

EFFECT OF PRE-FERMENTATION TECHNIQUES ON BLANC DU BOIS

A Thesis

by

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ABSTRACT

Blanc Du Bois accounts for a significant growth segment of the Texas Wine Industry as it thrives in the terroir and is disease resistant. Variability in the sensory profiles of Blanc Du Bois wines have been observed. This study examined pre-fermentation methods influencing sensory characteristics and volatile composition of Blanc Du Bois wines. The must was treated with PVPP, and hyper oxygenation prior to alcoholic fermentation. They were then rated by a descriptive analysis sensory panel. Panelists developed a lexicon of 24 aromas and 26 flavors. The wines were analyzed for the intensity of each attribute once panelists were trained with references for each attribute on a 16-point scale calibration. Gas chromatography mass spectrometry with dual sniff ports (GC-MSO) was used to assess the volatile composition. Data were analyzed with analysis of variance (ANOVA), principal component analysis (PCA), and partial least squares (PLS) regression.

Significant differences were observed in the wines by treatments and vintage, and correlations between attributes, volatiles, and wines were observed in PCA and PLS models. The first vintage was associated with floral, apricot, green, alcohol/fermented, buttery and sour attributes; and esters, organic acids, alcohols, and a hydrocarbon volatiles. The second vintage was associated with the fruity, citrusy, sweet, vinegar, and malty attributes, and hyper oxygenated and control wines associated with esters, and one alcohol, while the PVPP wine correlated mostly with alcohols and esters, two organic acids, miscellaneous sulfur compounds, and one hydrocarbon.

Results indicate that pre-fermentation treatments had minimal impact on the wines, both sensorily and chemically. Although pre-fermentation treatments were effective in improving overripe tropical fruit aroma and alcohol flavor and a reduction in acetic acid was observed, it did not have a large impact on sensory and chemical characteristics and may have little impact on wine quality and style.

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CHAPTER I

INTRODUCTION

The Texas wine industry currently contributes over \$2.27 billion in economic value to the State of Texas (Frank 2017) and is continuing to grow. While some regions of Texas have successfully produced high quality *Vitis vinifera* grape cultivars, the warm, humid climates commonly found in Texas due to the proximity of the Gulf of Mexico are not ideal due to higher disease pressure and accelerated fruit ripening leading to an imbalanced fruit chemistry and reduced fruit quality. The cultivation of wine grapes that are resistant or tolerant to diseases is of increasing importance.

American hybrid grape cultivars that are disease resistant or tolerant have been identified (Buzombo et al. 2006). Cultivars, such as Blanc Du Bois, have demonstrated the ability to tolerate Pierce's disease, black rot, and powdery mildew while maintaining high yield and high fruit quality potential (Buzombo et al. 2006). Approximately 156 acres of Blanc Du Bois wine grapes are being grown in the Texas Hill Country, Southeast Texas, the Gulf Coast, and North Texas (Office 2015). Blanc Du Bois grapes create a "spicy" and "fruity" white wine that has grown in popularity over the last decade, and can be made into various styles of wine including Madeira, sweet, semi-sweet, off-dry, and dry (Westover 2012). However, significant variability in flavor and aroma has been observed. Dreyer et al. (2013) examined fourteen Blanc Du Bois wines from three states using descriptive analysis (DA) and reported flavors ranging from citrusy, bitter, and greenwood/stemmy to sweet, fruity, and floral flavors. Those wines described as sweet, fruity, and floral were found to be of higher quality (Dreyer et al. 2013) and the observed

differences in flavor profiles were attributed to varied viticultural and winemaking techniques. Common training methods utilized for Blanc Du Bois include Vertical Shoot Positioning (VSP), Smart-Dyson, Watson, and Geneva Double Curtain (GDC) training systems (Westover 2013). Grapes are commonly harvested between 16° to 23° Brix, a pH of 3.2 to 3.6, and a titratable acidity (TA) of 4.8 to 10 grams per liter (Westover et al. 2013). Winemaking techniques include long and cold alcoholic fermentations to various levels of dryness with a variety of yeasts being used. Depending on the style of wine being made, malolactic fermentation and blending may be done (Westover et al. 2013).

Although a range of winemaking techniques and flavor profiles have been observed in Blanc Du Bois, little is known about what fermentation methods produce wines with a desirable sensory profile and volatile composition.

It is common knowledge that volatile and phenolic compounds greatly influence the color, aromas, and flavors of wine and that the presence and concentration of these compounds is affected by the genotype of grape, climate, soil composition, vineyard management practices, and pre- to post-fermentation techniques. Because pre-fermentative techniques, such as PVPP fining and hyperoxygenation, have been shown to improve the quality, color, flavor and aromatic volatile composition, sensory characteristic stability, and the storage/aging ability of wine, these strategies may have potential to improve Blanc Du Bois wine (Artajona et al. 1990; Parish et al. 2016).

CHAPTER II

LITERATURE REVIEW

Blanc Du Bois

Blanc Du Bois is an American hybrid white wine grape that was the outcome of crossing the Pierce's disease resistant hybrid grape, Florida D6-148, and Cardinal (Figure 1) (Mortensen 1987). Its name is a tribute to the French winemaker and grape grower Emile DuBois, who spearheaded grape growing efforts in Florida for over 20 years, planted more than 150 cultivars, and steadily won awards for his Florida wines (Mortensen 1987). Although Blanc Du Bois was originally crossed in 1968 at the University of Florida's Central Florida Research and Education Center by John A. Mortensen, it wasn't released for commercial production until 1987 (Mortensen 1987).

Blanc Du Bois berries are light green, round, juicy, with a muscat flavor, and a slipskin, and the clusters are of medium compactness (Mortensen 1987). Important characteristics of Blanc Du Bois are that it is well adapted to warm, humid climates, matures at a balanced rate, produces a high yield, and is resistant or tolerant to bacterial and fungal diseases on its own roots (Buzombo et al. 2006). The yield potential of Blanc Du Bois is reported as 3 to 8 tons per acre depending on the soil and vine-training method, which is often vertical shoot positioned, bilateral cordon, or Geneva double curtain training systems (Westover 2012). The diseases and pests it is reported to have tolerance to include powdery mildew, *Isariopsis* leaf blight, and Pierce's disease (PD), and additionally is tolerant of grape leaf folder insect pests, and nematodes unless on calcareous soils where it is recommended to graft it onto a PD tolerant rootstock (Mortensen 1987) (Westover 2012). Blanc Du Bois is, however, reported to be susceptible to anthracnose,

black rot, and ripe rot (Mortensen 1987). It is commercially grown in Georgia, South Carolina, Arkansas, Mississippi, Alabama, Louisiana, Texas, and Florida with Texas leading in production (Westover 2012).

Fermentation Techniques, Blending, and Storage

Blanc Du Bois wine is produced in a variety of wine styles and is often generally characterized as a “spicy” or “fruity” white wine. These styles include Madeira, sweet, semi-sweet, off-dry, and dry (Westover et al. 2013). An off-dry to semi-sweet wine style with aromas of tropical fruit and a citrus finish is a common style produced in Texas (Westover et al. 2013).

The most common fermentation techniques for Blanc Du Bois include long and cold alcoholic fermentations between 8.8°C and 13.3°C to produce a wine high in aromatics and with a refreshing crisp citrus to mineral finish (Westover 2012). A range of yeasts are used to produce diverse flavors and aromas. Depending on the style of wine being made, the percent of residual sugar may vary or chaptalization may be done. Some vintners opt to put the wine through secondary fermentation to reduce some of the bitterness or sourness of the wine. Fermentation is done in stainless steel tanks. Again depending on the style of wine being made, the complete fermentation process is done in the stainless steel tank or may be mostly done in stainless steel and then transferred briefly into oak barrels. Oak chips are also used to impart flavor. This is common of the dry or off-dry style of Blanc Du Bois. Racking and fining is the primary means of clarification for Blanc Du Bois although some vintners use filtration instead. It has been noted that cold stabilization is vital for this wine and is usually carried out between -2.22°C and 3.33°C (Westover et al. 2013).

Blanc Du Bois is made into both a varietal wine and a blended wine. Blending is done either after crushing, where the musts are combined and the varieties undergo co-fermentation, or it is completed in the winery after each variety has undergone its own fermentation. Winemakers blend as little as 75% Blanc Du Bois and 25% of a *Vitis vinifera* variety (Westover et al. 2013). Some *Vitis vinifera* varieties that have been blended with Blanc Du Bois include Trebbiano, Semillion, and Viognier (Westover et al. 2013). Some vintners observe that a more consistent and favorable wine flavor is created by blending the wine (Westover et al. 2013).

The most significant factor affecting wine quality and aging potential is temperature. The recommended storage temperature for most fresh, fruity wines is less than 10°C and this is commonly practiced for Blanc Du Bois (Jackson 2008) (Westover et al. 2013). It can occasionally be a challenge to maintain this temperature during transportation and distribution due to warm climate and the Texas heat. If fluctuations or rapid temperature changes occur, the seal between the cork and bottle may be loosened or impaired and oxygen ingress may occur. Oxygen exposure can affect the volatile content of the wine altering the aromas and flavors of the wine. The increase in temperature also accelerates the aging process, chemical reactions, and degradation of carbohydrates present in the wine (Jackson 2008). This results in a brown coloration, baked flavor, and loss in fragrance. For this reason, it is essential to have cold consistent storage conditions for Blanc Du Bois wine.

Wine Chemistry

The grape chemistry resulting from fruit ripeness and maturity dictates the style of wine produced and greatly affects the quality of the grapes, and thus the wine. A number of factors contribute to grape chemistry including the grape varietal, the weather the grapes are exposed to throughout growing season, soil composition, time of harvest, and pruning techniques. Ideally, the winemaker harvests the grapes with balanced chemistry by taking into consideration the °Brix, Titratable Acidity (TA), and pH of the grapes (Jackson and Lombard 1993). The sugar content of the grapes is effected by the species and cultivar, ripeness, and health of the grape and is essential to yeast growth and metabolism. The ideal soluble solids range to harvest white wine grapes is 17-24 °Brix (Jackson 2008).

The acidity of a wine is expressed as pH and TA. While pH is a measurement of the free acid in a wine, the TA is the measurement of both free and bound acids. The TA is indicative of what the perceived acidity will be of the wine in respect to taste (Plane, Mattick, and Weirs 1980). The pH of a must affects yeast activity during fermentation and protects the wine from bacterial spoilage. The ideal range to harvest white wine grapes is a pH of 3.2 to 3.4 with a TA of 6.0-9.0 g/L (Jackson 2008).

Blanc Du Bois grapes are typically harvested at 16° to 23° Brix, a pH of 3.2 to 3.6, and titratable acidity (TA) of 4.8 to 10 g/L (Westover et al. 2013).

PVPP Fining

Fining is an important part of the clarification and stabilization processes in winemaking. During this process, the insoluble particulates and compounds that are suspended in the wine are removed. This is typically done after fermentation and is achieved through the use of fining agents, racking, and filtration, and greatly increases

the clarity of the wine. A variety of additives can be used as fining agents such as egg whites, bentonite, casein, gelatin, sparkolloid, pectic enzymes, and Polyvinylpolypyrrolidone (PVPP). Each of these fining agents function in different ways either through electrostatic, adsorbent, enzymatic, or ionic actions. The main consideration in choosing a fining agent is the tannin content, color, and type/style of wine being made (Jackson 2008).

