentonite caused a decrease in the concentration of volatile aroma compounds but the use of a silica gel as a fining agent increased some of the volatile

compound concentrations. Neither Armada and Falqué (2007) nor Moio et al. (2004) conducted sensory testing on the wines to determine whether these differences yielded detectable sensory differences in the wines.

Using a trained sensory panel, Flores et al. (1991) compared the effects of ultrafiltration and bentonite fining on Riesling and Gewürztraminer wines. They used a 9-pt intensity scale and 10 panelists to rate several flavors and aromas. Overall, they found the control wine had the highest aroma intensities, with the ultrafiltered wines having the lowest aroma intensities. No differences in flavor intensity were reported. A sensory study by Castillo-Sánchez et al. (2006) reported that wines fined using PVPP, egg albumin, casein, and gelatin were rated higher than the unfined control by sensory panelists for organoleptic properties, especially in taste.

Another study (Lopez-Toledano et al. 2007) used kappa-carragenate and alginate gel beads to immobilize yeast cells to color correct sherry-type wines. These researchers found that the yeast immobilized on kappa-carragenate gels made a larger impact on wine color compared to yeasts immobilized on alginate gels. Sensory panelists evaluated the different treatments for color, aroma, and flavor with results indicating that sensory properties were negatively altered when high concentrations (3 and 5 g/L) of the yeast were used.

A study by Fischerleitner et al. (2003) compared the performance of different vegetable fining agents to animal-based agents. The vegetable protein fining agents, soya protein, lupin protein, and vegetable protein resulted in precipitation of proteins and phenolics that was comparable to animal protein fining agents (gelatin, casein, albumin, isinglass, and whey protein) in both red and white wines. All of the fining agents tested

reduced bitterness and improved color in the red wines compared to the unfined control. However, sensory testing revealed that the vegetable proteins also transferred some undesirable cooked vegetal flavors and aromas to the wines.

Maury et al. (2001) studied the effects of fining with different molecular weight gelatins on proanthocyanidin composition and sensory perception of red wines. They used a commercial gelatin, which contained a combination of several molecular weight gelatins, and two different isolated molecular weight fractions to fine four different red wines. Lower molecular weight gelatins were found to be more selective for tannins, with a reduction in perceived astringency in all treated wines compared to the unfined control wine.

An experiment conducted by Weetall et al. (1984) used immobilized tannic acid derivatives on several column types to stabilize wine and remove temperature-induced haze formations from wines. The immobilized tannic acid successfully removed the proteins and tannin-protein complexes that contribute to haze formation and wine instability.

Bentonite and isinglass have been used as fining agents in the wine industry but are suspected to sometimes affect sensory properties (Armada and Falqué 2007; Flores et al. 1991; Moio et al. 2004). Bentonite has been shown to affect volatile aroma compositions of white wines (Armada and Falqué 2007; Flores et al. 1991), but it has not yet been confirmed if the overall quality of the wine is negatively affected. Armada and Falqué (2007) found that bentonite decreased concentrations of terpenes, C13-norisoprenoids, and C6-alcohols compared to an unfined control. These types of volatile compounds contribute to a wine's varietal character, so a decrease in their concentration

could change the sensory profile of a wine. Flores et al. (1991) conducted a sensory study that found Gewürztraminer wine treated with bentonite was significantly different in chemical and cooked vegetal aromas than an unfined control.

A previous study conducted at Washington State University compared the effects of fining agents on the chemical and sensory properties of Washington State Gewürztraminer and Chardonnay wines (Sanborn 2008). These wines were fined using bentonite, isinglass, activated charcoal, whole milk, Sparkalloid, and wheat gluten. Using a trained sensory panel, no significant sensory differences were found between the Gewürztraminer wines fined with different agents. However, volatile analysis of the Gewürztraminer treatments revealed significant differences in the concentrations of most of the volatile compounds between fining agents. Specifically, the higher alcohols were significantly different between treatments, with wheat gluten resulting in the lowest concentrations compared to the other fining agents. The concentration of benzene ethanol, which is thought to have a honey/spice or floral aroma (Acree and Arn 2004), was also significantly impacted by the fining treatments, with the lowest concentrations present in the wines fines with Sparkalloid, wheat gluten, and bentonite and the highest concentration in the wine fined with isinglass. There were also significant differences in ester concentrations between Gewürztraminer treatments, and again wheat gluten had the greatest reduction on these compounds. Linalool was also quantified in the Gewürztraminer fining treatments, and it had the lowest concentration in the bentonite fined wine.

This study found many more differences in the Gewürztraminer wines than in the Chardonnay from the volatile analysis. In Chardonnay, spicy aroma and floral/honey

flavor were significantly different as measured by the trained panel. However, these sensory differences did not translate to chemical differences. The only volatile compound significantly different across fining treatments was ethyl dodecanoate, which was highest in the control wine and lowest in the bentonite wine. For the most part, wheat gluten reduced alcohol concentrations more than any other treatment, but the impact was not statistically significant. Possible reasons for the few differences observed may be due to the base wine. This study was conducted on wines obtained from a commercial winery. These wines had already been cold stabilized, possibly contributing to the lack of significant differences found between the different treatments.

V. Labeling and Fining

Both the European Union and Australia have recently changed food labeling legislation so that wines fined with potential allergens must disclose the use of these fining agents on their labels (European Union 2003). Due to this new legislation, Weber et al. (2007) studied whether or not any fining agent residues were present after proteinaceous fining agents were applied to four commercial German white wines (2 Rieslings, a Pinot blanc and a Pinot gris). The only fining agent that could be detected in all the wines, at <1 ppm, was dried egg white, which had been applied at a concentration of 200 ppm. Another study, conducted by Rolland et al. (2006) was undertaken to determine whether any commercially available fined wines could induce a serious allergic reaction (anaphylaxis) in susceptible individuals. As fining agents should be completely absent from bottled wines, with residues potentially triggering allergic reactions in susceptible individuals, the authors also measured fining agent residue in

finished wines. The researchers used 26 subjects who suffered from food allergies and tested 108 wines, with no subjects experiencing a severe allergic reaction from the wines.

Due to these changes in international labeling laws, winemakers in the United States must comply with these regulations if they intend to export their wine to any of the regulated countries. There has been speculation that more stringent labeling laws will eventually be placed upon wines made in the United States with respect to fining agents. Many winemakers and researchers are taking a proactive approach to find alternative fining agents that will be more "label friendly" and less off-putting to consumers. Current labeling laws by the BATF do not require U.S. winemakers to include any fining agents on wine labels to be sold in the United States (www.atf.gov/pub). The only additives that must be cited on U.S. wine labels at this time are sulfites (www.atf.gov/pub).

CHAPTER 3

MATERIALS AND METHODS

I. Winemaking

A. Grapes

One half ton of both Riesling and Gewürztraminer grapes were donated by Columbia Crest Winery (Paterson, WA). The Gewürztraminer grapes were harvested on September 25, 2008 and transported to Washington State University's (WSU) experimental winery (Pullman, WA) on September 26, 2008. The grapes were held at approximately 74ºF overnight and processed in the WSU winery the following day. The Riesling grapes were harvested October 9, 2008, transported to Washington WSU on October 10, 2008, held overnight at approximately 74ºF and processed in the experimental winery the next day. Some of the Riesling grapes appeared to be raisined, and showed signs of bunch rot but none of the grapes were culled.

B. Crush

The same procedure was followed for crushing the Riesling and Gewürztraminer grapes. The grapes were processed using a (F.LLI Rossi Pigiadiraspatrice Capri, Trestina, Italy) crusher and collected in food grade-plastic containers and transferred to a bladder press (Prospero Equipment Corp., Pleasantville, NY). The free-run juice was first collected and the grapes were then pressed using a maximum of 2 bar pressure. The juice was pumped into 15 gallon plastic containers for overnight settling at 23ºC. The Gewürztraminer grapes yielded approximately 60 gallons of juice, while the Riesling grapes yielded approximately 64 gallons of juice. Excessive browning was observed in

one of the 15 gallon containers of Riesling juice, resulting in 6 gallons of Riesling being discarded.

The pH of the juice was determined in triplicate using an Accumet AB15 Plus pH meter (Fisher Scientific, Pittsburgh, PA). Based on this pH, potassium metabisulfite (Brewcraft, Portland, OR) was added directly to the grapes in the bladder press to yield 25 ppm total SO_2. To measure SO_2 concentration, the Ripper method (Amerine and Ough, 1988) was used.

After the juice was allowed to settle overnight at 23ºC, all of the plastic containers were racked into two 55-gallon stainless steel fermentation vessels, one vessel for each wine variety. In order to determine the initial level of sugar prior to alcoholic fermentation, Brix measurements were taken in triplicate on the Riesling and Gewürztraminer juices using a refractometer (Novatech Pocket Pal Refractometer, Tokyo, Japan). The Gewürztraminer was inoculated with Lallemand's Enoferm QA23 (Montréal, Québec) yeast at the rate recommended by the manufacturer, 25 g/hL. The Gewürztraminer was initially placed in a cold room (50ºC) for fermentation, but the room could not be held at a high enough temperature to allow the fermentation. It was then moved to a room-temperature storage space after the fermentation had not started after 7 days.

A different yeast strain was used for the Riesling fermentation, as recommended by Lallemand for WA State wines. The Riesling juice was inoculated with Lallemand's Lalvin R-HST yeast at an inoculation rate of 40 g/hL. This level was higher than the manufacturer's recommendations as a high level of native yeasts were observed in the juice under a Leica DMLS microscope (Bartels and Stout, Inc., Bellevue, WA). A higher

inoculation rate was necessary to outcompete native yeast because the native yeast had already begun to thrive in the wine, so a more robust level of selected yeast was needed to ensure they would not be suppressed by the native yeast (Fugelsang and Edwards 2007)

The Riesling and Gewürztraminer fermentations were monitored daily by checking the Brix. Brix were measured by removing 50 mL samples from the fermentation tanks daily during alcoholic fermentation and measured using a hydrometer once the fermentation had commenced. Towards the end of the fermentation, when the wine was below 2 Brix (Bird 2005; Zoecklein et al. 1999), the residual sugar was measured using Clinitest tablets (Bayer Corp., Elkhart, IN), since hydrometers are not as accurate at low sugar levels. When the fermentations had reached dryness (less than 2 Brix as measured by the Clinitest), the wines were racked into 5-gallon glass carboys and placed in cold storage at 50ºF. The wines were racked into new carboys as sediment formed in the containers. The total concentration of SO_2 was measured in the wines immediately after alcoholic fermentation so that the concentration could be adjusted to maintain 30 ppm total. Potassium metabisulfite was added following each racking to prevent oxidation and microbial contamination. Following racking, the wines were stored at 40ºF until they were needed for experimental treatments and analyses. Once the alcoholic fermentations were complete, the level of ethanol in the wines was measured in triplicate using an ebulliometer (Braun-Knecht Heimann Co., San Francisco, CA).

II. Fining

A. Fining Trials

Fining trials were conducted to determine the optimal level of each fining agent to be used in the bulk fining treatments. Also, more fining agents were tested in the preliminary fining trial than were feasible in the bulk experiment in order to determine the most effective fining agents. Each fining agent was tested at five different concentrations in both wines, with a control sample (no fining agent applied) maintained for each wine. Table 1 lists all the fining agents tested, along with their recommended concentrations and methods of preparation for addition. Table 2 shows all of the fining agents used in the preliminary fining trials, along with their concentrations. All of the fining agents were prepared and added at levels recommended by their manufacturer's instructions, except for the soy milk powder, which was added at levels found in literature (Fischerleitner et al., 2003).