PVPP is a synthetic polymer that is effective in binding tannins and polyphenols into large macromolecular complexes that are then precipitated and removed through racking, filtration or centrifugation (Lamuela-Raventós, Huix-Blanquera, and Waterhouse 2001). These compounds are responsible for astringency, bitterness, herbaceousness, browning or pinking, and haziness found in some wines (Jackson 2008) (Parish et al. 2016). It extemporaneously precipitates and functions well in cooler temperatures (Jackson 2008). The effects of PVPP fining are dependent upon the grape varietal. A study on muscadine wine found that pre-fermentation treatment with an addition rate of 1.0 g/L PVPP resulted in no significant sensory differences although post-fermentation fining did (Sims, Eastridge, and Bates 1995). However, a separate study using an addition rate of 0.25/0.80 g/L PVPP for free run/pressed Sauvignon blanc grape juice found that pre-fermentation treatment resulted in some significant differences in aromatic volatile compounds (Parish et al. 2016).

Micro-oxygenation, Oxidation, and Hyper oxygenation

Micro-oxygenation

Micro-oxygenation is a winemaking technique where oxygen is introduced into wine at a controlled rate during various phases of the winemaking process. This process

was first developed by Patrick Ducournau, a winemaker from South East France, in the late 80's. It can be achieved in two ways: through the addition of large quantities of oxygen added abruptly at specific phases in the winemaking process, or through lower quantities added slowly and continuously during the winemaking process. During the quick addition method, oxygen is added in large amounts during must extraction, at the beginning of alcoholic fermentation, or after malolactic fermentation is complete prior to bottling. In the slow and continuous addition method, a large amount of oxygen is added initially during alcoholic fermentation but decreases as alcoholic fermentation finishes and then added again in lower quantities at the beginning of malolactic fermentation with the amount of oxygen decreasing to zero as malolactic fermentation finishes (Fargeton 2017). This technique has been shown to be advantageous in improving wine quality and aroma and flavor profiles. These advantages include an increase in color intensity, a reduction of bitterness and vegetable perception without actually decreasing the concentration of 3-isobutyl-2-methoxypyrazine (IBMP), a softening of tannins, an increase of fat and astringency for a more balanced mouthfeel, a better integration of oak characteristics, and a more stable wine with a higher resistance to oxidation during storage and aging (Fargeton 2017). However, the development of these benefits is greatly dependent on the cultivar of grape and the production of acetaldehyde in the right amount (Fargeton 2017). If too much acetaldehyde is produced, oxidation can occur causing wine faults or spoilage.

Oxidation

Oxidation is one of the most common wine faults that can occur during or after the winemaking process. It can result in a loss of color, aroma, and flavor by oxidation

of flavonoids such as anthocyanins, catechins, and epicatechins (du Toit et al. 2006).

Aroma and flavor faults can also be caused by the oxidation of ethanol into acetaldehyde and acetic acid. Although yeast metabolism can produce acetaldehyde and acetic acid as byproducts, their presence in wine is typically related to the oxidation of ethanol. Acetaldehyde is often described as pungent, fruity, green, and sour (Flament 2002), and can be linked to of acetic acid bacteria and surface film yeasts, which lead to acetic acid formation and spoilage. Concentrations of acetic acid above detection can cause wine to take on a pungent, sour, vinegar, and overripe fruit aroma and flavor (Flament 2002). For this reason, sulfur dioxide are often added by winemakers to prevent oxidation from occurring.

Hyperoxygenation

Hyperoxygenation is the addition of oxygen to a non-sulfited must to the point of saturation prior to fermentation (Guerzoni et al. 1981). After hyper-oxygenation, the must is allowed to settle at cold temperatures and is racked before continuing the wine-making process. Minimal sulfur dioxide is used throughout winemaking when using this technique. The resulting color of white wines after fermentation is lighter despite the oxidized must being a dark brown. This is due to the insoluble brown pigments as phenolic compounds precipitating out (Schneider 1998). The resulting wine has more stable sensory characteristics with less bitterness and perceivable astringency due to the removal of flavonoids, and is more resistant to browning and oxidative quality degradation post-fermentation while aging (Macheix et al. 1991; Schneider 1998). Typically, the removal of sufficient flavonoids can be achieved through hyper oxygenation with one oxygen saturation if the juice did not receive any skin contact, but up to three saturations are

needed if the juice did have contact with the skin (du Toit et al. 2006). Hyper-oxygenation affects the volatile composition and aroma and flavor profiles differently depending on the cultivar of grape, must composition, and quantity of oxygen (Cejudo-Bastante et al. 2011). Numerous studies have reported positive effects on the volatile composition and quality of a (Cejudo-Bastante et al. 2012), Chardonnay, Grenache, Mauzac, Moscatel of Alejandría, Penedés white wines (Macabeo and Parrellada) (Cheynier et al. 1989)(Cheynier et al. 1991), as well as other Spanish, and French white wines (Artajona et al. 1990). However, negative effects of hyper-oxygenation have been observed in Chardonnay, Chenin blanc, French Colombard, and Semillon wines due to drastic decrease in the flavonoid and phenolic content, a reduction in aromatic intensity, and less fruity flavor profile (Singleton, Zaya, and Trousdale 1980)(Dubourdieu and Lavigne 1990).

Sensory Evaluation

Sensory evaluation uses experimental design and statistical analysis to assess a consumer products with respect to the human senses of sight, smell, taste, touch and hearing (Meilgaard, Civille, and Carr 2007). Humans are used as the testing instruments to evaluate the product, their responses are recorded, and statistical analysis is conducted. The sensory analyst can then interpret these results to draw conclusions and make recommendations for action on the tested product. There are three main categories of analysis including discriminative testing, descriptive testing, and consumer evaluation (Meilgaard, Civille, and Carr 2007).

Descriptive analysis sensory evaluation involves a trained panel detecting and describing qualitative and quantitative sensory attributes of a product (Meilgaard,

Civille, and Carr 2007). Panelists are required to detect and describe perceived sensory attributes of a product that distinguish it from others including appearance, aroma, flavor, texture, or sound qualities and must differentiate and rate the intensity of each characteristic (Meilgaard, Civille, and Carr 2007).

Dreyer et al. (2013) conducted a sensory evaluation of Blanc Du Bois to assess the principal aromas and flavor profiles of the cultivar. The most common positive wine sensory attributes included apple, tropical fruit, peach, grapefruit, lemon, rose, honey, and sweetness. The negative attributes included greenwood/stemmy, phenolic/rubber, sour, and bitter and some citrus. Higher quality Blanc Du Bois was associated with sweet, fruity, and floral flavors (Dreyer et al. 2013).

Volatile Compounds

Wine is extremely complex in aromatics due to a large variety of volatiles. Over 800 wine aromatics exhibiting differences of polarity, solubility, and volatility have been recognized (Ortega-Heras, González-SanJosé, and Beltrán 2002). These volatile compounds are important for determining the style of the wine. The taste (flavor by mouth), mouth feel, color, and aroma, both pleasant and pejorative, of a wine can all be influenced by these compounds. They are derived from the grapes, vine stems, fermentation, and oak barrels or chips used during fermentation or aging, and can vary based on the cultivar of grape and vineyard (Jackson 2008). These compounds include alcohols, terpenes, organic acids, esters, phenolics, aldehydes, and more with each contributing different aromatic attributes to the wine (Ribéreau-Gayon et al. 2006).

The GC-MS-O is an analytical technique using gas chromatography, and mass spectrometry with an olfactory port to evaluate flavors and aromas, and identify and separate substances into volatile aromatic compounds. Once the volatiles are separated, they are transferred to the olfactory meter where a trained panelist sniffs the aromas as they exit the olfactory port. Using a software program, the panelist can record the aroma as they identify and quantify the attribute and its intensity.

A study analyzing the volatile composition of Blanc Du Bois found that esters and alcohols comprised the vast majority of the non-ethanol or methanol compounds present in the wine (Dreyer et al. 2013).

In the Texas Gulf Coast, Blanc Du Bois wines are often fermented cold ($<15^{\circ}\text{C}$) to obtain a “fruity” flavor profile. However, knowledge of the volatile compounds present in Blanc Du Bois wines that are responsible for desirable and undesirable flavor profiles, how these chemical compounds are influenced by hyperoxygenation, and PVPP additions prior to fermentation, and the correlation between these compounds and the flavor and aroma profile are unknown. This information could prove to be of importance to vintners in Texas and the South that produce Blanc Du Bois, as they are constantly aspiring to create a more enjoyable wine experience for consumers.

The objectives of this study are to investigate the impact of the pre-fermentation practices of PVPP fining and hyper-oxygenation on the aroma, flavor and volatile composition of Blanc Du Bois wine.

CHAPTER III

MATERIALS AND METHODS

Grapes and Juice

The Blanc Du Bois grapes and juice sources were chosen based on availability each year. In 2014, Blanc Du Bois grapes were collected from a commercial vineyard in Mission, TX, and in 2015, Blanc Du Bois juice was donated by Haak Winery in Santa Fe, TX. The grapes from Mission, TX were transported to the Department of Horticulture at Texas A&M University, College Station, TX immediately after harvest. The grapes were crushed, destemmed and pressed upon arrival and the juice was treated with potassium metabisulfite at 145.3 mg/L, then stored overnight at 10°C. The grapes used for the juice donated by Haak Winery were a blend of Blanc Du Bois grapes harvested in Santa Fe, TX and at Austin County Vineyards in Cat Spring, TX. After pressing, the juice was stored overnight at their winery at 12.8°C and shipped the following day to the Department of Horticulture at Texas A&M University, College Station, TX. Upon arrival, the juice was treated with potassium metabisulfite at a rate of 145.3 mg/L and stored at 1.7°C for a period of 5 days.

Juice Chemical Composition

Juice soluble solids were measured using a hand held refractometer (PCE Americas Inc., Jupiter, FL) with temperature correction. Titratable acidity was determined on a 5ml aliquot by titration against 0.1N NaOH to an endpoint of pH 8.2 and expressed as tartaric acid equivalents. The juice pH was measured using a Orion Dual Star pH/ISE meter (ThermoFisher Scientific Inc., Waltham, MA).

The must from 2015 had 19.1°Bx, pH 3.46, and a T.A. of 7.6g/L. The must produced from the Mission grapes had 18.2°Bx, pH 3.9, and a T.A. of 7.23g/L. Citric acid was added to both juices to lower their pH to 3.34 and 3.38, respectively.

Winemaking Treatments

The must was separated into 11.36L carboys. If 11.36L carboys were not available, 9 of the 3.79L carboys were used. The pre-fermentation treatments were conducted at 1.7°C to minimize any additional metabolic activity. The treatments consisted of polyvinylpyrrolidone (PVPP) fining added to the must at a rate of 0.25 g/L, followed by racking 72 hours later, hyperoxygenation (HOX) for twelve hours using an aquarium air pump, and a control. The Control and PVPP carboys were treated with potassium metabisulfite at a rate of 145.3 mg/L to prevent microbial spoilage and oxidation. The hyperoxygenated (HOX) carboy was treated with antifoam, a food grade silicone oil emulsion that reduces frothing, to prevent excessive foaming during aeration. An air stone attached on the end of food grade tubing connected to the air pump (Whisper 20; Tetra Spectrum Brands, Blacksburg, VA) was inserted in the HOX carboy and the must was aerated overnight for twelve hours at an airflow rate of 1L/min. The HOX and PVPP musts were allowed to settle at 1.7°C for 72 hours later. Racking was completed prior to alcoholic fermentation to remove solids.

Alcoholic fermentation was conducted at 7.2-8.9°C using R2 yeast (Scott Laboratories Inc., Petaluma, CA) inoculated at a rate of 0.25g/L. No additional nutrient additions were made prior to fermentation. Wines were fermented to dryness with the fermentation taking 127 days for the 2014 wine and 137 days for the 2015 wine. Dryness

was measured using a hydrometer to obtain a specific gravity 0.995 or less and confirmed, when needed, with a Clinitest urine glucose analysis test kit (Bayer, Whippany, NJ).

After the completion of alcoholic fermentation, the wine was racked and cold stabilized at 1.7°C for ten days to facilitate tartrate precipitation. The wine was racked again and fined using Sparkolloid at a rate of 0.5g/L for 14 days. The wine was racked immediately after fining. At each racking, metabisulfite was added 145.3 mg/L. The headspace of the carboys was sparged with nitrogen after each racking. The wine was then filtered using the Buon Vino Mini Jet Wine Filter (Buon Vino MFG., Cambridge, ON) with a number 3 filter pad and bottled into 375ml wine bottles. Green bottles were used for wine made in 2014 and clear bottles were used for the wine made from the 2015 juice. This allowed for an easy distinction between the two vintages. The bottles were stored at 8.9-11.1°C for 4 months until descriptive analysis could be completed.

Consensus Descriptive Analysis

Nine panelists were used in total for the descriptive analysis (DA) testing. The six panelists that evaluated the 2014 wine from Mission were graduate students with experience in Sensory Science Evaluation and in analyzing food and beverage products. This panel was comprised of two males and four females ranging in age from 22-28. The five panelists that tasted the 2015 wine were trained and had knowledge in analyzing coffee and a range of other food and beverage products. This panel consisted of one male and four females ranging in age from 25-70. Two panelists, including the panel leader, analyzed both wines.

To begin training, the panelists tasted Blanc Du Bois wines and similar Texas white wines to familiarize themselves. Because a lexicon for Blanc Du Bois does not exist, the first panel worked to develop one by listing all potential attributes for flavors and aromas during these tastings. The Le Nez Du Vin White Wine Aroma Kit (Carnoux en Provence, France) was used to aide panelists in identifying the aromas they recognized. They then worked through group discussions lead by the panel leader and came to a consensus in order to condense the list of 86 attributes to 24 aromas and 26 flavors (Table 1). Most of the references used were based on the World Coffee Research Sensory Lexicon as there are a large number of similar attributes found in coffee that are also found in wine. This provided replicable and quantifiable intensities for each attribute. Some of the aromas were referenced using the Le Nez Du Vin kit (Carnoux en Provence, France). Once the panelists determined which particular references best represented the attributes, they were then trained to calibrate themselves on each attribute's intensity using a 16-point anchored scale (0=none, 2.5=very mild, 5=mild, 7.5=mild-distinct, 10=distinct, 15=strong).

Both the 2014 and the 2015 wines were stored at ~6-9°C prior to DA. Wine was poured at ~4.5°C and analyzed between ~6-8°C. During training, each wine was poured as a 75-90ml sample and the glass was covered using a watch glass for 5 minutes prior to analysis to aide the panelists in the evaluation of aromas. Palate cleansing between samples was accomplished with distilled water and unsalted saltine crackers. The training sessions ranged from 30 minutes to an hour with a maximum of four wines per session.

The wines under study were evaluated in triplicate at 5-8°C. Testing sessions were conducted over six days, three consecutive days per week for two weeks, at the

Sensory Evaluation Laboratory at Texas A&M University. Three wines were tested per session. A 30ml sample was poured and covered with a watch glass 5 minutes before analysis. Glasses were labeled with a random three-digit code to prevent bias. Wines were evaluated in a randomized order each day of testing. Panelists were seated across from one another in a separate room from the preparation area where they individually analyzed the wine for flavor and aroma one at a time, noting the attributes and their intensities. After each sample, the results were discussed amongst the group in a discussion led by the panel leader until they arrived at a consensus profile. The data was then recorded in Excel.

Volatile Analysis

Volatile analysis was completed on the wines at the time of their DA. The samples were obtained during the DA to ensure that they were similar to those being analyzed by the trained panel. As with the DA, each of the three wine samples were to be collected over the course of the six testing days resulting in a total of 18 samples. Gas chromatography-mass spectrometry with an olfactory port (GC-MS-O) and AromaTrax software (ver. 8; Volatile Analysis, Grant, AL) were used to evaluate the volatiles of each wine. This technique identifies the volatile compounds in a given sample, determines the aromas and flavors associated with volatiles at parts per trillion, and assigns a chemical structure to each compound. The samples were obtained using a static headspace Solid-Phase Micro Extraction (HS-SPME) Portable Field Sampler with a 75 Carboxen/PDMS fiber (Supelco model 504831; Sigma-Aldrich, St. Louis, MO). For each extraction, a 30ml sample of wine was poured into a 237ml glass jar with a Teflon lid beneath a metal screw cap. The wine samples were poured at ~5-8°C and the headspace

was collected for two hours for each sample. The SPME fiber was inserted into the gas chromatograph (GC; Agilent Technologies 7920 series GC, Santa Clara, CA) injection port where the sample was desorbed at 280°C for 3 minutes. The sample was loaded into the first column (30m x 0.53mm ID/BPX5 [5% phenyl polysilphenylene-siloxane] x 0.5 µm, SGE Analytical Sciences, Austin, TX) of the multidimensional gas chromatograph. In the first column, the starting temperature was 40°C and increased subsequently at 7°C/minute reaching a maximum temperature of 260°C. The compounds then move into the second column (30m x 0.53mm ID [BP20-polyethylene glycol] x 0.50 µm, SGE Analytical Sciences) where they were separated by polarity. At a three-way valve, the GC column partitions into three different columns. Two going to the two humidified sniff ports with glass nose pieces and one going to the mass spectrometer (MS; Agilent Technologies 5975 Series MS, Santa Clara, CA). The sniff ports were then heated to a temperature of 115°C. A portion of the AromaTrax software (Micro Analytics-AromaTrax, Round Rock, TX) and sniff ports are used in discerning the flavors and aromas. Trained panelists use the AromaTrax program to accurately identify the aroma attributes in the Blanc Du Bois lexicon we developed.

Statistical Analysis

SAS statistical software (ver. 9.3; SAS Institute; Cary, NC) was used to analyze the descriptive analysis (DA) data using an alpha of ($P < 0.05$). The PROC GLM procedure was used to compute an Analysis of Variance (ANOVA) and least square means for the treatment effects of the wines for the flavor or aroma attributes, and volatiles. This allowed us to determine if there was an effect on attribute intensity in either flavor or

aroma for the different wine treatments for each year. For Analysis of Variance, we examine individually the relationships of the volatiles, flavors, and aroma attributes with the wine treatments and the vintages, or vintage year.

Principal component analysis (PCA) and partial least squares regression (PLS) were run using XLSTAT (ver. 2017, Addinsoft, New York, NY). The PCA biplots showed individually the correlations of the volatiles, flavor and aroma attributes amongst each other with respect to the vintage, or vintage, and wine treatment. This allowed us to visualize how the vintage and wine treatment might be effected by those correlations. The PLS biplots allowed us to visualize any similarities between the volatiles and the flavor or aroma attributes as well how the treatments segment within them. This attempts to predict the flavors or aromas using the volatiles. All attributes that were detected are shown on the PCA and PLS biplots.

CHAPTER IV

RESULTS AND DISCUSSION

Trained Panel Sensory Attributes

The definition, reference standards, and intensities for the aromas, flavors, basic tastes, and mouth feel descriptive sensory attributes used in this study to analyze Blanc Du Bois wine are shown in Table 1. These attributes are listed on a 16 point scale with 0 equaling none, or not detectable, and 15 equaling extremely intense.

Only two characteristics, overripe tropical fruit aroma and alcohol flavor, differed ($p < 0.05$) across treatments (Table 2). The hyperoxygenated wine had a stronger overripe tropical fruit aroma than the control and PVPP fined wines while the control had less alcohol flavor than the hyper-oxygenated and PVPP treatments ($p < 0.05$). Multiple attributes were significantly different among the wines across vintages at $p < 0.05$. These aromas include alcohol, floral, grapefruit, green, lemon, musty/dusty, overripe tropical fruit, pear, sour, stemmy, and yeasty/fermented. The flavors that differed by vintage were alcohol, floral, grapefruit, lemon, overripe tropical fruit, sour, sweet, vinegar, and yeasty/fermented. The panelists were also trained, and evaluated the wines for burnt aroma and flavor, caramelized aroma and flavor, crisp/clean aroma, green flavor, malty flavor, and woody flavor and aroma. However, as the intensity levels were zero and none of the attributes were detected in the wine samples, they are not reported here.

These results show that the vintage influenced the aroma and flavor attributes of Blanc Du Bois wine while the treatment had less of an effect on the attributes. This was expected among the vintages as the source vineyards are in distinctly different locations,

with varied soils and climates, and produced grapes with dissimilar chemistry. The results were not as expected in regards to the effect of the treatments as previous studies demonstrated more differences in sensory characteristics of wines as a result of these pre-fermentation treatments (Cejudo-Bastante et al. 2011; Parish et al. 2016).

Similar to the Dreyer et al. (2013) study, the panelists tended to use the lower end of the scale when describing the intensity ratings of the attributes. Across all wines, the most intense rating for an aromatic attribute was alcohol with an average intensity of 5.17, followed by yeasty/fermented (4.56), and sweet (4.16). The least intense aroma was buttery with an average intensity of 0.11. Alcohol was also the flavor attribute with the most intense rating with an average of 6.33. It was followed by yeasty/fermented with an average intensity of 4.99 and bitter at 4.11. Honey and buttery were the flavors with the least two intensities at 0.06. The most intense mouthfeel was cooling effect with an average intensity of 3.61. The least intense mouthfeel was effervescence at 1.72. Amongst the more desirable wine characteristics, the highest aromatic intensities were grape (3.67), floral (2.94), apple (2.61), and apricot (2.5) and the most intense flavor attributes were grape (3.11), sweet (2.94), crisp/clean (2.56), and apricot/peach (2.4).

Principal component analysis (PCA) was conducted to understand the relationship between the sensory descriptive attributes and the Blanc Du Bois wines. The PCA plots for aromatics and flavors can be seen in Figures 2 and 3, respectively. The PCA plot for aromatics (Figure 2) showed the attributes primarily in two main clusters. The fruity, citrusy, sweet, earthy, and malty attributes were on the left side of the plot and the floral, green, alcohol/fermented, sour, and buttery aromatics were on the right side of the plot. The opposing orientations of these groups indicates that wines located near the

right side of the plot can be described as high in these aromas and low in the aromas on the left side of the plot. 72.68% of the variability of the data is explained by the PCA with 57.73% accounted for by principal component F1. The wines were thought of as negative or positive along F1 based on the opposing orientation of the two attribute clusters along F1. There was a clear separation of the wines by vintage on the PCA plot for aromatics. Vintage 1 wines were correlated to the right side of the plot with floral, rose, pear, green, alcohol/fermented, leathery, sour and buttery aromatics. The vintage 2 wines correlated with sweet, fruity, citrusy, malty and earthy attributes on the left side of the plot. The vintage 1 control correlated most with buttery, while the vintage 1 hyperoxygenated wine correlated more with alcohol, yeasty, floral attributes. The vintage 1 PVPP wine had a closer correlation to leathery, green, floral, rose, and pear. The vintage 2 control correlated with musty/dusty, citrus fruits, stemmy, malty, sweet, and vinegar. Over-ripe tropical fruit, honey, apple, and rubber were more correlated to the vintage 2 hyperoxygenated wine, while the vintage 2 PVPP wine was more strongly correlated to the rubber, and apricot/peach aromatics. The strongest correlation observed was between the lemon and grapefruit aromas. The strongest negative correlation was between alcohol and grapefruit aromas.