The preliminary fining trial was conducted in replicate for each fining agent. To conduct the preliminary fining trials, wine samples (100 mL) were placed in 120 mL glass jars (Wheaton Science Products, Millville, NJ), with the fining agents added to the wine samples at each concentration listed in Table 2. Following fining agent addition, the wines were thoroughly mixed and placed in 13ºC, approximately the same temperature at which the bulk fining trial would be performed. The samples were monitored visually for sediment build-up at the bottom of the jars, and after two weeks, the point at which all the samples appeared clear, the turbidity of all the samples was measured using a Klett meter. Some wines appeared clear before two weeks, but no turbidity measurements were taken until all wines were clear. Initially turbidity was measured using a Klett-Summerson Photoelectric Colorimeter (Klett MFG, Co., Inc., NY) because a turbidimeter was not available. However, a turbidimeter (Hach 2100P,

Table 1. Fining agents, composition, supplier, preparation method and recommended dosage of fining agents used in the preliminary fining trial on Riesling and Gewürztraminer.

Fining Agent	Composition	Supplier	Preparation Prior to Addition	Recommended Dosage
Blankasit	silica sol	Erbsloh	Shake solution well before addition.	30-100ml/100 ml of wine
FlavoClair	silica sol modified with oak heart wood tannin	Erbsloh	Shake solution well before addition.	50-100 ml/100 ml of wine[1]
Soy Milk Powder	dehydrated soy milk, de-fatted, 90% protein	Moscow, ID Food Co-op	Dissolve powder in warm water.	0.4-1.6 mg/100 ml of wine
Plantis AF	pure plant protein (gluten free)	Enartis	Dissolve in water acidified with 2-4 g/l of citric acid in a ratio of 1:10, stirring continuously.	10-30 mg/100 ml of wine
Plantis Clar	hydrolyzed gluten and pea protein	Enartis	Dissolve in water in 1:10 ration, stirring continuously.	5-20 mg/100 ml of wine
Plantis Fine	hydrolyzed gluten and pea protein	Enartis	Dissolve in water in 1:10 ration, stirring continuously.	5-20 mg/100 ml of wine

[1] Dosages for soy milk powder were based on soy milk protein levels used in Fischerleitner et al. (2003).

Table 2. Fining agents and their concentrations (mg/100ml wine) or volumes (µl /100 ml wine) used in preliminary fining trials of Riesling and Gewürztraminer. Fining agents were prepared as described in Table 1 and concentrations were selected at even intervals surrounding the manufacturers' recommended dosages.

Fining Agent	Level 1	Level 2	Level 3	Level 4	Level 5
Bentonite	50	75	100	125	150
Soy milk powder	0.72	1.44	2.16	2.52	3.24
Plantis Clar	5	10	15	20	25
Plantis Fine	5	10	15	20	25
Plantis AF	10	15	20	25	30
Flavoclair	50 µl	65 µl	80 µl	95 µl	105 µl
Blankasit	30 µl	50 µl	70 µl	90 µl	110 µl

Loveland, CO) was acquired and used to measure the turbidity of the fining trial and then used during the large scale fining study as NTUs are the standard turbidity unit used in the wine industry.

From these preliminary fining trials, the lowest fining agent dosage that yielded a change in turbidity, expressed as Klett units, was selected as the dosage level for the large scale fining treatments. Due to volume constraints, not all of the fining agents tested in the preliminary fining trial could be used in the large scale fining study. Thus the fining agents that showed the greatest reduction in wine turbidity were selected. Plantis Clar and Flavoclair were not used in the large scale fining study, and Plantis AF was only used on the Gewürztraminer wine because the larger volume of Gewürztraminer allowed an additional fining agent to be applied.

After fining agents and their dosage levels were selected from the preliminary fining trial, the wine samples, at the selected dosages, were subjected to a heat stability test (Pocock and Rankine, 1973) to determine wine stability under extreme storage conditions. The samples were heated for 6 hours at 80ºC, cooled and then visually examined for the presence or absence of cloudiness or sediment. Only binary data was collected from this experiment (i.e. presence or absence of cloudiness or haze), not the extent of cloudiness or haze. The samples were also measured for turbidity.

B. Bulk Fining Treatments

Upon the completion of the preliminary fining trials, the selected fining agents were added to the bulk wine at the dosages listed in Table 7. For each wine, one carboy (five gallons) of wine was subjected to each fining treatment, while two to three carboys were reserved as the control (unfined) treatment (10 gallons of Riesling and 15 gallons of

Gewürztraminer). To each carboy, the fining agents were added, mixed and allowed to settle for 7 days at 50ºF, a time selected based on the results from the fining trial. Fining agents were not applied to wines in replicate. On the seventh day, the turbidity levels were determined in triplicate for all the treatments. To measure turbidity, samples were removed from the 5-gallon carboys. When the turbidity level measured <10 NTU (Weiss et al. 2001), the wines were racked into clean carboys. Fining treatments required different time periods to reach <10 NTU in both varietals. At this point, the wines were racked off the fining agents, and potassium metabisulfite was added to maintain a total concentration of SO_2 of approximately 30 ppm. The alcohol levels were then measured in triplicate.

Potassium metabisulfite was checked prior to filtration, and adjusted to maintain a total concentration of SO_2 at approximately 30 ppm. Wines were filtered through a 0.45 μm filter (Vitipore II plus filter, Millipore, Billerica, MA) directly into clear glass bottles (750-ml Bordelaise BVS 30 H 60, Vitro, Monterrey, Mexico) and sealed with screwcap closures (Stelvin, Alcan Packaging, Montreal, Canada) using a screwcapping machine (Technovin, Saxon, Switzerland). The final turbidity values were measured in triplicate from a single bottle of each treatment. After bottling the wines were stored at 50ºC with minimal light exposure until they were needed for sensory and analytical testing. Following 4 months of storage, the bottled wines were subjected to heat stability testing (described above).

III. Sensory

A. Consumer Acceptance Panel

Consumer acceptance panels were performed on Riesling and Gewürztraminer for all fining treatments at the WSU Sensory Facility. Two panels were conducted, one for the Riesling wines, and a second for the Gewürztraminer wines one week later. Consumers were recruited from WSU and the surrounding community through e-mail notices and advertisements placed around campus and in the WSU Daily Evergreen. For the Riesling panel, 79 consumers (32 male and 47 female) participated while 100 consumers (46 male and 54 female) participated in the Gewürztraminer panel. The consumers for both sessions ranged in age from 21 to over 60 and were occasional (2-3 times per month) to frequent (at least once per week) wine consumers. Minimal information was provided to consumers about the study to avoid imparting any bias on the panel, and all consumers completed an informed consent form, approved by the Institutional Review Board at WSU.

Wine samples were presented using a random serving order with a complete block design, with all wines presented one at a time. During the Riesling panel, to each consumer, five wine samples were presented and during the Gewurztraminer panel, six samples were presented to each consumer. Each wine sample was served under white lights at room temperature in a covered tulip-shaped clear ISO/INAO wine glass and labeled with a 3-digit random code. Consumers were provided with filtered, distilled water and unsalted top Saltine crackers (Kraft Foods, East Hanover, NJ) to cleanse their palate between samples.

Consumers evaluated each wine for overall acceptance and acceptance of appearance, aroma, flavor, and mouthfeel using a 7-point hedonic scale, with 1= dislike very much and 7= like very much. Consumers were provided with definitions of each

attribute to minimize confusion (Table 3). The panel data were collected electronically using Compusense®*five* software (Guelph, ON).

B. Trained Panel

A trained descriptive analysis panel was held to evaluate the sensory attributes of the wines treated with different fining agents. Panelists were recruited from WSU and the surrounding community. The final panel consisted of 12 panelists, 10 women and 2 men ranging in age from 21 to 60. All panelists received non-monetary incentives for their participation. The panelists were given minimal information about the panel to avoid imparting any bias on the panel results.

For Riesling evaluations, the panelists were trained over 11 1-hour sessions for a total of eleven hours of training. The panelists were first trained to evaluate the Riesling wines, after which time they evaluated the Riesling treatments. Following the Riesling evaluations, the panelists underwent an additional 3 hours of training for the Gewürztraminer wines. Thus, for the Gewürztraminer wines, the panelists were trained for 14 hours. The panel was divided in this manner because the Gewürztraminer wines had additional sensory attributes to evaluate.

A list of all the attributes evaluated for each wine, as well as the attribute standards, are shown in Table 4. These attributes were selected from a preliminary focus group consisting of 8 experienced wine tasters. The intensities of all attributes were evaluated using an unstructured 15-cm line scale anchored with ‘low’ at 1 cm and ‘high’ at 14 cm. The data were collected with Compusense®*five* software.

Table 3. Sensory attributes and definitions provided to consumers in-booth for acceptance panels of Riesling and Gewürztraminer wines. All attributes were evaluated using a 7-pt hedonic scale where 1=dislike very much and 7=like very much.

Sensory Attribute	Definition
Appearance	Color & clarity of the wine.
Aroma	Volatile compounds that are detected through the sense of smell
Flavor	The sensory impression created in the mouth by the combination of taste (sweet, sour, salty, etc.) and aroma. Example of flavors: vanilla, lemon, etc.
Mouthfeel	A chemical interaction in the mouth that produces a physical sensation, such as astringency and alcohol burn.

Table 4. Sensory attributes and standards evaluated by the trained panel in their evaluations of the Riesling and Gewürztraminer wines.

Sensory Attribute	Training Standard (in 100 ml of white wine[1])
Fruity Aroma & Flavor	15 ml syrup from canned pears & 7.5 ml syrup from canned peaches (Safeway Select, Pleasanton, CA)
Floral Aroma & Flavor	8 mg/L nerol (Sigma Aldrich, St. Louis, MO)
Vegetal Aroma & Flavor	1 inch^2 piece of fresh bell pepper
Yeast Aroma & Flavor	1 tsp dried baker's yeast (Hodgson Mill, Effingham, IL)
Alcohol/Chemical Aroma & Flavor	30 ml ethanol (JT Baker, Phillipsburg, NJ)
Sweet Taste	4g/L sucrose (JT Baker, Phillipsburg, NJ)
Sour Taste	4 g/L tartaric acid (Sigma Aldrich, St. Louis, MO)
Ethanol Burn	30 ml ethanol (JT Baker, Phillipsburg, NJ)
Lychee Aroma & Flavor[2]	30 ml syrup from canned lychees (Walong Markering Inc, Buena Park, CA)

[1]Bulk white wine used for standard preparation was Franzia Refreshing white wine (Ripon, CA).
[2]This attribute was only evaluated in the Gewürztraminer wines.

On the first day of training, panelists were instructed on how to use the 15-cm line scale. Proper evaluation techniques for smelling and tasting were also demonstrated. Panelists were also instructed to thoroughly cleanse their palates between each sample with water and unsalted saltine crackers and to expectorate samples into cuspidors rather than swallowing the samples. The panelists were then introduced to the Riesling aroma standards. The standards were evaluated and discussed as a group to ensure the panel was in agreement that all the prepared standards were accurate examples of each aroma attribute and were appropriate intensities.