The PCA plot for flavor attributes can be seen in Figure 3. The attributes are primarily displayed in two main clusters, with the exception of effervescence and apple, which did not load highly on the F1 dimension. Alcohol, yeasty/fermented, sweet, floral, apricot/peach, pear astringent, and sour attributes are clustered together on the right side of the plot and were opposite vinegar, grape, lemon, and musty/dusty attributes found on the left side of the plot. The opposing orientations of these groups indicates that wines

located near the right side of the plot can be described as high in these flavors and low in the flavors on the left side of the plot. 66.08% of the variability of the data is shown here with 46.32% accounted for by principal component F1. The wines were thought of as negative or positive along F1 based on the opposing orientation of the two attribute clusters along F1, suggesting that as alcohol, yeasty/fermented, sweet, floral, apricot/peach, pear, astringent and sour flavors increase, vinegar, grape, lemon, and musty/dusty flavors decrease. Bitter, honey, overripe tropical fruit, grapefruit, and stemmy flavors were grouped together on the upper left section of the plot and opposite cooling effect, apricot/peach, crisp/clean, astringent, and floral flavors, indicating that these two clusters are inversely related. A separation of the wines by vintage is indicated by the PCA plot of wine flavor attributes (Figure 3). Vintage 1 wines can be found on right side of the plot and vintage 2 wines on the left side of the plot. Alcohol, yeasty/fermented, sweet, rose, leathery, and mouth drying attributes were related to the vintage 1 hyperoxygenated wine. The vintage 1 control corresponded with rose, mouth drying, leathery, and pear, while the vintage 1 PVPP wine corresponded to floral, astringent, sour, crisp/clean, and apricot/peach flavors. Overripe tropical fruit, honey, grapefruit, bitter and stemmy flavors were associated with the vintage 2 hyper-oxygenated wine. Vinegar, grape, lemon, musty/dusty, and buttery flavors corresponded with the vintage 2 control while the vintage 2 PVPP wine was more strongly correlated to buttery, rubber, and musty/dusty. The flavors with the strongest correlation were mouth drying and rose with the strongest negative correlation found between sour and grape.

Volatile Analysis

Using the GC-MS-O, 36 volatiles were identified. The majority of these volatiles, 27, were esters or alcohols. 16 esters were identified with a number of them as ethyl esters. These ethyl esters may be attributed to yeast metabolism byproducts (Swiegers et al. 2009) (Antonelli et al. 1999). A number of the corresponding organic acids to these esters were found as well, including acetic, hexanoic, octanoic, and nonanoic acids. 11 alcohols were identified including ethanol, as expected in wine. Of the remaining volatiles, two were aromatic hydrocarbons, two miscellaneous sulfur compounds, and one aldehyde. Table 3 defines the volatile aromatic compounds identified.

The control and treated wines were similar in terms of volatile analysis. Only one volatile compound, acetic acid, was significantly different across treatments ($p < 0.05$) (Table 3). Acetic acid is indicative of oxidation, aroma and flavor faults, and wine spoilage and although it can be produced by yeast metabolism, it can also be caused by lactic acid and acetic acid bacteria. Acetic acid was higher in concentration in the PVPP wines than in the control and hyper-oxygenated wines. However, a number of volatiles were significantly different among the wines across vintages at $p < 0.05$ including acetic acid; acetic acid, 2-phenylethyl ester; acetic acid, hexyl ester; styrene; butanedioic acid, diethyl ester; ethanol; octanoic acid, methyl ester; carbon disulfide; and sulfur dioxide (DOT) (Table 3).

PCA was conducted to better comprehend the connection between the volatile compounds and the wines (Figure 4). 71.51% of the variability of the data is shown here with 49.02% accounted for by principal component F1. Vintage 1 wines are clustered together in the lower left quadrant. The volatiles found in this area were varied with a mix of esters, organic acids, alcohols, and an aldehyde and hydrocarbon. These included 4-

ethyl-2-methoxy-phenol (C35), 2-(hexyloxy)-ethanol (C36), dl-Limonene (C18), hexanoic acid (C20), benzaldehyde (C30), and 2-hexenoic acid, ethyl ester (C12). Vintage 1 hyper-oxygenated wine was located at the top of the quadrant nearest octanoic acid (C22). The vintage 1 control was closest to 4-ethyl-2-methoxy-phenol (C35). The vintage 1 PVPP wine was nearest to 2-(hexyloxy)-ethanol (C36). Vintage 2 control and hyper oxygenated wines were grouped in the top two quadrants. All but one of the volatiles found surrounding them were esters. The one non-ester was isoamyl alcohol (C23) nearest the vintage 2 control. The vintage 2 hyper-oxygenated wine was grouped close to ethyl acetate (C34), acetic acid, ethyl ester (C33), and 1-butanol, 3-methyl-, formate (C11). Vintage 2 PVPP was the most distinct and was found independent of the other wines in its own quadrant separate. There were a large number of volatiles clustered near it with the majority being esters and alcohols and a few organic acids, hydrocarbons and miscellaneous sulfur compounds. These included acetic acid (C3), acetic acid, 2-phenylethyl ester (C5), 2,6-bis (1,1-dimethylethyl) -4-methylphenol (C28), 1-butanol, 3-methyl (C1), nonanoic acid (C32), 1-butanol, 3-methyl-, acetate (C2), and nonanal (C8).

Partial least squares (PLS) regression was administered to understand the correlation between volatile compounds, sensory attributes, and the wines. There was a clear separation of wines by vintage in both the PLS for aromas (Figure 5) and for flavor attributes (Figure 6). Vintage 1 wines were gathered tightly together near rose, buttery, green, and pear aromas and clustered tightly to 4-ethyl-2-methoxy-phenol (C35), 2-(hexyloxy)-ethanol (C36), and dl-Limonene (C18) (Figure 5). Vintage 1 control perfectly aligned with buttery aroma and 4-ethyl-2-methoxy-phenol (C35). This is simply because it was the only wine found to have a buttery aroma and 4-ethyl-2-methoxy-phenol (C35).

Vintage 1 PVPP and hyper oxygenated wines were very close to buttery and rose aromas and 2-(hexyloxy)-ethanol (C36) and 4-ethyl-2-methoxy-phenol (C35) as well. Vintage 2 control and hyper oxygenated wines are close and are grouped near rubber, overripe tropical fruit, apple, and honey aromas, and near isoamyl alcohol (C23), and esters such as ethyl acetate (C34), acetic acid, ethyl ester (C33), dodecanoic acid, ethyl ester (C31), decanoic acid, ethyl ester (C17), and 1-butanol, 3-methyl-, formate (C11). Again, vintage 2 PVPP is shown isolated in the lower right hand quadrant away from the other wines and aromas. It is perfectly in line with nonanoic acid (C32), as it is the only wine that contains it, and is tightly grouped with 1-Butanol, 3-methyl (C1), acetic acid, ethyl ester (C13), and 2,6-bis (1,1-dimethylethyl)-4-methylphenol (C28). It is difficult to say what is causing this isolation as there are many volatiles around it, including alcohols, esters, organic acids, miscellaneous sulfur compounds, and a hydrocarbon.

The PLS for flavors, wines, and volatiles (Figure 6) shows the vintage 1 wines together on the right side of the biplot near rose, leathery, apricot/peach, astringent, and floral flavors, and near octanoic acid (C22), 4-ethyl-2-methoxy-phenol (C35), dl-Limonene (C18), and 2-(hexyloxy)-ethanol (C36). The vintage 1 hyper oxygenated wine was the only wine found to have rose flavor and near octanoic acid (C22) and 4-ethyl-2-methoxy-phenol (C35). The vintage 1 control is in line with 4-ethyl-2-methoxy-phenol (C35) and near rose and leathery flavors. Vintage 1 PVPP is near apricot/peach, astringent, and floral flavors and next to 2-(hexyloxy)-ethanol (C36). All vintage 2 wines are located on the left side of the biplot, again with vintage 2 PVPP isolated. Vintage 2 PVPP is located near effervescence flavor and closely correlated to a number of volatiles

including alcohols such as 1-butanol, 3-methyl (C1), decanol (C16) and 2,6-bis (1,1-dimethylethyl)-4-methyl phenol (C28), esters such as 1-butanol, 3-methyl, acetate(C2), acetic acid, hexyl ester (C5), and acetic acid, ethyl ester (C13), and a couple of organic acids including nonanoic acid (C32), and acetic acid (C3). Vintage 2 control and hyper oxygenated wines are grouped together near buttery, rubbery, overripe tropical fruit, honey, grapefruit and bitter flavors, and near isoamyl alcohol (C23), and esters such as ethyl acetate (C34), acetic acid, ethyl ester (C33), dodecanoic acid, ethyl ester (C31), decanoic acid, ethyl ester (C17), and 1-butanol, 3-methyl-, formate (C11). Vintage 2 hyper oxygenated wine is located in line with honey flavors as it was the only wine found with it and is near overripe tropical fruit, grapefruit, and bitter flavors. Vintage 2 control was the only wine found with buttery flavor and is near rubbery flavor.

The grouping of these attributes and volatiles are fairly similar to the known aromatic descriptors for these volatiles (Table 3).

CHAPTER V

CONCLUSIONS

The descriptive analysis panel developed an attribute list for Blanc Du Bois wines consisting of 22 aromas, 22 flavors, and four mouthfeels. Significant differences were observed in aromas and flavors across vintages ($p < 0.05$). These included aromas and flavors for alcohol, floral, grapefruit, lemon, overripe tropical fruit, sour, and yeasty/fermented attributes, aromas for green, musty/dusty, pear, and stemmy attributes, and sweet and vinegar flavors. Only two attributes were found to be significantly different by treatment ($p < 0.05$) including overripe tropical fruit aroma, and alcohol flavor.

Principal component analysis revealed two distinct aroma and flavor profiles for each vintage. Vintage 1 wines were floral, pear, apricot/peach, green, sour, yeasty/fermented, and alcoholic and contrasted with vintage 2 wines which were fruity, citrusy, honey, vinegar, rubbery, and musty dusty. There were a few attributes that were associated with one vintage and the corresponding aroma or flavor was found in the other vintage. These include buttery aroma found in the vintage 1 control wine, buttery flavor found only in the vintage 2 control wine, apricot/peach and sweet aromas found in vintage 2 wines, and apricot/peach and sweet flavor found in vintage 1 wines. PCA also showed three different volatile profiles. Vintage 1 wines were a blend of esters, organic acids, alcohols, and a hydrocarbon. Vintage 2 hyper oxygenated and control wines were mostly comprised of esters and a single alcohol, while vintage 2 PVPP wine was comprised of a large number of alcohols and esters, two organic acids and miscellaneous sulfur compounds, and one hydrocarbon. Some of these volatiles were aligned with the expected sensory attributes such as the ethyl esters.

Partial least squares regression reinforced the PCA findings by showing three different volatile and sensory attribute profiles for the wines. Vintage 1 wines were again associated with buttery, floral, rose, pear, green, sour, yeasty/fermented, and alcoholic aromas with the corresponding flavors excluding buttery and consisted of a range of volatiles including esters, alcohols, organic acids, and a single hydrocarbon and aldehyde. Vintage 1 wines were also shown near astringent and crisp/clean mouthfeels. Vintage 2 hyper-oxygenated and control wines were shown with sweet, honey, fruity, citrusy, rubber, stemmy, and musty/dusty aromas and contained mostly of esters and a single alcohol. These wines were shown to have many of the corresponding flavors except musty/dusty and sweet flavors and with the addition of bitter and buttery flavors. Vintage 2 PVPP wine was isolated away from the majority of the aromatics and was shown near a number of alcohols, esters, a couple organic acids and miscellaneous sulfur compounds, and a single hydrocarbon. The same isolation was exemplified in flavors showing the nearest mouthfeel effervescence.