The second training session reviewed the aroma attributes and standards presented in the first training session, with sweet, sour and ethanol ('burn') standards introduced. The panelists also evaluated and discussed the fined wine samples. The third training included a review and discussion of all the aroma, taste and mouthfeel attribute standards previously introduced. The panelists then evaluated five Riesling samples for these attributes and then there was a discussion of each sample evaluated.

The flavor attribute standards were introduced during the fourth training session, and the panelists evaluated and discussed these standards as well as two fined Riesling samples. During this session, two combination standards were also introduced; a sweet and sour standard and a floral and fruity standard, the dosage levels for these standards were the same as the solitary standards listed in Table 4. These were provided as additional practice to panelists who could then evaluate both of these attributes in one standard, as opposed to alone in the standard solutions. These attributes could enhance each other when they are evaluated in the same sample, so the combined standards helped panelists practice focusing on a single attribute in a more complex matrix.

In the fifth training session, the panelists reviewed all the standards and then evaluated and discussed the five fined Riesling wines for all of the attributes. The panelists had a practice evaluation using the computers during the sixth training session in order to learn how to use the software and acclimate to the sensory facility before the final evaluations. After this practice evaluation, the panelists received feedback on their sample evaluations in the form of the panel means and standard deviation from which information the panelists could determine their deviation from the panel means. The panelists also evaluated identical samples and following these evaluations, discussed these two samples to demonstrate their own replicate error. Following the seventh training session, the final evaluations of the Riesling wines took place.

Following the evaluation of the Riesling wines, the panelists received three additional training sessions for the additional Gewürztraminer attribute of lychee aroma and flavor (Table 4). The panelists evaluated and discussed six Gewürztraminer fined wine samples. During the ninth training session, the panelists reviewed all the standards and evaluated and discussed the six Gewürztraminer samples again. The tenth session was a practice session using the computers in the sensory facility. The panelists received feedback on the panel means and their standard deviations from the sample evaluations on the computers during the eleventh training session. Following this session, the panelists conducted the final evaluations of the Gewürztraminer wines.

IV. Volatile Analysis

A. GC/MS Methodology & Equipment specifications

The gas chromatography-mass spectrometry (GC/MS) analysis was performed using an Agilent Technologies 6890 series gas chromatograph coupled with a mass

spectrophotometer MS 5975C (inert XL MSD) and equipped with a 6890N GC split/splitless injector. The GC chromatographic column was a HP-5MS (5% Phenyl Methyl Siloxane) (30 m x 0.248 mm x 0.25µm film thickness) (Agilent Technologies Inc., Santa Clara, CA) connected to the split/splitless injector with helium as the carrier gas. Data collection was performed by Chemstation software version E.02.00.493.

The GC/MS analyses were automated using a CTC CombiPal autosampler (Zwingen, Switzerland), which was programmed using a CycleComposer software (version A.01.04, Agilent Technologies Inc., Santa Clara, CA). The autosampler was equipped with two sample trays, a temperature controlled agitator tray and a fiber-conditioning device.

The same GC/MS methodology was used as previously described by Sanborn (2008) for the volatile analysis of WA State white wines. A polydimethylsiloxane/divinylbenzene (PDMS/DVB) coated fiber for an automated holder was conditioned for 30 minutes at 250ºC. This fiber was then used to sample the headspace of 2 ml wine samples with 0.65 g (6 M) NaCl in 4 ml amber glass vials, capped with Teflon-coated silicon septum. Twelve samples were placed in the autosampler tray to await sampling, giving the samples reasonable time to equilibrate at ambient temperature. Sample vials were taken from the autosampler tray to the agitator to perform the headspace extraction for 45 min at ambient temperature. The fiber was desorbed into the GC port, with an injector temperature of 200ºC. The fiber was desorbed into the splitless injection port for 5 minutes. The oven program was as follows: 33ºC for 5 minutes, 5ºC/min to 50ºC, 2ºC/min to 225ºC, with 13 min hold time at 225ºC. The MS detector temperature was maintained at 230ºC, with all data collected

in SCAN mode from a mass range of 35 to 550 m/z. Compounds were identified using retention times that were identified by running pure standard volatile compounds and confirmed using the NIST library (version 2.0d) in the Chemstation software.

The control wines were first analyzed using GC/MS to determine aroma compounds that could be quantified in the wines. In addition, compounds commonly found in Riesling and Gewürztraminer wines as identified in the literature (Ribéreau-Gayon et al. 2000; Jackson 2000; Flores et al. 1991; Webster et al. 1993) were also targeted for quantification. The compounds that were identified for quantification in both wines are listed in the results and discussion portion of this study.

Pure volatile standards of all the target compounds were analyzed using the GC/MS at several concentrations with the internal standards 1-pentanol and 1-dodecanol (Sigma Aldrich) at concentrations of 10 ppm and 0.5 ppm, respectively to create a calibration curve for quantification of compounds in the wines. The internal standards were used to create reliable calibration curves and minimize experimental error. These particular standards were selected based on their reliable recovery using the GC/MS and because of their general absence in wine. The calibration curves used the ratios of the volatile standard peak areas to the peak area of one of the internal standards as the independent variable. The use of internal standards has been shown to help minimize variability in peak areas caused by column noise or replicate variability (Fan et al. 2007; Sanborn 2008; Webster et al. 1993). All the volatile standards were analyzed in duplicate at six different concentrations (Sanborn 2008).

The wines were analyzed in triplicate with the internal standards used to quantify all the volatile compounds present in each treatment. The compounds were identified

based on the retention times determined from the pure standards, and confirmed using the NIST library.

V. Statistical Analysis

All statistical analyses were conducted using SAS version 9.1 (Cary, NC) computer software. An alpha value of 0.05 was used for all calculations and Fisher's LSD was used as a method for means separations between significant treatments. For the analytical measurements (TA, pH, SO_2, ethanol, and turbidity), two-way ANOVA was employed for each wine, where repetition and fining agent were the independent variables and the analytical measurements were the dependent variables.

For all sensory panels, data were collected electronically using Compusense®*five* software (Guelph, Ontario). Two-way ANOVA was used to determine if any significant differences were found during the consumer acceptance panel, with consumer and fining agent being the independent variables and acceptance being the dependent variable. For the trained panel, data from each attribute were subjected to three-way ANOVA, considering panelist, fining agent, replicate and their interactions to determine if there were any significant differences between any of the wine fining agents. To determine differences in volatile compound compositions between the fining agents, a one-way ANOVA was performed.

CHAPTER 4

RESULTS AND DISCUSSION

I. Winemaking

Riesling and Gewürztraminer juice and final wine were evaluated chemically to characterize the must and wine, as well as monitor the fermentations. For both wines during fermentation, the Brix was measured daily to track the progress. The Gewürztraminer wine displayed an extended lag phase prior to the start of fermentation as initially, the fermentation room was too cool and the yeast were not active (Figure 3). This fermentation was moved to a warmer room on Day 7, with the fermentation starting on Day 8. The remainder of the fermentation proceeded without any problems.

The Riesling wine initially had a faster fermentation rate than anticipated due to a higher yeast inoculation rate (Figure 4). Some bunch rot was observed on the Riesling grapes, a sign of microbial contamination by yeast and mold. Bunch rot results in a higher level of native microflora present on the grapes (Ribéreau-Gayon et al. 2000). The Riesling juice was also allowed to settle a day longer than anticipated, allowing some microbial growth in the juice containers. Because of these factors, when the juice was inspected under a microscope, a high level of native yeasts were observed, leading to the decision to increase the recommended yeast inoculation rate in order to suppress the activity of native yeast present in the wine (Fugelsang and Edwards 2007). Thus, the faster fermentation rate may be attributed to the higher yeast inoculation rate that was applied. Despite these conditions, the fermentation was successful and no more unfavorable microbial growth was observed in the Riesling as evaluated by the absence of off-flavors or off-odors. No worrisome changes in appearance were observed,

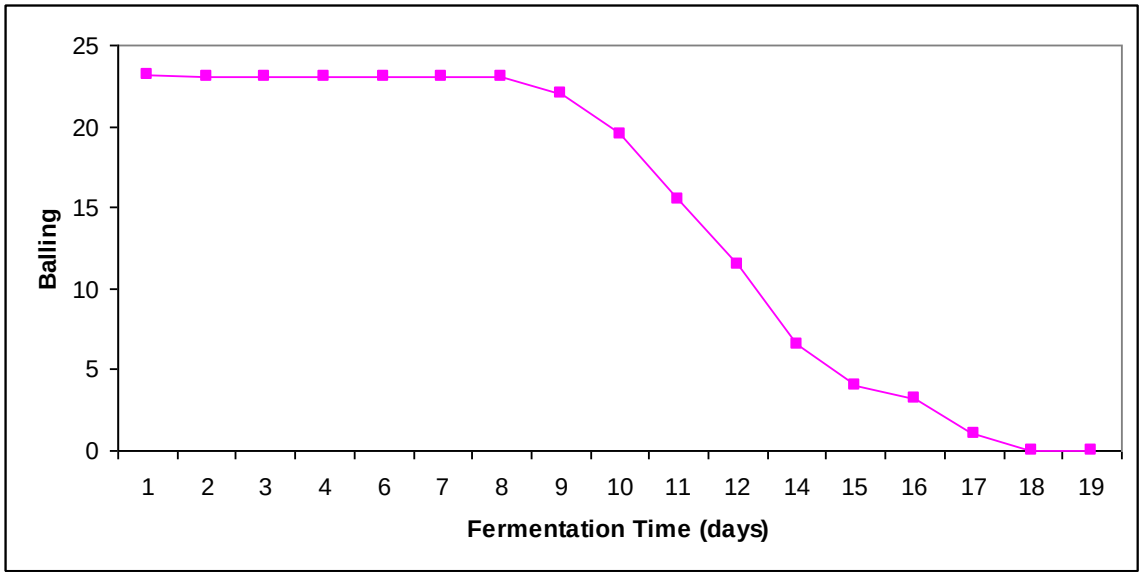

Figure 3. Balling changes over time (days) during fermentation of Gewürztraminer wine. Fermentation conducted at 21ºC (±2 ºC) and each data point represents the mean value of three measurements.

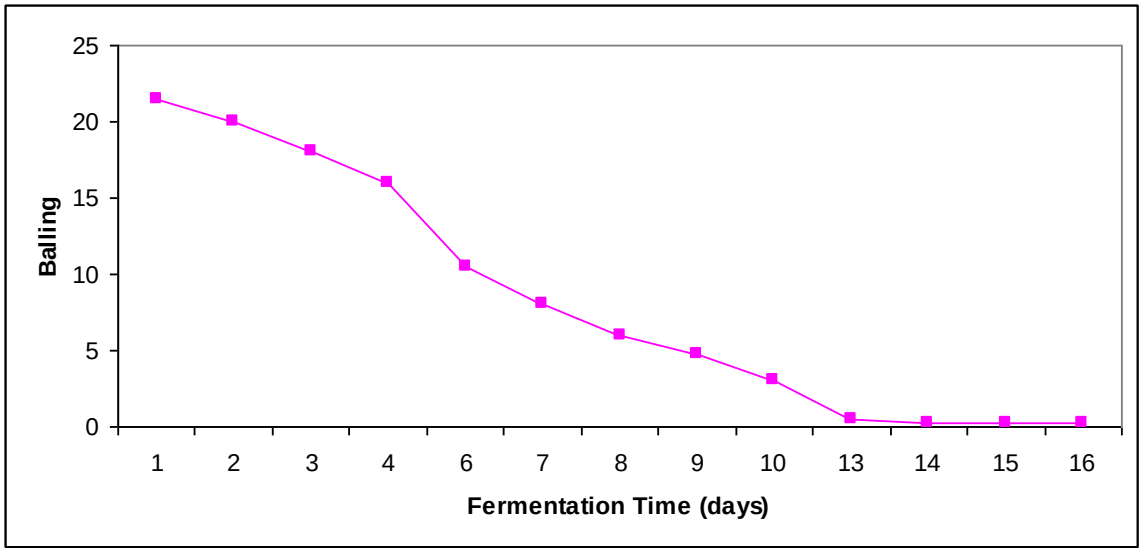

Figure 4. Balling changes over time (days) during fermentation of Riesling wine. Fermentation was conducted at 21ºC (±2 ºC) and each data point represents the mean value of three measurements.

including the growth of mold or mildew as an indication of contamination.