Results indicate that in this study pre-fermentation treatments had a minimal impact on the final wines, both sensorily and chemically. Thus, although pre-fermentation treatments were effective in improving overripe tropical fruit aroma and alcohol flavor and a reduction in acetic acid was observed, it did not have a large impact on sensory and chemical characteristics and will potentially have little impact on wine quality and style. However, as an acetic acid reduction was observed in the hyper-oxygenated wines, this pre-fermentation treatment could possibly improve the wine's storage and aging ability.

This research could be used to improve the overall aroma and flavor of Blanc Du Bois wines. The wines in vintage 1 were shown to have the flavor profile of a higher quality wine based on previous research by Dreyer et al, which showed higher quality Blanc Du Bois wines to have sweet, fruity, apricot/peach, floral, and rose flavor profiles and lower quality wines to have citrusy, green, stemmy, and bitter flavor profiles. As this study indicates a large impact on the final wines by vintage, both sensorily and chemically, it is likely that the vintage or location of vineyard had a greater effect on creating the desired flavor profile than the pre-fermentation technique administered. Further examination should be conducted to determine if the impact was a result of the vintage or vineyard location for grape sources. This could be achieved by repeating this experiment with multiple vintages from the same vineyard to determine if it is the vintage or vineyard location that is causing this difference in creating a desirable or undesirable flavor profile. Additional research analyzing Blanc Du Bois winemaking and viticultural techniques should be conducted with respect to wine quality and consumer preference to determine optimal practices.

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APPENDIX A

TABLES

Table 1. Definition and reference standards for Blanc Du Bois descriptive flavors (F), aromatics (A), basic taste, and mouthfeel sensory attributes and their intensities where 0=none; 15=extremely intense. Adapted from World Coffee Research (2016) and Le Nez Du Vin (Carnoux en Provence, France).

Sensory Attribute	Definition	Reference
Alcohol	A colorless, pungent, chemical-like aromatic associated with distilled spirits or grain products.	Everclear grain alcohol = 6.0 (A)
Apple	A sweet, light, fruity, somewhat floral aromatic commonly associate with fresh or processed apples.	HEB Apple Juice = 4.0 (A); 6.0 (F) Le Nez du Café n.17 “apple” = 5.0 (A) Fuji Apple = 7.0 (A); 8.0 (F)
Apricot/Peach	The floral, perfuming, fruity, sweet, slightly sour aromatics associated with apricots or peaches.	HEB dried apricot = 5.0 (A); 7.0 (F) Fresh peach pit = 5.0 (A) Le Nez du Café n.16 “apricot”= 7.5 (A) Libby’s Peach Slices, canned = 8.0 (A); 7.0 (F)
Bitter	The fundamental taste factor associated with a caffeine solution.	0.02% caffeine solution = 3.5 0.035% caffeine solution = 5.0 0.05% caffeine solution = 6.5 0.06% caffeine solution = 8.5

Table 1. Continued.

Sensory Attribute	Definition	Reference
Burnt	The dark brown impression of an over-cooked or over-roasted product that can be sharp, bitter, and sour.	Alf's red wheat Puffs = 8.0 (A); 3.0 (F)
Buttery	Sweet, dairy-like aromatic associated with natural butter	Land O'Lakes unsalted butter = 8.0 (A); 7.0 (F)
Caramelized	A round, full-bodied, medium brown, sweet aromatic associated with cooked sugars and other carbohydrates. Does not include burnt or scorched notes.	Kraft caramels = 3.0 (A); 6.0 (F)
Crisp/Clean	The refreshing sensation that gives a crisp mouth feel.	Sprite = 6.0 (F)
Floral	A sweet light, slightly fragrant aromatic associated with flowers.	Welch's white grape juice, diluted 1:1 with water = 5.0 (F) Geraniol = 7.5 (A)
Grape	The sweet, fruity, floral, slightly sour, musty aromatic commonly associated with grapes.	Green seedless grapes = 5.0 (F) Muscat beauty grapes = 7.0 (F)
Grapefruit	The citric, sour, bitter, astringent, peel, sharp, slightly sweet aromatic associated with grapefruit.	Le Nez du Vin no. 2 "grapefruit" = 5.0 (A) Grapefruit = 8.0 (F)
Green	An aromatic characteristic of fresh, plant-based material. Attributes may include leafy, vines, unripe, grassy, and peapod.	Fresh parsley water = 9.0 (A); 6.0 (F)

Table 1. Continued.

Sensory Attribute	Definition	Reference
Honey	Sweet, light brown, slightly spicy aromatic associate with honey.	Honey = 4.0 (A); 7.0 (F) Le Nez du Vin no. 27 “honey” = 7.0 (A)
Leathery	Aromatics associated with tanned animal hides.	Leather = 6.0(A)
Lemon	The citric, sour, astringent, slightly sweet, peel and somewhat floral aromatic associated with lemon.	Fresh lemon juice = 3.0 (A); 5.0 (F) Le Nez du Vin no. 1 “lemon” = 6.0 (A)
Malty	The light brown, dusty, musty, sweet, sour and or slightly fermented aromatic associated with grains.	Nestle malted milk original = 3.0 (A); 5.0 (F)
Musty/Dusty	The aromatic associated with dry, closed-air spaces such as attics and closets. May have elements of dry, musty, papery, dry soil, or grain.	Kretschmer wheat germ = 5.0 (A)
Overripe Tropical Fruit	The sweet, slightly sour aromatic characteristic of tropical fruit past their optimum ripeness.	Overripe cantaloupe melon = 6.0 (A); 7.0 (F)
Pear	The sweet, slightly floral, musty, woody, fruity aromatic associated with pears.	Libby’s pear halves, canned = 6.0 (A); 5.0 (F)
Rose	A sweet, slightly musty/dusty floral fragrance associated with fresh or dried roses.	Rose petals = 7.0 (A)

Table 1. Continued.

Sensory Attribute	Definition	Reference
Rubber	A dark, heavy, slightly sharp, and pungent aromatic associated with rubber.	A&W rubber bands = 5.0 (A)
Sour	The fundamental taste factor associated with citric acid.	0.015% citric acid solution = 1.5 0.050% citric acid solution = 3.5
Stemmy	The fundamental aromas associated with grape vines and stems.	Blanc Du Bois stem shavings = 2.0 (A) Blanc Du Bois broken stem = 5.0 (A)
Sweet	The fundamental taste factor associated with sucrose.	2.0% sucrose solution = 2.0
Vinegar	The aromas and flavors associated with vinegar.	White distilled vinegar = 2.0 (A); 3.0 (F)
Woody	The sweet, brown, musty, dark, aromatic associated with a bark of a tree.	Diamond shelled walnuts = 4.0 (A); 4.0 (F) Popsicle sticks = 7.5 (A)
Yeasty/ Fermented	The pungent, sweet, slightly sour, sometimes yeasty, alcohol-like aromatic characteristic of fermented fruits or sugar or over-proffer dough.	Red Star quick rise instant dry yeast = 9.0 (A)
<u>Mouthfeel</u> Astringent	The chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as puckering/dry and associated with tannins or alum.	Lipton tea, 1 bag = 6.0 (F)

Table 1. Continued.

Sensory Attribute	Definition	Reference
Cooling Effect	A nose and mouth feel associated with coolness.	Listerine cool mint mouthwash = 15 (F)
Effervescence	The bubbling or fizzing sensation present in a liquid.	Ozark sparkling original water = 7.0 (F)
Mouth Drying	A drying, puckering, or tingling sensation on the surface and/or the edge of the tongue and mouth.	0.05% Alum solution = 2.5 (F) 0.07% Alum solution = 3.5 (F) 0.09% Alum solution = 4.5 (F)

Table 2. Blanc Du Bois sensory attributes least square means for vintage, control, hyperoxygenation, and PVPP Blanc Du Bois wine treatments.

Attributes	Vintage			Treatment			RMSE ^d	Sum of Squares	DF	
	<i>P</i> -value ^c	1	2	<i>P</i> -value ^c	Control	HOX				PVPP
<u>Aromas</u>										
Alcohol	0.00	6.4 ^b	4.0 ^a	0.86	5.2	5.3	5.0	1.10	24.86	4
Apple	0.12	2.2	3.0	0.98	2.6	2.7	2.6	0.94	2.72	4
Apricot/Peach	0.95	2.5	2.5	0.48	2.9	1.8	2.8	1.63	4.10	4
Buttery	0.22	0.2	0.0	0.13	0.3	0.0	0.0	0.29	0.67	4
Floral	0.01	3.4 ^b	2.5 ^a	0.48	2.7	3.2	3.0	0.63	3.77	4
Grape	0.61	3.5	3.8	0.75	3.6	3.5	3.9	0.98	1.45	4
Grapefruit	0.001	0.0 ^a	1.5 ^b	0.87	0.9	0.7	0.8	0.68	11.13	4
Green	0.04	0.4 ^b	0.0 ^a	0.24	0.2	0.3	0.0	0.35	0.93	4
Honey	0.08	0.0	0.7	0.74	0.5	0.5	0.2	0.69	2.12	4
Leathery	0.31	1.1	0.4	0.48	0.2	1.0	1.0	1.22	4.38	4
Lemon	0.003	0.1 ^a	1.7 ^b	0.97	0.9	0.8	0.9	0.82	15.08	4
Malty	0.14	0.2	0.6	0.94	0.4	0.3	0.4	0.54	4.42	4
Musty/Dusty	0.02	1.3 ^a	2.8 ^b	0.78	2.3	2.0	1.9	1.04	18.75	4
Overripe Tropical Fruit	0.004	0.0 ^a	2.6 ^b	0.03	0.8 ^a	2.7 ^b	0.4 ^a	1.47	47.65	4
Pear	0.03	2.6 ^b	2.0 ^a	0.37	2.1	2.5	2.3	0.48	2.58	4
Rose	0.10	1.4	0.0	0.21	0.1	1.7	0.2	1.59	17.15	4
Rubber	0.17	0.4	1.2	0.08	1.7	0.5	0.3	1.08	11.32	4
Sour	0.02	3.4 ^b	2.5 ^a	0.48	3.0	3.2	2.7	0.65	5.48	4
Stemmy	0.03	1.8 ^a	2.8 ^b	0.17	2.7	2.3	1.8	0.80	9.35	4

Table 2. Continued.

Attributes	Vintage			Treatment			RMSE ^d	Sum of Squares	DF ^e	
	<i>P</i> -value ^c	1	2	<i>P</i> -value ^c	Control	HOX				PVPP
<u>Aromas (con't)</u>										
Sweet	0.19	3.8	4.5	0.85	4.0	4.3	4.1	0.93	3.21	4
Vinegar	0.10	0.2	0.8	0.23	0.9	0.2	0.4	0.74	7.33	4
Yeasty/Fermented	<0.0001	6.8 ^b	2.4 ^a	0.95	4.5	4.7	4.5	1.09	74.86	4
<u>Flavors</u>										
Alcohol	<0.0001	7.9 ^b	4.8 ^a	0.02	5.6 ^a	6.7 ^b	6.7 ^b	0.65	54.4	4
Apple	0.56	2.1	1.8	0.26	2.4	1.3	2.1	1.13	4.43	4
Apricot/Peach	0.44	2.6	2.3	0.34	2.8	2.0	2.5	0.94	3.04	4
Bitter	0.79	4.0	4.2	0.42	4.2	4.5	3.6	1.09	2.41	4
Buttery	0.06	-0.1	0.2	0.57	0.1	0.0	0.0	0.21	0.37	4
Crisp/Clean	0.05	3.0	2.1	0.15	2.4	2.2	3.1	0.79	6.20	4
Floral	0.004	3.2 ^b	1.5 ^a	0.93	2.2	2.3	2.4	0.97	11.83	4
Grape	0.07	2.5	3.7	0.75	3.4	2.8	3.1	1.21	8.85	4
Grapefruit	0.001	0.0 ^a	1.9 ^b	0.22	0.8	1.5	0.5	0.95	19.25	4
Honey	0.54	0.0	0.1	0.41	0.0	0.2	0.0	0.24	0.18	4
Leathery	0.06	0.7	0.1	0.13	0.2	0.8	0.1	0.62	3.32	4
Lemon	<0.0001	-0.2 ^a	3.3 ^b	0.23	1.7	1.2	1.8	0.64	47.05	4
Musty/Dusty	0.08	1.0	1.9	0.78	1.7	1.3	1.3	0.98	15.97	4
Override Tropical Fruit	0.02	-0.1 ^a	1.4 ^b	0.06	0.0	1.5	0.4	1.07	15.34	4
Pear	0.33	2.2	1.7	0.71	1.8	2.2	1.9	0.83	2.03	4

Table 2. Continued.