In addition to monitoring the Balling level of the Riesling and Gewürztraminer during fermentation, other analytical measurements were taken, including titratable acidity (TA), pH, SO_2 concentration, and ethanol concentration (Table 5). The pH was significantly different between the wine and juice for both varietals. In Gewürztraminer, the pH was significantly higher in the wine than in the must while the opposite relationship was observed in the Riesling. The fermentation process causes changes in wine composition, such as the production of weak acids that can shift wine pH from the initial value in the must. Carbon dioxide is generated during the fermentation process, and can form weak acids in the wine. Different varietals are composed of different concentrations and types of acids (tartaric, malic), so the fermentation process may affect acid composition differently in each varietal. The SO_2 concentration most likely varied slightly from the values presented in Table 5 as it was constantly being adjusted through the addition of potassium metabisulfite to maintain a level of 20 ppm total.

TA measurements were also taken of both wines. In the wines, the TA was measured before and after the fining treatments were applied to determine if the fining agents impacted the level of acid in the wine (Table 6). For both the Riesling and Gewürztraminer wines, the TA decreased after settling, fining agent addition and cold stabilization. During cold stabilization, tartaric acid crystallizes out of the wine and is removed by subsequent filtration. The removal of the tartrate crystals may have been responsible for the decrease in TA. Since the fining agents react with compounds in the wine via electrostatic interaction, adsorption, or bond formation, it was hypothesized that the concentration of acids in the fined wines may be decreased compared to the unfined

Table 5. Brix, pH and total SO_2 measurements of Riesling and Gewürztraminer must and wine prior to fining agent addition. RS is abbreviated for residual sugar and was measured with Clinitest tablets. Mean values of triplicate measurements are shown followed by the standard deviation in parentheses. No standard deviation value indicates a standard deviation of zero. Within each row, values followed by different letter superscripts are significantly different at $p<0.05$.

	Gewürztraminer		**Riesling**	
	Juice	**Wine**	**Juice**	**Wine**
pH	3.46^{a}	3.65^{b}	3.24^{c}	3.13^{d}
Brix	23.2	<0.2% RS	21.5	<0.2% RS
TA (g acid/100 mL sample)	0.41	0.63	0.76	1.02
Total SO_2 (mg/L)	NA	24.5	NA	18.7

Table 6. Titratable acidity measurements (TA; expressed as g/L) of the Riesling and Gewürztraminer wines following application of fining agents. Mean values of triplicate measurements are shown. Within each column, values followed by different letter superscripts are significantly different at $p<0.05$.

	Gewürztraminer	Riesling
Treatment	**TA (g acid/ 100mL wine)**	**TA (g acid/ 100mL wine)**
Control (Unfined)	0.52^{c}	0.94^{a}
Bentonite	0.49^{c}	0.88^{d}
Soy Milk Powder	0.69^{a}	0.90^{c}
Plantis Fine	0.54^{bc}	0.91^{bc}
Plantis AF	0.55^{bc}	NA
Blankasit	0.59^{b}	0.92^{ab}

wine. This phenomenon was in fact observed in all the wines except for the Gewürztraminer fined with soy milk powder. The fining agents were in contact with the wines while they were also being cold stabilized, so the fining agents could have decreased the level of acidity in the wines. The soy milk powder could also contain some acids that could have leached into the wine while they were in contact with one another (Liu and Chang 2004; Xu and Chang 2009). The differences in TA between wine treatments were not very large so they are most likely due to the occurrence of some level of secondary fermentation in the carboys or to differences in tartrate precipitation and stabilization, rather than the impact of fining agents. Because the wines were not treated with fining agents in replicate, we can not conclusively determine the exact source of variation between the treatments, as it could also be the result of experimental error. The lack of replicate treatments prevents this study from having the ability to claim all the results seen were statistically repeatable.

II. Fining Trials

A. Preliminary Fining Trial

After the wines had completed fermentation, a preliminary fining trial was conducted to determine the appropriate fining agents and dosages to be applied in the bulk fining treatment study. Figure 5 depicts a plot of fining agent concentration versus turbidity to demonstrate how the fining agent dosage was selected for the bulk fining treatments. The lowest fining agent dosage that yielded the lowest turbidity was selected for the bulk treatment (Bird 2005). At this dosage, there is enough fining agent present to bind to the available protein molecules in the wine, removing the unstable proteins with minimal excess fining agent applied to the wine.

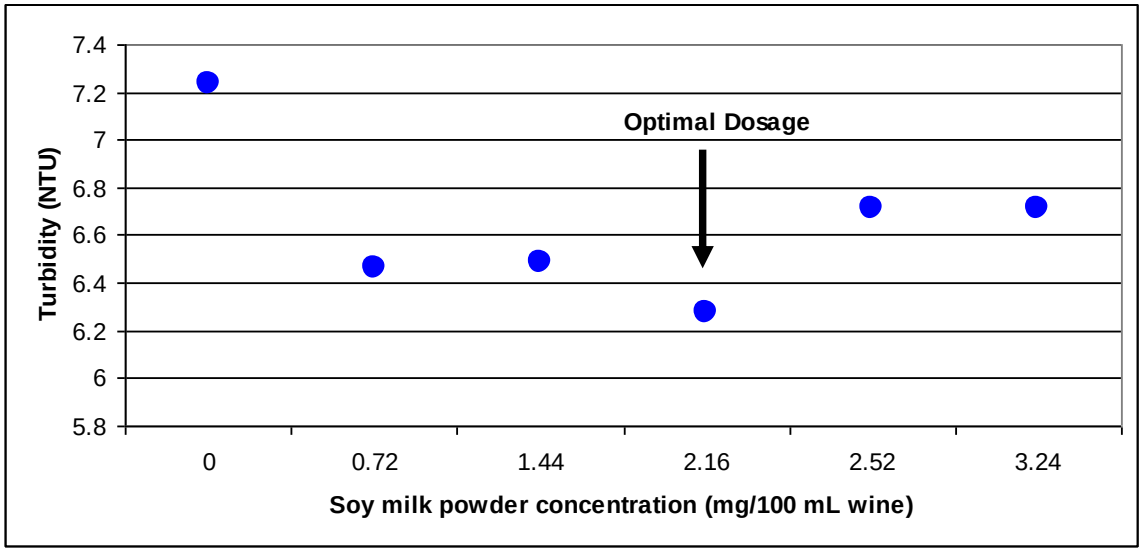

Figure 5. Graphical representation of determination of optimal soy milk powder (SMP) to add to Gewürztraminer wine during a preliminary fining study. In determining the optimal fining agent dosage, five concentrations of SMP were added to a sample of wine, and allowed to settle for 14 days before their turbidities were measured to determine the lowest fining agent dosage that resulted in a wine with the lowest turbidity. Turbidity was expressed as NTU. From the above example, the optimal dosage of SMP required for fining was 2.16 mg/100 mL of wine. The concentration range of SMP was determined from Fischerleitner et al. (2003).

The fining agents and dosages that yielded the best results in the preliminary fining trial are listed in Table 7 along with the resulting turbidities of the wines following the application of the fining agents. The fining trial samples that used the selected fining agents and dosages were subjected to a heat stability test (Pocock and Rankine 1973) to determine protein stability under adverse storage conditions. After the wines were heated for six hours at 80ºC and allowed to cool, they were visually inspected for haze formation and their turbidities were measured. None of the Riesling samples showed any visual signs of haze, but did have slightly higher turbidity levels as a result of the heat treatment. The Gewürztraminer treated with soy milk powder, Plantis AF, and the untreated wine all showed some slight haze formation and their turbidities measured over 10 NTU. For all fining agents, NTU measurements increased following heat treatment.

B. Bulk Fining Treatment

The unfined Gewürztraminer and Riesling wines had initial turbidities of 493 NTU and 37.3 NTU, respectively. Gewürztraminer wines tend to have more problems with haze formations and thus the large difference between the two wines in initial turbidities was anticipated (Waters et al. 2005). The Gewürztraminer in the present study had a higher initial turbidity compared to the WA State Gewürztraminer wine from the previous fining study (Sanborn 2008). The Gewürztraminer in the previous study had an initial turbidity of 211 NTU; however this wine had been rough filtered prior to the application of the fining agents thus reducing its initial turbidity, which may have contributed to the lack of many significant differences in the study.

For both wines, the application of fining agents reduced the final turbidities of the wines compared to the unfined controls. In the Riesling, the unfined control had the

Table 7. Dosages of fining agents applied to Riesling and Gewürztraminer wines and their turbidity levels, expressed as NTU following 14 days of settling. Turbidity values (NTU) of each wine following the heat stability test are also presented. Mean values of triplicate measurements are shown. Within each column, for each wine varietal, values followed by different letter superscripts are significantly different at $p<0.05$.

Wine	Fining Agent	Dosage	Turbidity (NTU)	Heat Stability[A] (NTU)
Gewürztraminer	Control	NA	7.24^{a}	17.93^{b}
	Bentonite	150mg/100ml	0.45^{f}	0.86^{f}
	Soy Milk Powder	2.16mg/100ml	6.28^{b}	16.73^{d}
	Plantis Fine	15mg/100ml	5.66^{c}	16.83^{c}
	Plantis AF	30mg/100ml	5.43^{d}	15.37^{e} (0.06)
	Blankasit	30µl/100ml[B]	4.66^{e}	19.50^{a}
Riesling	Control	NA	3.15^{a}	5.96^{c}
	Bentonite	100mg/100ml	0.26^{e}	0.46^{e}
	Soy Milk Powder	3.24mg/100ml	2.36^{c}	10.57^{a}
	Plantis Fine	25mg/100ml	1.24^{d}	3.00^{d}
	Blankasit	30µl/100ml[B]	2.56^{b}	6.22^{b}

A-Heat Stability was measured as described by Pocock and Rankine (1973).
B-Blankasit was added as a volume as suggested by the manufacturer. No concentration of fining agent was provided by the manufacturer.

highest turbidity while bentonite yielded the lowest turbidity level ($p<0.05$; Table 8). Soy milk powder yielded wine with the second highest turbidity level compared to the unfined control. Although all of the wines had turbidities well below 10 NTU at bottling, the unfined control Riesling exhibited some visible precipitation following approximately 6 months of storage at 4ºC.