Attributes	Vintage			Treatment			RMSE ^d	Sum of Squares	DF ^e	
	<i>P</i> -value ^c	1	2	<i>P</i> -value ^c	Control	HOX				PVPP
<u>Flavors (con't)</u>										
Rose	0.08	0.7	-0.2	0.31	0.1	0.7	-0.1	0.86	5.40	4
Rubber	0.29	0.5	1.1	0.21	1.3	0.8	0.2	1.07	6.13	4
Sour	<0.0001	5.4 ^b	2.5 ^a	0.74	3.7	4.0	4.1	0.82	36.14	4
Stemmy	1.00	2.8	2.8	0.73	3.0	2.7	2.7	0.82	0.44	4
Sweet	0.03	3.6 ^b	2.3 ^a	0.48	2.8	2.7	3.4	1.03	11.05	4
Vinegar	<0.0001	0.0 ^a	2.0 ^b	0.22	0.8	1.0	1.2	0.35	18.41	4
Yeasty/Fermented	<0.0001	7.4 ^b	2.6 ^a	0.43	5.4	5.0	4.6	0.90	99.43	4
<u>Mouthfeel</u>										
Astringent	0.20	3.4	3.1	0.42	3.0	3.3	3.3	0.43	0.76	4
Cooling Effect	0.65	3.7	3.5	0.19	3.5	3.3	4.0	0.61	1.51	4
Effervescence	0.82	1.8	1.7	0.52	1.6	1.7	1.9	0.57	1.34	4
Mouth Drying	0.78	3.1	3.0	0.85	3.0	3.2	3.0	0.61	0.17	4

^{a, b} Mean values within a row and attribute followed by the same letter are not significantly different ($P > 0.05$).

^c *P*-value from analysis of variance tables.

^d Root Mean Square Error.

^e Degrees of Freedom.

Table 3. Blanc Du Bois aromatic chemical compounds least square means for vintage, control, hyperoxygenation, and PVPP Blanc Du Bois wine treatments and aroma descriptors.

Code	<u>Volatile</u>	P-value ^c	<u>Vintage</u>		P-value ^c	<u>Treatment</u>			RMSE ^d	Sum of Squares	DF ^e	Aroma Descriptor
	Aromatic Compound		1	2		Control	HOX	PVPP				
C1	1-Butanol, 3-methyl	0.28	0.0	190404.7	0.35	8,563	0	277,044	364,241.8	460960217430	3	Strong, pungent, slight fruity-winey
C2	1-Butanol, 3-methyl-, acetate	0.16	21559.2	786189.3	0.38	107,067	173,712	930,844	1,106,462	5.1435619E+12	3	Strong fruity, pear, banana
C3	Acetic acid	0.003	17402.7 ^a	336383.4 ^b	0.03	121,018.2 ^a	46,935.5 ^a	362,725.5 ^b	194,833.5	785137315838	3	Sharp, sour, vinegar, overripe fruit
C4	Acetic acid, 2-phenyl-ethyl ester	0.0003	0.0 ^a	174830.1 ^b	0.81	99,099	92,337	70,809	78,577.2	140164171418	3	Floral, rose, honey, fruity
C5	Acetic acid, hexyl ester	0.03	20313.1 ^a	386707.4 ^b	0.24	193,728	34,692	382,112	338,744.2	967064868918	3	Fruity, green, apple, pear
C6	Benzene ethanol	0.10	85068.8	225680.9	0.64	105,842	160,880	199,403	172,546.9	115506937617	3	Floral, rose, fresh, sweet
C7	Hexanoic acid, ethyl ester	0.87	2052433.9	1891989.7	0.11	1,278,548	1,024,310	3,613,777	2,176,459	2.4562389E+13	3	Sweet, pineapple, fruity, waxy

Table 3 continued.

Code	Volatile		Vintage			Treatment			RMSE ^d	Sum of Squares	DF ^e	Aroma Descriptor
	Aromatic Compound	P-value ^e	1	2	P-value ^e	Control	HOX	PVPP				
C8	Nonanal	0.06	17845.2	289069.6	0.45	131,474	58,277	270,621	288,915.5	470651728089	3	Waxy, citrus, orange peel, fresh
C9	Octanoic acid, ethyl ester	0.48	8869163.2	10794882.7	0.54	9,956,009	7,921,707	11,618,353	5,674,745	5.7821706E+13	3	Waxy, sweet, fruity, winey
C10	Styrene	0.0010	0.0 ^a	66068.1 ^b	0.20	34,638	13,688	50,777	33,938.1	23792399946	3	Sweet, balsamic, floral-strong aroma
C11	1-Butanol, 3-methyl-, formate	0.71	3804.1	6054.9	0.10	0	14,789	0	12,945.9	897595932	3	Sharp, green, apple, winey
C12	2-Hexenoic acid, ethyl ester	0.10	1856.9	0.0	0.83	1,121	1,194	471	2,261.1	17418195.00	3	Rum-like, fruity, green, sweet
C13	Acetic acid, ethyl ester	0.56	304355.4	523231.9	0.27	152,272	233,531	855,578	795,849.3	1.9919507E+12	3	Ethereal, fruity, sweet, grape
C14	Butanedioic acid, diethyl ester	0.03	13790.6 ^b	0.0 ^a	0.43	5,078	3,350	12,258	12,232.4	1123557102	3	Mild, fruity, cooked apple, ylang
C15	Butanoic acid, ethyl ester	0.98	66643.8	65602.4	0.21	38,947	33,196	126,227	97,187.2	32615886651	3	Fruity, sweet, juicy

Table 3 continued.

Code	Volatile		Vintage		P-value ^c	Treatment			RMSE ^d	Sum of Squares	DF ^e	Aroma Descriptor
	Aromatic Compound	P-value ^c	1	2		Control	HOX	PVPP				
C16	Decanal	0.69	7221.9	12797.1	0.63	3,636	7,197	19,196	29,204.6	937404855	3	Orange-peel, citrus
C17	Decanoic acid, ethyl ester	0.11	1325137.8	5327897.0	0.41	5,427,671	3,057,275	1,494,607	5,005,367	1.1915878E+14	3	Sweet, waxy, fruity, apple
C18	dl-Limonene	0.22	14559.9	0.0	0.48	17,237	2,304	2,299	24,315.5	1846273206	3	Citrus, herbal, terpenic
C19	Ethanol	0.001	61372.3 ^a	2874233.1 ^b	0.67	1,192,252	1,302,417	1,908,740	1,485,447	3.7391073E+13	3	Strong, alcoholic, medicinal
C20	Hexanoic acid	0.08	26213.1	7639.0	0.57	12,259	13,894	24,626	21,613.6	2094134291	3	Fatty, cheesy, sour
C22	Octanoic Acid	0.51	34406.0	22960.8	0.37	46,269	18,080	21,701	36,659.6	3412091010	3	Fatty, waxy, rancid, cheesy
C21	Linalool	0.22	1965.1	14985.9	0.55	10,920	593	13,912	21,667.1	1348790599	3	Fruity, citrus, floral, sweet, rose
C23	Isoamyl alcohol	0.21	15301.0	43992.2	0.70	16,514	34,580	37,850	46,755.5	5288665381	3	Fusel, alcoholic, whiskey, fruity

Table 3 continued.

Code	Volatile		Vintage		P-value ^c	Treatment			RMSE ^d	Sum of Squares	DF ^e	Aroma Descriptor
	Aromatic Compound	P-value ^c	1	2		Control	HOX	PVPP				
C24	Octanoic acid, methyl ester	0.005	3022.1 ^b	0.0 ^a	0.22	956	870	2,708	1,954.5	54008034.2	3	Waxy, green, sweet orange, vegetal/herbal
C25	1-Hexanol	0.85	14427.2	17820.2	0.15	5,956	0	42,416	38,149.6	6379534125	3	Chemical, green, wine, fruity
C26	Carbon disulfide	0.01	0.0 ^a	51186 ^b	0.47	25,563	11,883	39,334	38,194.4	14050737526	3	
C27	Nonionic acid, ethyl ester	0.20	362.8	14276.8	0.51	6,899	0	15,060	22,026.5	1553231688	3	
C28	2,6-bis (1,1-dimethyl ethyl) -4-methyl phenol	0.26	0.0	48558.0	0.44	10,610	0	62,227	88,209.9	23908836243	3	Mild, phenolic, camphor
C29	Sulfur dioxide (DOT)	0.0003	0.0 ^a	171964.6 ^b	0.75	92,008	66,920	99,019	76,892.2	136490840584	3	
C30	Benzaldehyde	0.06	8941.8	0.0	0.26	9,240	0	4,173	9,340.2	616722348	3	Strong, sweet, bitter, almond, cherry
C31	Dodecanoic acid, ethyl ester	0.14	0.0	102804.2	0.57	40,340	98,791	15,075	139,481.4	69685574639	3	Sweet, waxy, soapy, floral

Table 3 continued.

Code	Volatile		Vintage			Treatment			RMSE ^d	Sum of Squares	DF ^e	Aroma Descriptor
	Aromatic Compound	P-value ^c	1	2	P-value ^c	Control	HOX	PVPP				
C32	Nonanoic acid	0.33	0.0	5567.3	0.39	0	0	8,351	11,810.1	418435206	3	Waxy, dirty, cheesy, dairy
C33	Acetic acid, ethyl ester	0.25	18670.1	135930.7	0.22	28,005	203,896	0	211,388.1	208466077355	3	Ethereal, fruity, sweet, grape, cherry
C34	Ethyl acetate	0.58	44552.3	106395.1	0.18	226,421	0	0	231,899.2	222276560094	3	Ethereal, fruity, sweet, grape, rum-like
C35	4-ethyl-2-methoxy-phenol	0.33	1468.0	0.0	0.39	2,202	0	0	3,114.1	29092824.0	3	Spicy, smoky, medicinal, clove, vanilla
C36	2-(hexyloxy)-ethanol	0.30	3070.1	0.0	0.41	0	309	4,296	6,060.4	111323148.8	3	

a, b Mean values within a row and aromatic compound followed by the same letter are not significantly different ($P > 0.05$).

c P-value from analysis of variance tables.

d Root Mean Square Error.

e Degrees of Freedom.

Table 4. Principal component analysis factor loadings for Blanc Du Bois descriptive aromatics, flavors, and basic taste sensory attributes, and eigenvalues and variability explained.