A study by Cabello-Pasini et al. (2005) also found that bentonite was the most effective fining agent for removing unstable proteins from white wines when compared to protein-based fining agents. The authors concluded that bentonite was successful because it is negatively charged, and therefore highly attracted to unstable proteins, which are generally positively charged. This explanation may also explain why the plant-based protein fining agents used in the present study were not as effective at producing a heat stable wine as bentonite was. These agents (Plantis Fine, Plantis AF, and soy milk powder) are positively charged, and thus are not as effective at removing wine proteins. A study by Maury et al. (2003) using plant-based fining agents (white lupin powder, wheat gluten, and hydrolyzed wheat gluten) found that the unfined control wine had the smallest decrease in turbidity as a result of gravity settling compared to all wines treated with fining agents.

In Gewürztraminer, different fining agents also yielded different results (Table 9). Wine fined with bentonite had the lowest turbidity, just as observed in the Riesling wine ($p<0.05$). However, in the Gewürztraminer wines, the wine fined with Blankasit had the highest turbidity, even higher than the unfined control. The Gewürztraminer fined with Blankasit had the longest contact time with the fining agent at 22 days because the turbidity did not reach below 10 NTU until this time. This long contact time suggests

Table 8. Turbidity measurements (NTU), days of contact and heat stability of Riesling wines following bulk fining. Values with different letter superscripts are significantly different at $p<0.05$. Days of contact refers to number of days the fining agent was in contact with the wine. Haze formation represents presence (+) or absence (-) of haze following storage at 80ºC for 6 hours.

Treatment	Turbidity (NTU)	Days of Contact	Haze Formation
Control	3.05^{a}	7	+
Bentonite	0.19^{e}	7	-
Soy Milk Powder	1.40^{b}	7	+
Plantis Fine	0.25^{d}	7	+
Blankasit	0.27^{c}	7	+

Table 9. Turbidity measurements (NTU), days of contact and heat stability of Gewürztraminer wines following bulk fining. Within the turbidity column, mean values of triplicate measurements are presented. Values with different letter superscripts are significantly different at $p<0.05$. Days of contact refers to number of days the fining agent was in contact with the wine. Heat stability is represented by presence (+) or absence (-) of haze formation following storage at 80ºC for 6 hours.

Treatment	Turbidity (NTU)	Days of Contact	Haze Formation
Control	0.66^{c}	13	+
Bentonite	0.33^{f}	7	-
Soy Milk Powder	0.87^{a}	13	+
Plantis Fine	0.60^{d}	13	+
Plantis AF	0.53^{e}	13	+
Blankasit	0.82^{b}	22	+

that Blankasit did not bind with the proteins in the Gewürztraminer as efficiently as the other fining agents to remove the unstable proteins from the wine. The presence of these unstable proteins would thus result in a higher turbidity level compared to the unfined wine. It is also possible that the dosage determined in the preliminary fining trial was not as effective for the bulk treatment, as it is difficult to accurately replicate all bulk fining parameters, which is necessary for accurate dosage determination (Weiss et al. 2001). If the dosage determined in the preliminary study was too high, excess Blankasit could remain in the wine without binding to any wine proteins, increasing the turbidity from that of the unfined control wine (Bird 2005).

As performed in the preliminary fining trial, the heat stability test was performed on the finished wine. Results showed haze formation in all the wines except for those treated with bentonite. Although the heat stability test involves extreme temperature abuse, these results suggested that all the wines except those treated with bentonite could potentially develop protein hazes during storage. There are several possible reasons why these results differed from the heat stability testing conducted on the preliminary fining trial samples. First, the wines in the bulk fining study had different contact times than those in the preliminary fining trial, which could have generated differences in the amount of protein remaining in the wines. The second reason is that the samples from the preliminary fining trial were not decanted before they were subjected to the heat stability test. Thus there may have been some sediment formation that was generated during the heat stability test that was undetected due to the presence of sediment already in the jars. In addition, the smaller wine volumes used in the preliminary fining trials

could have also allowed for more thorough mixing of the fining agents, resulting in more complete contact between the wine and the fining agents.

Wine color was measured using the L*a*b* method to determine if there were differences in color (Pérez-Caballero et al. 2003). The L* component of this measurement indicated the amount of black present in a sample where L* of 0 indicates completely black. The a* component represents the level of green or magenta (negative values indicate green and positive values indicate magenta) while the b* component represents yellow and blue intensity with negative values indicating more blue and positive values indicating more yellow.

Significant differences were found between treatments for all three color components in both the Riesling and the Gewürztraminer wines. For the Riesling, the wine fined with Plantis Fine and bentonite had the highest L* value ($p<0.05$), indicating that it was the lightest in color, while the control unfined wine had the lowest L* value (Table 10), indicating it was the darkest in color. This result was expected because fining agents can remove some colors from wines (Lopez-Toledano et al. 2007). Because the unfined wines did not have a fining agent applied, they did not have the removal of any colored compounds, possibly resulting in a darker wine.

Principal components analysis of the color values is shown in Figure 6. PC 1 was defined primarily by the L* color component while PC 2 was more defined by the contrast between a* and b* color values. The Plantis Fine and bentonite fined wines were the wines most strongly correlated with the L* color component. The Blankasit-fined Riesling was defined by its higher L* and b* values and its negative relationship with the a* parameter. These results indicate that it was the most green wine in

Table 10. Color values of Riesling wines where L* indicated lightness of color (L* of 0 indicates black), a* represented the amount of green or magenta (negative values indicate green, positive values indicate magenta), and b* represented the position between yellow and blue (negative values indicate more blue, while positive values indicate more yellow). Within a column, values with a different letter superscript are significantly different at $p<0.05$.

Treatment	L*	a*	b*
Control	98.30^{c}	-0.54^{a}	6.00^{b}
Bentonite	98.93^{a}	-0.647^{ab}	5.26^{c}
Soy Milk Powder	98.43^{c}	-0.587^{ab}	6.34^{a}
Plantis Fine	99.0^{a}	-0.70^{b}	4.93^{d}
Blankasit	98.77^{b}	-0.88^{c}	5.89^{b}

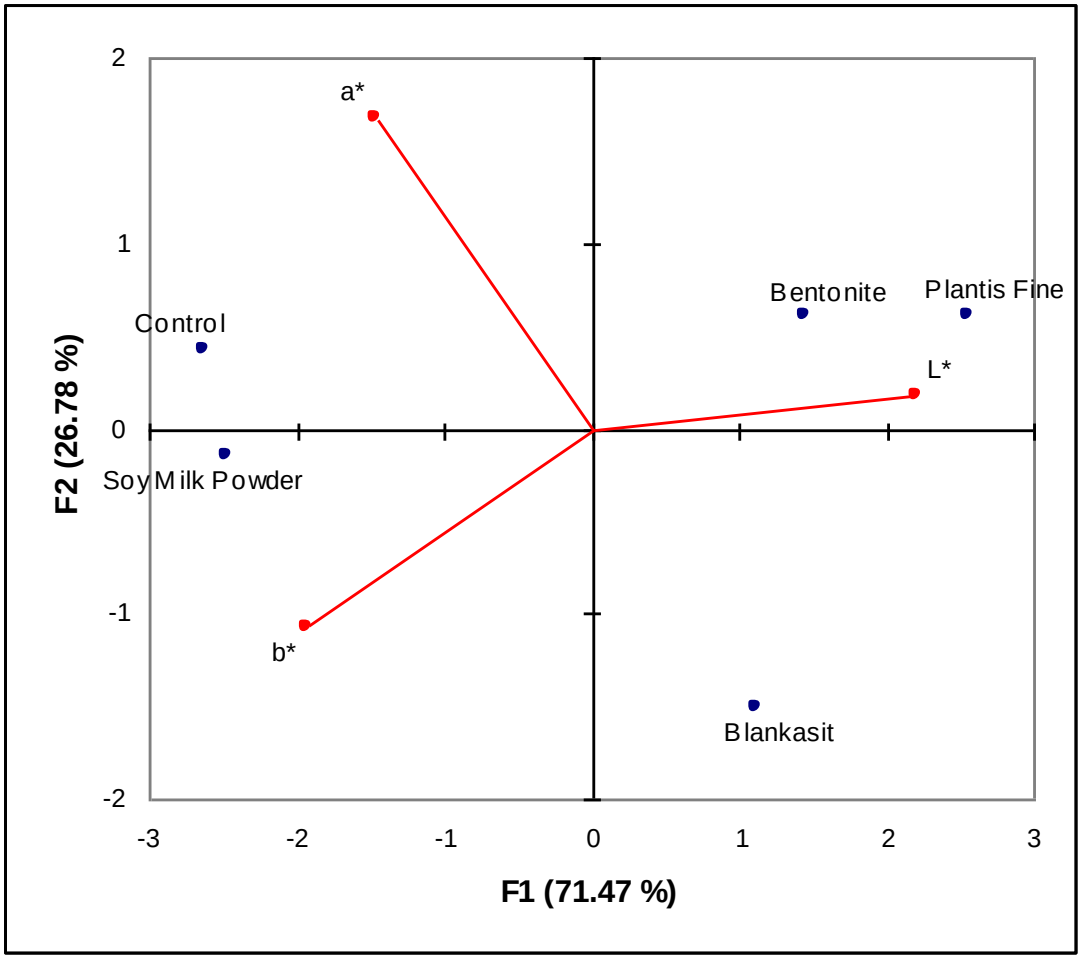

Figure 6. Principal component analysis (PCA) of L*a*b* color evaluation in Riesling wines.

appearance. The control unfined wine and the soy milk powder fined wines were both positively correlated with the a* and b* parameters and also negatively correlated with the L* parameter, suggesting that these wines were darker than the rest of the treated wines. The control wine was slightly more strongly associated with the a* parameter, while the soy milk powder wine was slightly more correlated with the b* parameter, meaning the control had more magenta or reddish hues in its color, while the soy milk powder fined wine had a slightly more yellow hue.

The soy milk powder was likely not as effective at removing color as the bentonite fining as soy milk powder itself is susceptible to oxidation and color changes that could decrease its capability to remove color compounds. The PCA plot (Figure 6) demonstrates the true magnitude of difference in color between all the wines as a result of all three color parameters in conjunction, which better demonstrates how the human eye perceives the color of the wines which were noticeably different upon visual examination.

In the Gewürztraminer wines, differences were also observed between fining agents (Table 11). Principal components analysis of the color values is shown in Figure 7. PC 1 was defined primarily by the b* color component while PC 2 was defined by the contrast between the L* and a* color components (Figure 7). The wines treated with soy milk powder and Plantis Fine were most strongly associated with the L* parameter, indicating they are the lightest in color. Both these wines are also weakly correlated with the a* parameter, meaning they have some slight magenta or reddish tones to their color.

The Plantis AF fined wine was most strongly correlated with the a* parameter, indicating it has the most magenta/red hue of all the wines, although it did not appear red

Table 11. Color values of Gewürztraminer wines where L* indicated lightness of color (L* of 0 indicates black), a* represented the amount of green or magenta (negative values indicate green, positive values indicate magenta), and b* represented the position between yellow and blue (negative values indicate more blue, while positive values indicate more yellow). Within a column, values with a different letter superscript are significantly different at $p<0.05$.

Treatment	L*	a*	b*
Control	98.70^{e}	-0.78^{d}	5.68^{a}
Bentonite	99.0^{b}	-0.60^{ab}	4.24^{e}
Soy Milk Powder	99.13^{a}	-0.683^{bc}	4.403^{d}
Plantis Fine	99.0^{b}	-0.66^{ab}	4.73^{c}
Plantis AF	98.80^{d}	-0.59^{a}	4.75^{c}
Blankasit	98.90^{c}	-0.76^{cd}	5.35^{b}

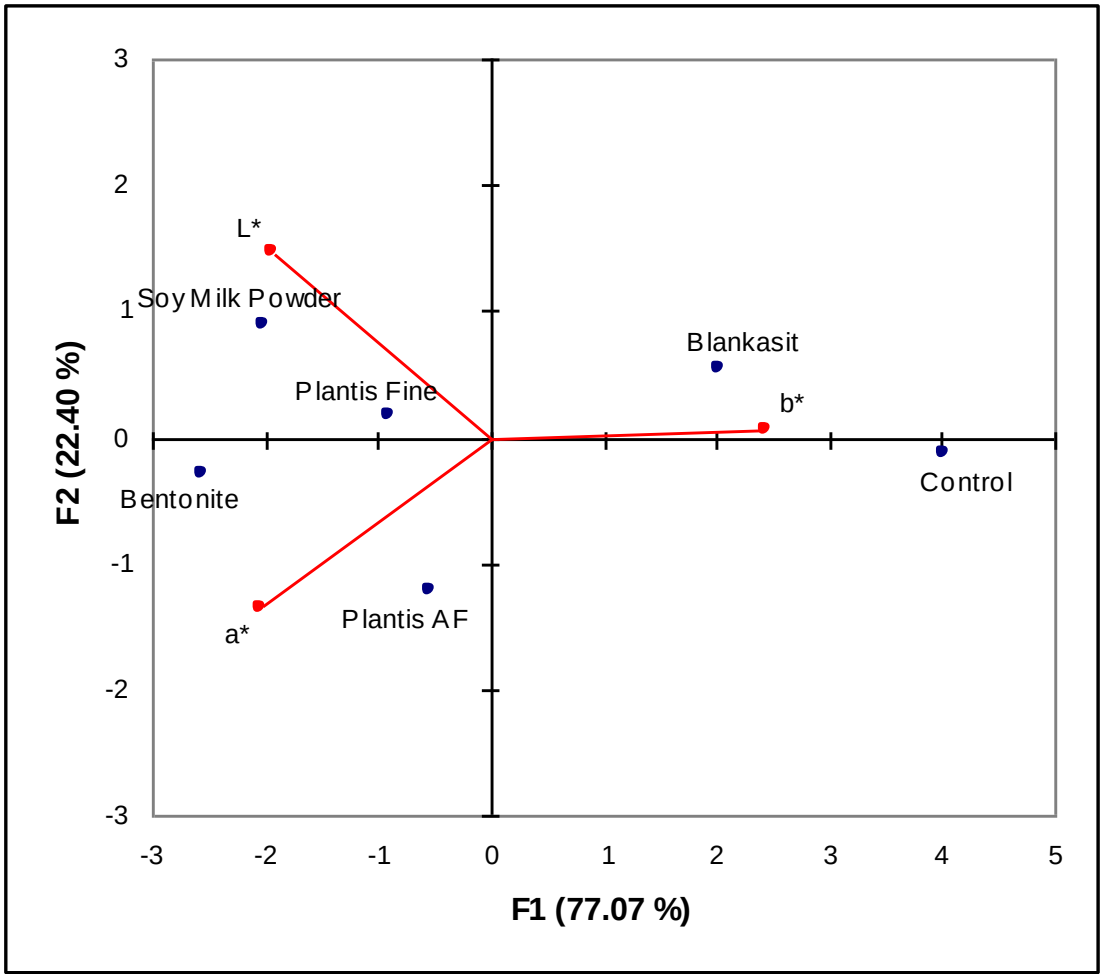

Figure 7. Principal Component Analysis (PCA) of L*a*b* color evaluation in fined Gewürztraminer wines.

visually. The wine fined with bentonite, on the other hand, was characterized by a negative relationship with the b* parameter, indicating that its hue was less yellow (more towards the blue end of the spectrum) than the other wines. The bentonite-fined wine was also weakly positively correlated with the L* and a* parameters.

The Gewürztraminer wines fined with Blankasit and the unfined control were the most strongly associated with the b* parameter, which shows they had a more yellow hue than the other wines. The control had a slightly stronger correlation with the b* parameter than the Blankasit-fined wine. These two wines were also negatively correlated with both the L* and a* parameters, indicating that they were both darker with more green hues compared to the other treatments. Dark colors in white table wines are generally considered undesirable by winemakers and consumers (Razmkhab et al. 2002). Razmkhab et al. (2002) also found significant color differences, measured by absorbance at 420 nm, between unfined wines and wines fined with dehydrated yeast cells. However, for the Gewürztraminer wines in this study, although the color differences were visually detectable, these differences did not significantly impact consumer acceptance of any of the wines.

It is important to note that the lack of replication of the fining treatments is a weakness of this study. Due to this fact, all measurements presented in this study are actually repeated measures of a single experimental unit and not true triplicate measurements of different experimental units. Consequently, all results presented must be viewed with caution as it can not be concluded with certainty that these results are statistically repeatable and not the result of experimental error. However, because the trends and results found did not deviate drastically from those found in previous studies

in literature, this study can be used as a valuable preliminary study of novel fining agents and their behavior in WA State white wines.

III. Sensory Evaluation of Gewürztraminer and Riesling Fined Wines

A. Acceptance Panel

Consumer acceptance panels were held to determine the acceptability of the Gewürztraminer and Riesling wines fined using the different fining agents. The consumers rated their overall acceptance of the wine, as well as acceptance of the wines' appearance, aroma, flavor, and mouthfeel.

The mean acceptance ratings for each attribute for all the Gewürztraminer fining treatments are shown in Table 12. No significant differences were found between any of the Gewürztraminer wines. However, the Gewürztraminer fined with Plantis AF was rated the highest for overall acceptance, but was not rated highest for any single attribute and the wine fined with Plantis Fine was rated the lowest for overall acceptance ($p<0.05$). The wine treated with Blankasit was rated the highest by consumers for acceptance of appearance and flavor, while the control unfined wine was rated the highest for aroma and mouthfeel acceptance. The soy milk powder fined Gewürztraminer was rated lowest for appearance and mouthfeel acceptance and the bentonite-fined wine was rated lowest for aroma, and the Plantis Fine wine was rated lowest for flavor acceptance. As this was a consumer acceptance panel, personal preference played a large role in consumers' decisions on acceptability thus it was not unexpected that significant differences in acceptance were not observed (Meilgaard et al. 2007).

In evaluating the acceptance of Riesling wines, consumers showed a significant difference in acceptability of appearance between wines fined with different agents

Table 12. Mean consumer (n=100) overall acceptance ratings and acceptance ratings of appearance, aroma, flavor and mouthfeel of Gewürztraminer wines fined using five different agents. Evaluations were collected along a 7-pt hedonic scale with 1=dislike very much and 7=like very much.

Treatment	Appearance	Aroma	Flavor	Mouthfeel	Overall Acceptance
Control	5.6	5.1	4.4	4.6	4.5
Bentonite	5.5	4.6	4.3	4.4	4.3
Soy Milk Powder	5.4	4.9	4.3	4.4	4.3
Plantis Fine	5.6	4.8	4.3	4.4	4.2
Plantis AF	5.5	5.0	4.4	4.4	4.5
Blankasit	5.7	4.8	4.5	4.4	4.4

(Table 13). The appearance of the control wine (unfined) was significantly less accepted compared to the other fining treatments ($p<0.05$). No significant differences in the acceptance of the other attributes were observed. As determined analytically, the significant differences in color and turbidity correlate with the results of the consumer acceptance panel. The unfined Riesling displayed the highest L* value, indicating that it was the darkest in color, together with the highest b* value, indicating that it was a golden color. These results are supported by results published by Lopez-Toledano et al. (2007), who found that consumers prefer wines that are lighter in color compared to those darker in color. In previous fining studies, differences in acceptance have been reported between wines fined with different fining agents. Fischerleitner et al. (2003) found that vegetable protein fining agents transferred unfavorable flavors and aromas to red and white wines. These flavors and aromas were detectable by trained sensory panelists who not only evaluated wines for specific sensory attributes but also evaluated wines for quality using a 5-point scale, where 0 indicated negative qualities and 5 indicated positive qualities. The wines fined with vegetable protein fining agents were rated lower than wines fined with soyaprotein, lupin protein, gelatin, albumin, casein, isinglass and whey protein along the 5-point scale, indicating a lower quality wine.

B. Trained Panel

A trained sensory panel was used to evaluate the intensity of aroma, flavor and mouthfeel sensory attributes of the Riesling and Gewürztraminer wines. In the Riesling wines, the trained panelists found no significant differences in any of the attributes evaluated (Table 14). No significant differences were found between replicate evaluations of the wines.

Table 13. Mean consumer (n=79) overall acceptance ratings and acceptance ratings of appearance, aroma, flavor and mouthfeel of Riesling wines fined using four different agents. Evaluations were collected along a 7-pt hedonic scale with 1=dislike very much and 7=like very much. Values within a column marked with a different letter superscript were significantly different at $p<0.05$.

Treatment	Appearance	Aroma	Flavor	Mouthfeel	Overall Acceptance
Control	5.2^{a}	4.5	4.1	4.3	3.9
Bentonite	5.4^{b}	4.9	4.2	4.5	4.3
Soy Milk Powder	5.4^{b}	4.9	4.3	4.3	4.2
Plantis Fine	5.5^{b}	4.9	4.2	4.3	4.2
Blankasit	5.7^{b}	4.7	4.3	4.3	4.3

Table 14. Mean intensity ratings along a 15-cm unstructured line scale of aroma, flavor, taste and mouthfeel attributes of Riesling wines fined using different fining agents, as evaluated by a trained panel (n=11).

Sensory Attribute	Control	Bentonite	Soy Milk Powder	Plantis Fine	Blankasit
Aroma Attributes					
Floral	9.4	8.8	9.2	8.7	9.6
Fruity	8.3	8.3	8.3	8.2	8.7
Vegetal	3.8	3.8	3.7	3.8	3.6
Alcohol/Chemical	7.8	7.8	7.5	8.0	7.8
Yeasty	2.6	3.3	2.8	3.0	2.7
Taste and mouthfeel attributes					
Sweet	7.4	7.3	7.5	7.4	7.3
Sour/Acid	8.3	8.4	8.4	8.0	8.3
Ethanol Burn	6.9	6.3	6.7	6.9	6.4
Flavor attributes					
Floral	8.6	7.9	8.4	8.5	8.4
Fruity	7.9	7.9	8.1	8.0	8.2
Vegetal	3.7	3.4	3.1	3.2	3.2
Alcohol/Chemical	8.4	8.1	7.6	8.1	7.9
Yeasty	2.4	2.7	2.5	2.5	2.3

Due to the presence of significant panelist effects observed in this study, these results should be interpreted with caution. Stone and Sidel (2004) state that in order to effectively describe attribute differences between products using untrained panels, descriptive techniques require seven to ten hours of training. In the present study, panelists participated in nine hours of training, with feedback provided during the training process. However, a significant panelist effect was still observed. The panelist effect in this study is likely due to differences in panelist perception or differences in scale usage by the panelists.