Attributes	F1	F2
<u>Aromas</u>		
Sweet	-0.836	-0.258
Sour	0.917	0.129
Floral	0.740	-0.616
Rose	0.669	-0.597
Apricot/Peach	-0.299	0.601
Pear	0.604	-0.738
Grapefruit	-0.957	-0.017
Lemon	-0.941	-0.006
Apple	-0.710	-0.573
Overripe Tropical Fruit	-0.659	-0.281
Grape	-0.188	-0.443
Honey	-0.727	-0.417
Leathery	0.584	-0.215
Musty/Dusty	-0.934	-0.104
Malty	-0.892	0.057
Stemmy	-0.894	-0.002
Yeasty/Fermented	0.954	-0.129
Green	0.776	-0.066
Buttery	0.359	0.830
Rubber	-0.563	-0.127
Alcohol	0.983	0.033
Vinegar	-0.821	0.004
<u>Flavors</u>		
Bitter	-0.454	0.853
Sour	0.992	-0.034
Sweet	0.811	0.073
Floral	0.959	-0.155
Rose	0.567	0.255
Apricot/PeachF	0.484	-0.563
Pear	0.439	0.750
Stemmy	-0.297	0.338
Yeasty/Fermented	0.949	0.125
Apple	0.006	-0.553
Overripe Tropical Fruit	-0.648	0.664
Lemon	-0.887	-0.390
Grapefruit	-0.887	0.322
Grape	-0.950	-0.220
Honey	-0.560	0.759

Table 4. Continued.

Attributes	F1	F2
Leathery	0.671	0.486
Musty/Dusty	-0.809	-0.391
Buttery	-0.377	-0.496
Rubber	-0.214	-0.348
Crisp/Clean	0.713	-0.533
Alcohol	0.925	0.252
Vinegar	-0.930	-0.166

Mouthfeel

Astringent	0.658	-0.092
Cooling Effect	0.229	-0.504
Effervescence	-0.004	-0.471
Mouth Drying	0.567	0.255

EigenvaluesAromas

Eigenvalue	12.700	3.290
Variability (%)	57.729	14.955
Cumulative (%)	57.729	72.684

Flavors

Eigenvalue	12.043	5.138
Variability (%)	46.321	19.763
Cumulative (%)	46.321	66.083

Table 5. Principal component analysis factor loadings for aromatic chemical compounds, and eigenvalues and variability explained.

Code	Aromatic Chemical Compound	F1	F2
C1	1-Butanol, 3-methyl	0.895	-0.366
C2	1-Butanol, 3-methyl-, acetate	0.939	-0.217
C3	Acetic acid	0.977	-0.183
C4	Acetic acid, 2-phenylethyl ester	0.664	0.704
C5	Acetic acid, hexyl ester	0.970	-0.126
C6	Benzene ethanol	0.918	0.122
C7	Hexanoic acid, ethyl ester	0.480	-0.830
C8	Nonanal	0.991	-0.029
C9	Octanoic acid, ethyl ester	0.359	-0.060
C10	Styrene	0.970	0.120
C11	1-Butanol, 3-methyl-, formate	-0.205	0.632
C12	2-Hexenoic acid, ethyl ester	-0.720	-0.380
C13	Acetic acid, ethyl ester	0.786	-0.542
C14	Butanedioic acid, diethyl ester	-0.595	-0.669
C15	Butanoic acid, ethyl ester	0.539	-0.797
C16	Decanal	0.715	-0.451
C17	Decanoic acid, ethyl ester	0.208	0.797
C18	dl-Limonene	-0.543	-0.268
C19	Ethanol	0.926	0.347
C20	Hexanoic acid	-0.612	-0.378
C21	Linalool	0.850	-0.055
C22	Octanoic Acid	-0.326	0.015
C23	Isoamyl alcohol	0.322	0.405
C24	Octanoic acid, methyl ester	-0.586	-0.666
C25	1-Hexanol	0.601	-0.736
C26	Carbon disulfide	0.976	0.130
C27	Nonionic acid, ethyl ester	0.958	-0.181
C28	2,6-bis (1,1-dimethylethyl) -4-methyl phenol	0.936	-0.306
C29	Sulfur dioxide (DOT)	0.876	0.441
C30	Benzaldehyde	-0.571	-0.422
C31	Dodecanoic acid, ethyl ester	0.192	0.866
C32	Nonanoic acid	0.884	-0.378
C33	Acetic acid, ethyl ester	-0.090	0.701
C34	Ethyl acetate	0.011	0.380
C35	4-ethyl-2-methoxy-phenol	-0.439	-0.176
C36	2-(hexyloxy)-ethanol	-0.333	-0.566
<u>Eigenvalues</u>			

Table 5. Continued.

Code	Aromatic Chemical Compound	F1	F2
Eigenvalue		17.648	8.094
Variability (%)		49.023	22.483
Cumulative (%)		49.023	71.506

Table 6. Partial least squares regression model quality of aromatic volatile compounds Blanc Du Bois descriptive sensory attributes, and wine.

Model quality:		
Index	Comp1	Comp2
<u>Aromas</u>		
Q ² cum	0.240	0.346
R ² Y cum	0.461	0.637
R ² X cum	0.460	0.702
<u>Flavors</u>		
Q ² cum	0.147	0.227
R ² Y cum	0.368	0.596
R ² X cum	0.464	0.711

APPENDIX B

FIGURES

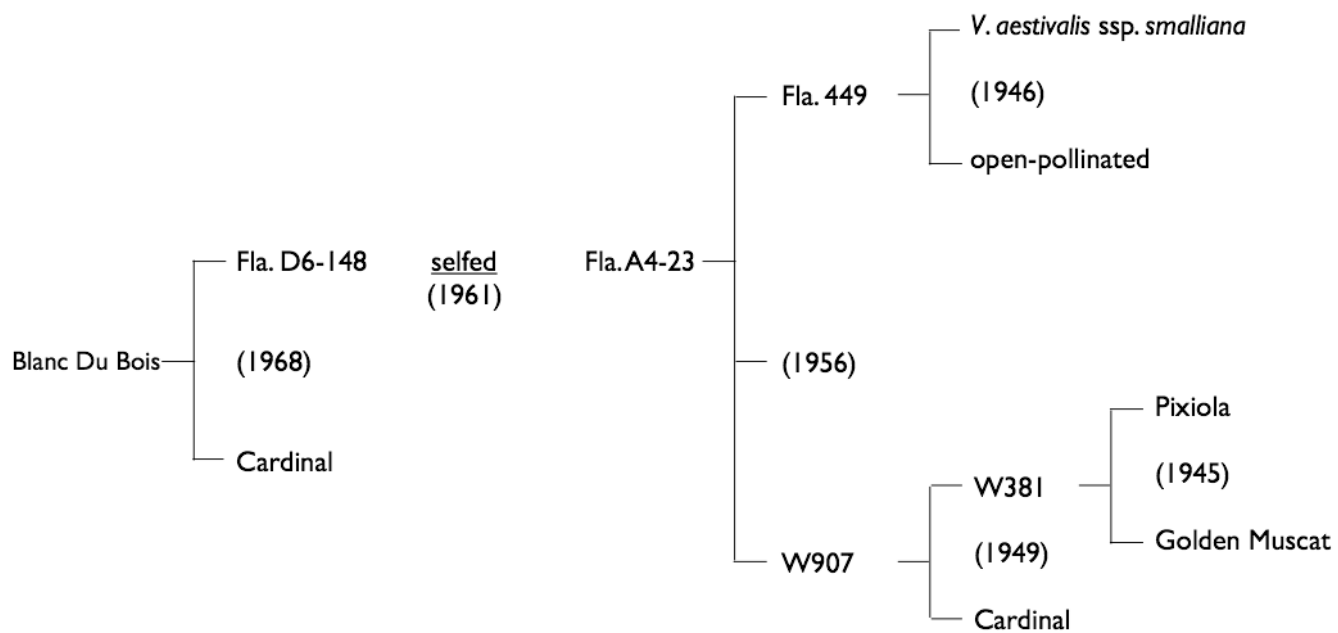


Figure 1. Pedigree of Blanc Du Bois with year of pollination in parentheses.

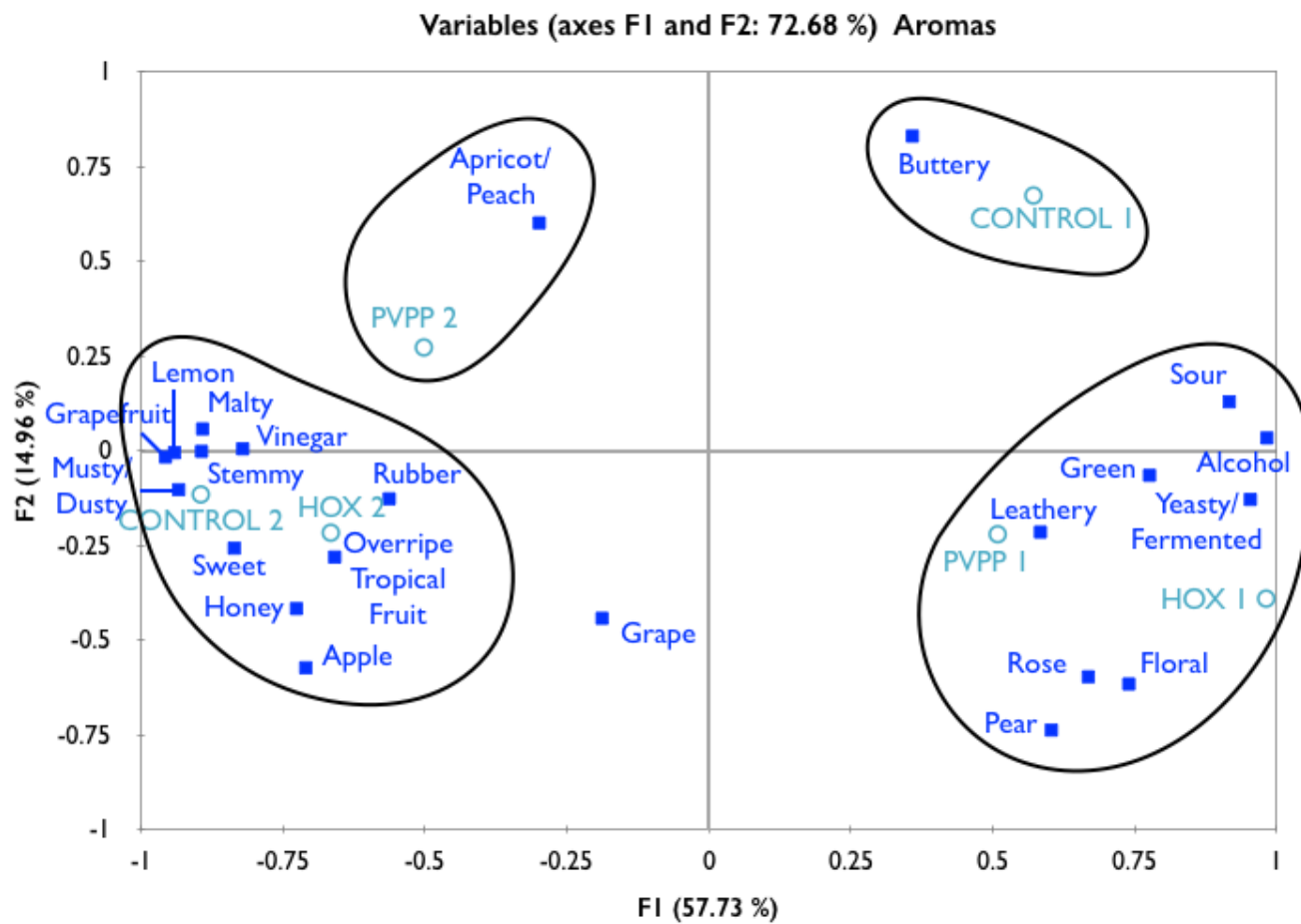


Figure 2. Principal component analysis of trained descriptive aroma attributes and wine treatments