Individuals also have different levels of sensitivity to odors or have unique anosmias to odorants that cannot be eliminated through training (Deibler and Delwiche 2004). Through initial screening of the panelists, it was determined that none of the panelist had anosmias to the compounds evaluated in this study although differences in sensitivity between panelists were observed. The number of odors an individual can identify at any one time depends on their inherent ability as well as their level of experience (Amerine and Roessler 1976). Experience can be gained through extensive training, but individual ability cannot be taught. The most common reason that an individual is unable to identify an aroma is due to lack of knowledge and experience with that particular odor. A typical wine contains approximately 200 odorants, and each of these odors has the potential to mask another, have little effect on one another, or act synergistically and intensify each other (Amerine and Roessler 1976). This abundance of aromas in wines makes it difficult for panelists to focus on a single aroma attribute and detect small differences that can be found through chemical analysis.

The Gewürztraminer wines were evaluated after the Riesling wines, and the panelists received an additional two hours of training prior to their evaluations (Table 15). No significant differences were found between replicate evaluations, while a significant panelist effect was observed. The only attribute found to significantly differ between treatments was the floral flavor, with the control and Blankasit having the highest floral flavor compared to the other fining agents ($p<0.05$). The volatile compounds associated with floral flavor and aromas are generally highly volatile, and therefore more likely to be lost or bound by fining agents, explaining why the unfined wine had the highest floral flavor compared to the fined wines. Blankasit also had a high floral flavor compared to the rest of the fining agents, and since it had the longest fining agent contact time, it is proposed that it was unable to bind all unstable proteins and perhaps did not bind as many volatile compounds either compared to other fining agents. Another study by Armada and Falqué (2007) also found that a silica-based fining agent increased many volatile concentrations in Albariño wines (another aromatic varietal), so more work should be done to determine the mechanism of action between silica and volatile compounds.

Previous studies have shown some sensory differences between wines fined with different agents. Flores et al. (1991) compared bentonite-fined Gewürztraminer and Riesling wines to an unfined control. In the Gewürztraminer, cooked vegetative attributes were significantly higher and chemical flavor was significantly lower in the control wine compared to the bentonite-fined wines. However, no differences were found between the Riesling wines, similar to the results in the present study. A previous fining study conducted on WA State Gewürztraminer and Chardonnay found few sensory

Table 15. Mean intensity ratings along a 15-cm unstructured line scale of aroma, flavor, taste and mouthfeel attributes of Gewürztraminer wines fined using different fining agents, as rated by a trained panel (n=11). Within a row, values with different letter superscripts were found to be significantly different at $p<0.05$.

Sensory Attribute	Control	Bentonite	Soy Milk Powder	Plantis Fine	Plantis AF	Blankasit
Aroma attributes						
Floral	9.6	9.0	8.7	9.2	9.4	9.1
Fruity	8.6	8.2	8.0	8.3	8.5	8.6
Vegetal	2.9	2.7	2.9	2.8	2.7	2.8
Alcohol/Chemical	8.0	7.9	8.0	8.3	7.3	7.6
Yeasty	2.2	2.3	2.2	2.3	2.2	2.2
Lychee	7.2	6.5	6.5	6.6	7.1	7.5
Taste and mouthfeel attributes						
Sweet	7.2	6.9	6.8	6.5	7.1	7.1
Sour/Acid	7.0	7.4	7.4	7.5	7.0	7.1
Ethanol Burn	7.6	6.6	7.0	6.8	6.6	6.9
Flavor attributes						
Floral	9.1^{a}	8.4^{b}	8.0^{b}	8.3^{b}	8.2^{b}	8.6^{ab}
Fruity	7.8	7.7	7.6	7.7	7.7	7.8
Vegetal	2.6	2.6	2.6	2.7	2.4	2.7
Alcohol/Chemical	8.7	8.5	8.4	8.6	8.0	8.4
Yeasty	2.1	2.2	2.2	2.2	2.2	2.3
Lychee	6.5	5.8	5.6	6.0	5.9	6.0

differences between the wines fined with different fining agents (Sanborn 2008). In Gewürztraminer, no significant sensory differences were shown between wines treated with different fining treatments including activated carbon, bentonite, wheat gluten, isinglass, whole milk, sparkalloid and an unfined control. In Chardonnay, differences were observed by trained panelists between wines treated with different fining agents, with the whole milk and wheat gluten-fined wine producing a wine with higher spicy aroma and floral/honey flavor.

The lack of significant differences in the present study found by the trained sensory panelists is likely due to the low concentrations of many of the volatile compounds in the wines, as well as the small differences in volatile concentrations between treatments. Most of the volatile compounds in the wines were present at concentrations well below their detection thresholds. A major exception was ethyl acetate, associated with nail polish remover aroma (Acree and Arn 2004), which was present at a concentration much higher than its detection threshold. In the present study, the panelists were trained to detect a fruity aroma that was presented as pear or peach aroma with the aroma standard. It is possible that panelists could have detected a difference in vinegar or nail polish remover aroma had they been trained to detect and evaluate this attribute.

However, the lack of more lengthy training may also have contributed to the absence of significant differences between treatments for the sensory attributes in the wines. Significant differences were observed between Gewürztraminer treatments, which had the benefit of additional training, which may have been beneficial for the panelists in the evaluation of the Riesling treatments as well.

IV. Volatile Analysis

To select and screen the volatile compounds that would be quantified in the wines, unfined Riesling and Gewürztraminer wines were initially analyzed using GC/MS. Literature was also consulted to determine appropriate compounds to track between different fining treatments (Ribéreau-Gayon et al. 2000; Jackson 2000; Flores et al. 1991; Webster et al. 1993).

Following the initial screening, pure aroma standards of the selected compounds were analyzed with the added internal standards, 1-pentanol and 1-dodecanol to generate the calibration curves (Table 16). These internal standards were selected based on their unique retention times (they did not co-elute with other compounds in the wine) and their absence from the wines. The internal standards were added to each wine sample prior to analysis by GC/MS so that the selected volatile compounds in each sample could be quantified. Most of the linear coefficient values (r^2) were greater than 0.90, indicating a strong linear relationship between concentration and resulting peak area. Low linear coefficient values were observed for 3-methyl-1-butanol and ethyl dodecanoate. The lower linear coefficient for 3-methyl-1-butanol was attributed to its low boiling point and similar retention time to that of ethanol. Whereas the lower linear coefficient for ethyl dodecanoate was attributed to its tendency to stick to the column, causing build-up and carry-over between replicate runs.

The volatile compounds determined in the Gewürztraminer wines are shown in Table 17. Overall, the fining agents tended to decrease the concentrations of the volatile compounds present, with the exception of the soy milk powder. Soy milk powder served to increase the level of many of the volatile compounds ($p<0.05$).

Table 16. Volatile compounds quantified in wine samples via gas chromatography/mass spectrometry (GC/MS) and their calibration curve equations, where x is the log of the volatile concentration (mg/L) and y is the log of the ratio of volatile's peak area to the internal standard's peak area. The internal standard 1-pentanol was used to generate calibration curves for the first half of the chromatogram (up to RT = 25 min, compounds ethyl acetate to hexyl acetate) and 1-dodecanol was used for RT from 25 to 60 min. These calibrations curves were used to quantify volatile compounds in both Riesling and Gewürztraminer wines.

Compound	Retention Time (min)	Calibration Range (mg/L)	Calibration Curve Equation	r^2
ethyl acetate	3.32	0.5-10	y = 1.01x - 2.3582	0.97
2-methyl-1-propanol	3.61	0.5-10	y=.8605x-2.7456	0.98
3-methyl-1-butanol	6.55	0.5-10	y=.8101x-1.8053	0.85
2-methyl-1-butanol	6.65	0.5-10	y=0.7111x-1.6953	0.98
ethyl butanoate	8.72	0.5-10	y=0.7151x-0.8144	0.98
1-hexanol	11.89	0.5-10	y=0.8311x-1.1476	0.99
3-methyl-1-butanol acetate	12.27	0.5-5	y = 0.7174x - 0.3684	0.97
ethyl hexanoate	19.88	0.5-10	y=0.64x+0.1383	0.96
hexyl acetate	20.74	0.5-10	y=0.6086x+0.201	0.94
benzene ethanol	27.85	0.5-10	y=1.0659x-1.1751	0.98
ethyl octanoate	34.22	0.5-10	y=0.7546x+0.6042	0.93
2-phenylethyl acetate	37.94	0.5-10	y=0.8406x+0.027	0.96
Beta-damascenone	46.63	0.5-10	y=0.7835x+0.5206	0.92
ethyl decanoate	47.82	0.5-10	y=0.7227x+0.747	0.91
ethyl dodecanoate	60.01	0.5-10	y=0.8103x+0.6501	0.83

Table 17. Volatile compound concentrations (mg/L) found using gas chromatography/mass spectrometry (GC/MS) in Gewürztraminer wines fined using different fining agents. Values within a row that have a different letter subscript are significantly different at $p<0.05$. ND (not detected) represents a concentration that was below the level of quantification.

Volatile Compound	Control	Bentonite	Soy Milk Powder	Plantis Fine	Plantis AF	Blankasit
ethyl hexanoate	0.13^{bc}	0.11^{c}	0.25^{ab}	0.13^{bc}	0.13^{bc}	0.34^{a}
ethyl octanoate	0.96	0.66	1.55	1.16	0.97	1.17
ethyl decanoate	0.28	0.14	0.43	0.30	0.18	0.27
ethyl dodecanoate	0.01^{a}	ND^{c}	0.004^{ab}	0.003^{bc}	0.002^{c}	0.001^{c}
ethyl acetate	56.70	73.95	73.32	69.54	69.14	45.18
benzene ethanol	24.79	26.66	37.38	22.52	24.59	20.68
3-methyl-1-butanol	224.80^{b}	235.80^{b}	217.60^{b}	196.70^{b}	195.20^{b}	599.10^{a}
1-hexanol	0.28^{b}	0.30^{b}	0.57^{ab}	0.29^{b}	0.30^{b}	0.88^{a}
2-methyl-1-butanol	8.65	ND	13.53	10.89	ND	32.96
3-methyl-1-butanol acetate	0.20	0.22	0.40	0.20	1.79	0.55
phenylethyl acetate	0.04	0.05	0.08	0.05	0.05	0.05

Four specific volatile compounds significantly differed between fining agents ($p<0.05$). The volatile compound that was present in the highest concentration in the Gewürztraminer was 3-methyl-1-butanol, which is associated with a whisky/malt or burnt aroma (Acree and Arn 2004). This compound is one of the most common higher alcohols present in wine and is impactful on the sensory profile of wines and wine spirits (Amerine and Roessler 1976). 3-Methyl-1-butanol was significantly higher in the wine treated with Blankasit compared to all other treatments and the control. This was likely due to the fact that Blankasit had a longer contact time in the Gewürztraminer than any other fining agent, and probably did not remove as many unstable proteins or volatile compounds as other treatments did.

Three other compounds significantly differed between wines treated with different fining agents. A significant difference in the concentration of 1-hexanol was found between treatments. This compound is associated with a green or unripe aroma (Acree and Arn 2004). 1-Hexanol originates from the grape tissue itself so it is commonly present in wines, especially if any unripened grapes were used to make the wine (Ribéreau-Gayon et al. 2000). The concentration of 1-hexanol in the Gewürztraminer treated with Blankasit was significantly higher than all other treatments except for the wine fined with soy milk powder, likely due to the lack of strong binding between the fining agent and volatile compounds and proteins in the wine. Ethyl hexanoate concentrations were also significantly different between Gewürztraminer treatments, and was highest in the Blankasit and soy milk powder fined wines ($p<0.05$). Ethyl hexanoate contributes a fruity, apple peel aroma (Acree and Arn 2004). The final compound that varied significantly in concentration in the Gewürztraminer wines was

ethyl dodecanoate. Ethyl dodecanoate has a leafy aroma (Acree and Arn 2004) and was not detectable (ND) in the bentonite-fined wine, while it was found in the highest concentration in the control and soy milk powder fined wines.

Ethyl hexanoate and ethyl dodecanoate are ethyl acetates of fatty acids, which are synthesized by yeast during alcoholic fermentation (Ribéreau-Gayon et al. 2000). These compounds tend to decrease in concentration during ageing because they are easily hydrolyzed (Ribéreau-Gayon et al. 2000). They are associated with sweet, pleasant odors that contribute to a favorable white wine bouquet. Published aroma descriptors for all aromas targeted for quantification in this study can be found in Table 19 along with their odor detection threshold values. Differences in volatile composition between fining treatments can be contributed to the differences in binding affinity of the fining agents with different wine compounds. For instance, bentonite is negatively charged, while the protein-based fining agents are primarily positively charged, so they attract different compounds (Zoecklein 1999).

For all fining treatments of the Gewürztraminer wines, a relatively high concentration of ethyl acetate was found compared to the levels of other volatile compounds in the wines. This compound is associated with a nail polish remover or vinegar aroma (Acree and Arn 2004); however no significant concentration differences were found for this particular compound. Ethyl acetate is the most common ester found in wine (Ribéreau-Gayon et al. 2000). It is formed by yeast activity and also by acetic bacteria during aging.

Benzene ethanol was also present in the Gewürztraminer wines at a higher concentration than most other compounds without significant differences between

Table 19. Volatile aroma compounds quantified in Riesling and Gewürztraminer wines, their published aroma descriptors and odor threshold detection values.

Volatile Compound found in Riesling and Gewürztraminer wines	**Published Aroma Descriptor[a]**	**Odor Threshold**
ethyl acetate	vinegar, nail polish remover	0.008 ug/L (in water)[b]
2-methyl-1-propanol	wine, solvent, bitter	8.2 x10^{8} molecules/cm^{3} (in air)[b]
2-methyl-1-butanol	malt	0.04 mg/L (in air)[b]
3-methyl-1-butanol	whiskey, malt, burnt	30 mg/L (in wine)[c]
ethyl butanoate	apple	1.50 x10^{10} molecules/cm^{3} (in air)[b]
1-hexanol	green	5.20 mg/L (in water)[b]
3-methyl-butanol acetate	banana, fruity	1.50 mg/L (in wine)[d]
2-methyl-1-butanol acetate	fruit	1.50 mg/L (in wine)[d]
ethyl hexanoate	apple peel, fruit	0.08 mg/L (in wine)[d]
benzene ethanol	honey, spice, rose, lilac	900 mg/L (in wine)[d]
Linalool	flower, lavender	6.0 ug/L (in water)[b]
ethyl octanoate	flower, fat	1.15 mg/L (in water)[b]
2-phenylethyl acetate	rose, honey, tobacco	1.8 mg/L (in wine)[d]
Nerol	sweet	0.5 mg/L (in wine)[e]
L-α-terpineol	oil, anise, mint	1.0 mg/L (in wine)[e]
ethyl decanoate	grape	1.10 mg/L (in other)[b]
ethyl dodecanoate	leaf	0.64 mg/L (in other)[b]
Hexyl acetate	fruit, herb	2.0 ug/L (in water)[b]
β-damascenone	apple, rose, honey	0.002 ug/L (in water)[f]

[a] Acree and Arn (2004)
[b] Fazzalari (1978)
[c] Guth (1997b)
[d] Peinado et al. (2004)
[e] Zea et al. (2001)
[f] Buttery (1993)

treatments. This compound lends an aroma of spice, honey, rose, or lilac (Acree and Arn 2004). In Riesling and several other white wine varietals (Cayuga white, Vidal blanc, and Seyval blanc), Chisholm et al. (1994) found benzene ethanol to be a major odor-active compound. Sanborn (2008) also found benzene ethanol in both the WA State Chardonnay and Gewürztraminer wines. Perhaps because benzene ethanol was present at high concentrations, which lends a floral aroma to wine, panelists were able to detect small differences in floral character between wine treatments, even though there were not significant differences in the concentration of benzene ethanol.

While the Riesling wines had more quantified volatile compounds that were detectable than the Gewürztraminer, two compounds showed significant differences between treatments, ethyl decanoate and ethyl dodecanoate (Table 18). Both the ethyl decanoate (grape aroma) and the ethyl dodecanoate (leaf aroma) were significantly higher in the unfined control wine compared to the other fining treatments, suggesting that the application of fining agents reduces the concentration of these compounds. However, the concentrations of these compounds were below odor threshold detection values in all treatments (Table 19). Thus while these compounds did significantly differ between treatments, these differences did not manifest in the sensory profile of the wines. The other ethyl esters were also higher in the unfined control ($p>0.05$) suggesting that these compounds may be removed by the application of fining agents.

The compounds that were present in the Riesling at the highest concentrations were 3-methyl-1-butanol, ethyl acetate, and benzene ethanol, similar to those compounds in the Gewürztraminer wines. In general, most of the volatile compounds were present in the wines in very low concentrations.

Table 18. Volatile compound concentrations (mg/L) found using gas chromatography/mass spectrometry (GC/MS) in Riesling wines fined using different fining agents. Values within a row that have a different letter subscript are significantly different at $p<0.05$. ND (not detected) represents a concentration that was below the level of quantification.

Volatile Compound	Control	Bentonite	Soy Milk Powder	Plantis Fine	Blankasit
ethyl acetate	75.78	66.54	61.75	72.69	63.16
ethyl butanoate	0.04	0.04	0.05	0.04	0.04
ethyl hexanoate	0.18	0.15	0.19	0.14	0.15
ethyl octanoate	1.30	0.58	0.93	0.83	0.84
ethyl decanoate	0.54^{a}	0.17^{b}	0.32^{b}	0.26^{b}	0.30^{b}
ethyl dodecanoate	0.05^{a}	0.001^{b}	0.01^{b}	0.003^{b}	0.01^{b}
beta-damascenone	0.02	0.02	0.02	0.02	0.03
phenylethyl acetate	0.08	0.06	0.06	0.07	0.06
3-methyl-1-butanol acetate	0.29	0.27	0.32	0.26	0.27
hexyl acetate	ND	ND	ND	ND	ND
2-methyl-1-butanol	5.17	5.80	15.44	14.70	5.68
3-methyl-1-butanol	217.83	205.26	227.82	201.82	192.97
1-hexanol	0.50	0.48	0.56	0.46	0.48
benzene ethanol	19.74	15.83	21.17	22.19	18.69

The differences found in the volatile analysis of the wines were not reflected in the sensory evaluation results. This phenomenon is most likely because the concentrations of the volatile compounds that did differ were insufficient to be detected by either smell or taste. Also, there are likely additional compounds that were not targeted for quantification in this study that were contributing to differences in the sensory profiles of the wines. Hence, the volatile analysis presented does not directly reflect the results of the sensory analysis. It is also difficult to predict how the different volatile compounds enhance or suppress perception of one another in the wine matrix. The volatile compound odor threshold values can be altered when aromas are mixed together. In these mixtures, volatile aroma compounds can be classified into one of three different categories of relative importance based on its behavior: impact, contributing, or insignificant compounds (Jackson 2000). Impact compounds are the volatile compounds that have a distinct aroma and lend varietal character or pronounced aroma to a wine. Contributing compounds are those volatiles which add to overall wine complexity, and they are also important for the aging process and add to the bouquet during aging (Jackson 2000). Since most of the volatile compounds in the wines in this study were present at concentrations below threshold values (Table 19), their combined aroma is more impactful and perceptible to panelists than a single aroma compound. If they were alone in solution, their aroma would be insignificant, however when they are combined they contribute to the overall wine bouquet (Jackson 2000).

CHAPTER 5

CONCLUSIONS AND FUTURE WORK

I. Conclusions

As was previously mentioned, the lack of replicate fining agent treatments makes the results of this study applicable as a preliminary study for future work, but the results can not be viewed as statistically repeatable. There was a great deal of valuable information gained from the extensive work in this study that will provide a foundation for future fining studies in WA State white wines.

From the information gained in this study, it was determined that fining agents impact the chemical and sensory properties of white wines. However, these effects were minimal and did not have a significant impact on the acceptability of the wines as determined by consumers. The fining agents applied in this study improved the stability of Riesling during storage, as was observed by the formation of sediment in the unfined Riesling wine following 6 months of storage at 4ºC. Sediment is a characteristic that would be unacceptable to a majority of consumers so the application of fining agents in this study was effective for improving consumer acceptability, even if those results were not reflected in the initial consumer acceptance panel.

Bentonite proved to be the most effective fining agent on both the Riesling and Gewürztraminer wines as evaluated by turbidity measurements and heat stability testing (Pocock and Rankine 1973). Bentonite was also the only fining agent that did not generate sediment or haze as a result of the heat stability test. The commercially available plant-based fining agents evaluated in this study did not appear to be as effective as bentonite in stabilizing white wine, but they also did not negatively impact

the sensory properties of the white wine. However, before these alternative fining agents can be competitive with bentonite, additional experimental work needs to be performed. Although they did not transfer negative sensory properties to the wines, they were also less effective wine clarifiers and stabilizers, as witnessed by the heat stability testing and changes over storage. Thus additional studies are recommended to improve the protein absorption capabilities of these fining agents. Also, bentonite is currently inexpensive and easily obtainable, whereas plant-based fining agents are only available through a few select suppliers and are more expensive than bentonite. While bentonite does have several advantages, it does present challenges during wine addition, such as large lees volumes and lengthy hydration time. Thus the development of a fining agent that is more easily added to wine is desirable.

II. Discussion of future studies

From this study, additional information can be gleaned about the effects of fining agent application on white wines. In order to determine the impact of ageing on the wines and the effectiveness of the fining agents over time, the wines remaining from this study should be stored and allowed to age for at least a year, and then evaluated again using both sensory and chemical methods. It is possible that small differences in the young wines may become more noticeable and impactful over time. During aging, unstable proteins can bind with one another or with volatile compounds, resulting in the formation of sediment in the bottled wines or the removal of specific volatile compounds. Other published fining studies cited in this thesis have not evaluated sensory properties of the wine before and after aging. Allowing the wines to age may also reveal differences in the effectiveness of the fining agents over time. The results of the heat stability test in

this study suggest that some of the wines, with the exception of the wine treated with bentonite, may form hazes over time. As any haze formation would negatively impact the quality of the wines, the winemaker needs to be confident that the fining agent that (s)he applies will not result in haze formation during aging.

Other future studies should include an investigation of just noticeable differences (JND) in wine aroma caused by changes in the volatile composition of volatile compounds present in wine near the odor detection threshold values. The change in JND in volatile compounds, both in a single mixture and mixtures with other volatile compounds, should be evaluated. The results of this study and the previous study in WA State white wines (Sanborn 2008) showed that changes in volatile composition did not necessarily yield corresponding sensory differences. Additional sensory studies should be conducted to determine how the degree of change in volatile compound concentration impacts aroma and flavor perception by trained or untrained panelists. Also, this fining agent study did not determine any fining agents that resulted in wines that were unacceptable to consumers. An additional study examining the level of sediment or haze that makes wines unacceptable to consumers would be of interest to the wine industry.

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