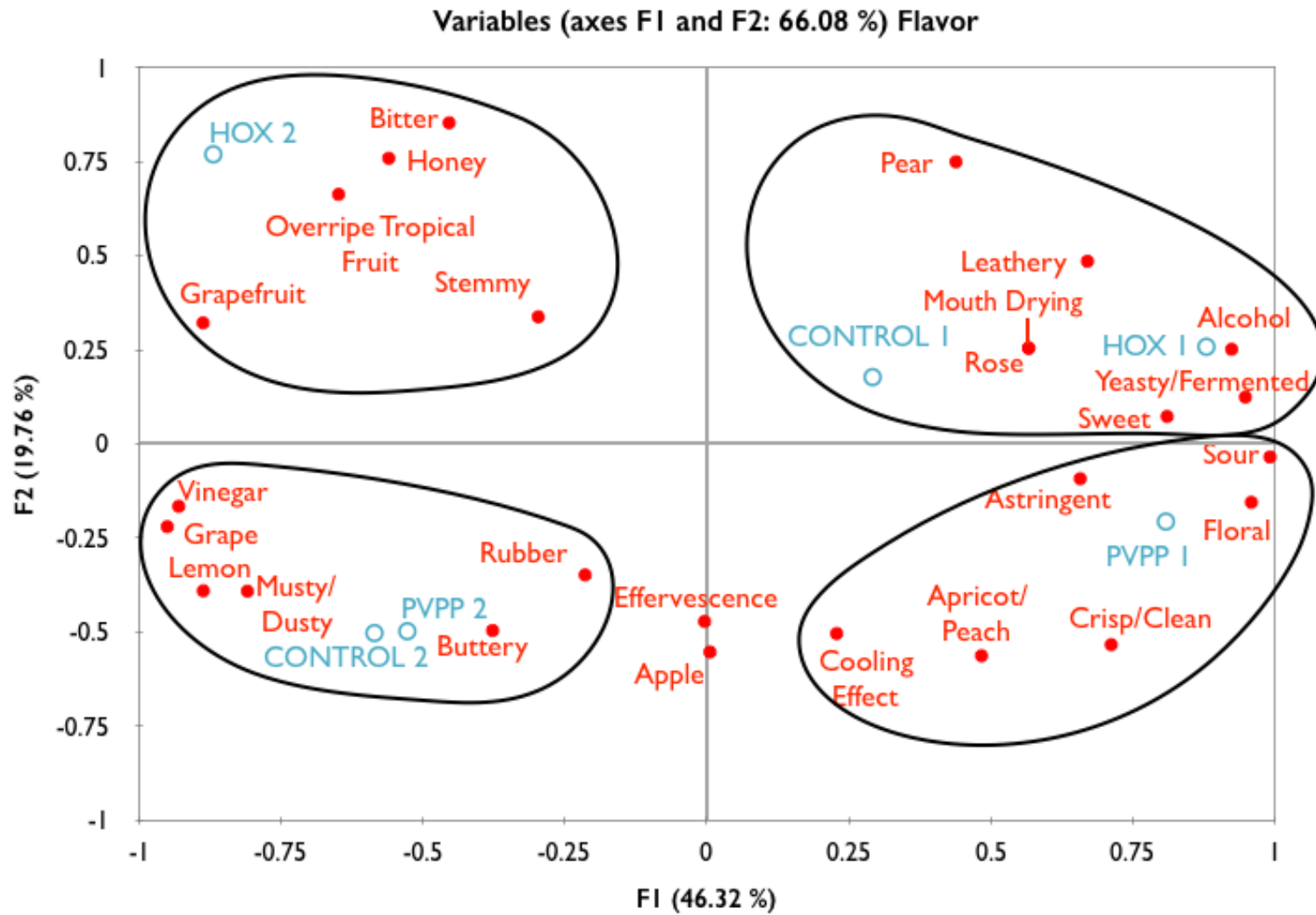


Figure 3. Principal component analysis of trained descriptive flavor attributes and wine treatments

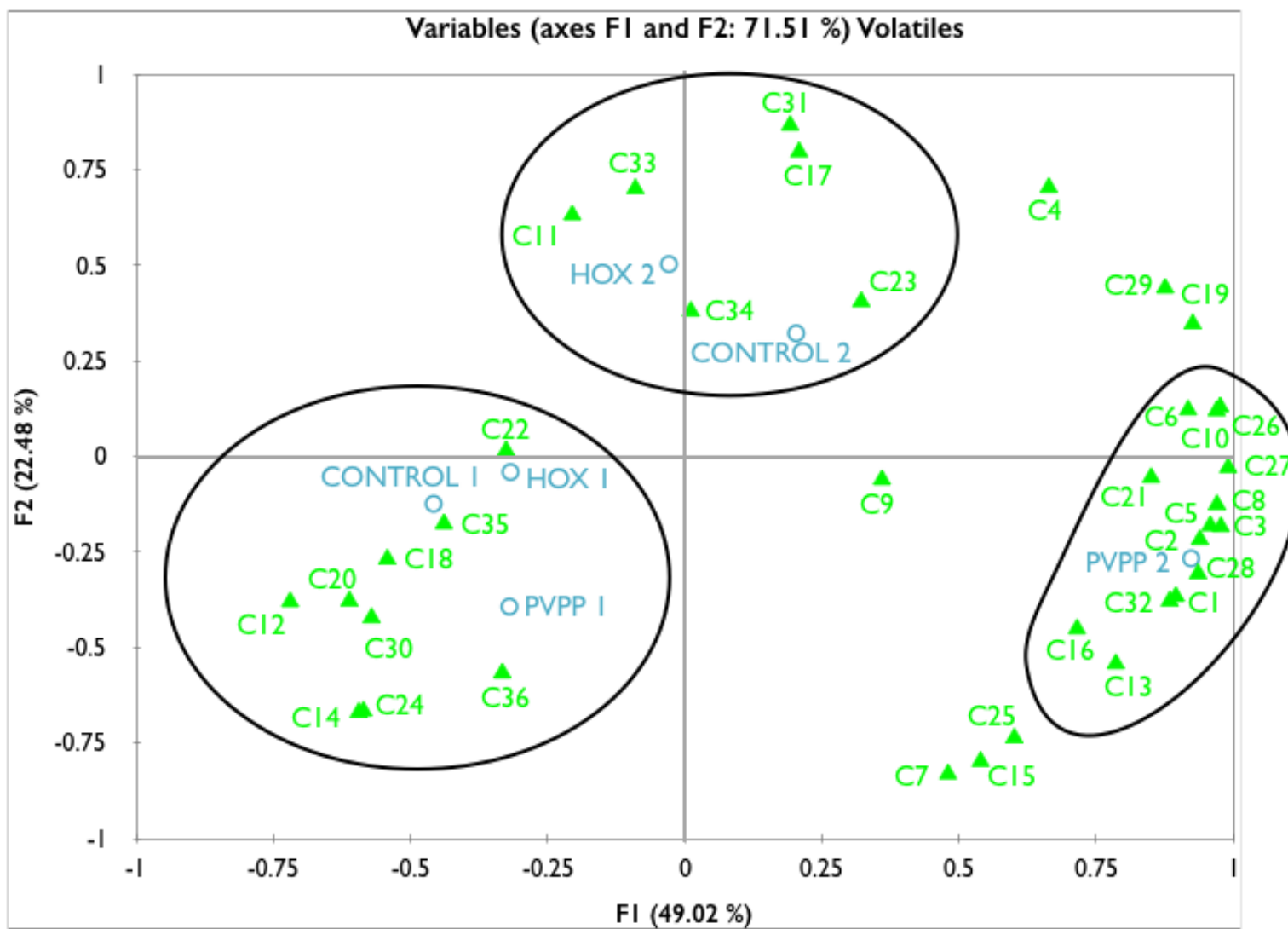


Figure 4. Principal component analysis of aromatic volatile compounds and wine treatments.

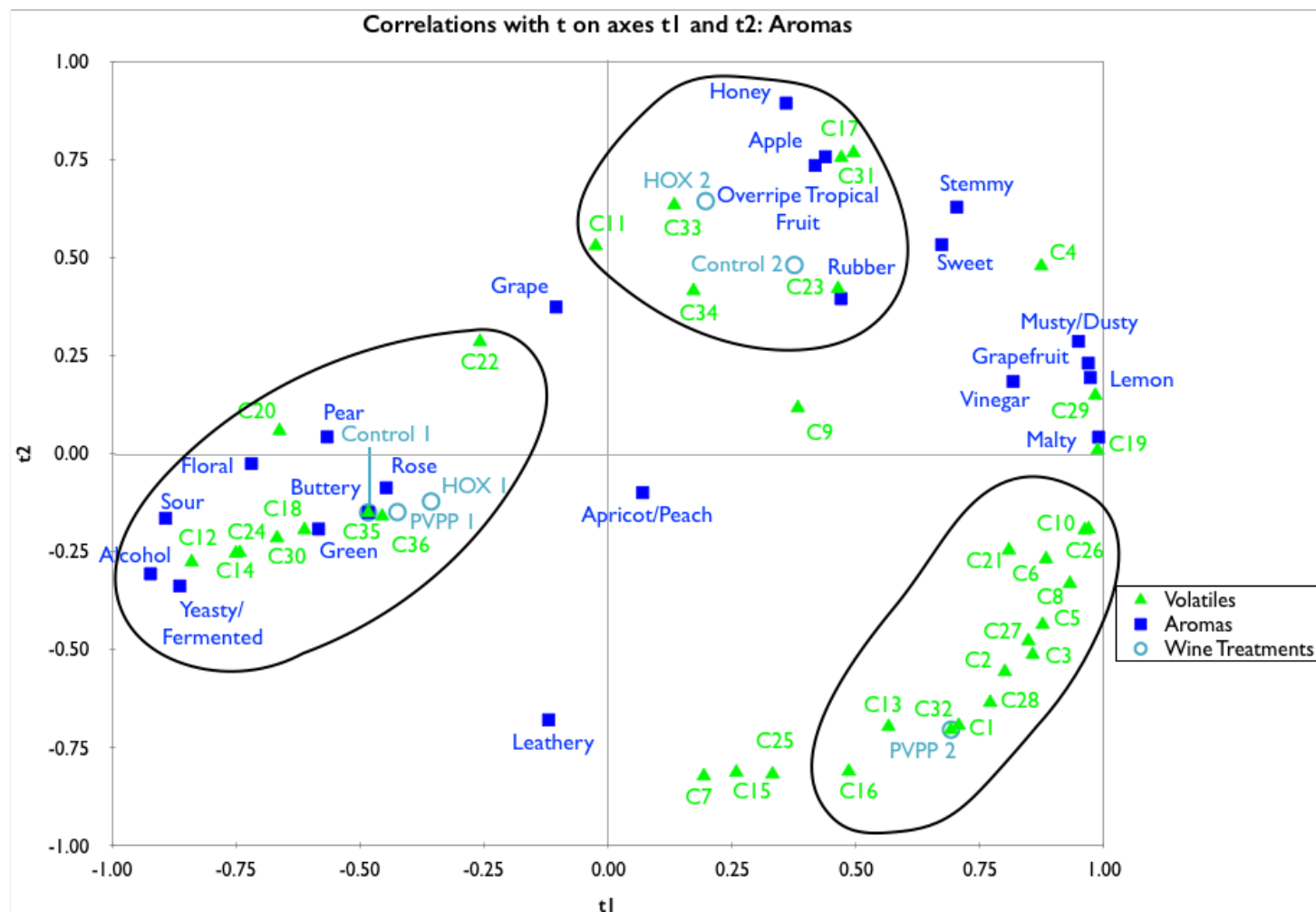


Figure 5. Partial least squares regression of aromatic volatile compounds, aroma attributes, and wine treatments.

