# INFLUENCE OF WINE COMPONENTS ON THE CHEMICAL AND SENSORY QUALITY OF WINES

By

CHARLES DIAKO

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

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
School of Food Science

MAY 2016

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of CHARLES DIAKO find it satisfactory and recommend that it be accepted.

_____

Carolyn F. Ross, Ph.D., Chair

_____

Charles G. Edwards, Ph.D.

_____

John K. Fellman, Ph.D.

_____

Marc A. Evans, Ph.D.

## ACKNOWLEDGMENTS

My biggest debt of gratitude goes to my advisor, Dr. Carolyn F. Ross for accepting me into her program and giving me the opportunity to work on wine research. Coming into her program as a novice in wine research, her patience, constructive criticism of my work and her expectation of excellence have helped me build a wealth of knowledge and skills in food and wine research. My sincere appreciation also goes to my committee members: Dr. Charles G. Edwards, Dr. John Fellman and Dr. Marc Evans for their advice and support which helped me to finish this work. I have learned life lessons from each of my advisory committee during this period of mentoring including developing a passion for whatever I'm doing, keeping things simple and keeping a balance between fun and hard work.

I will also like to express my gratitude to Dr. Kevin Vixie and Professor Kevin Cooper of the Department of Mathematics and Statistics for taking a special interest in my research and for all their useful suggestions and time. Special thanks also go to Dr. Boon Chew, Dr. Girish Ganjyal, Jodi Anderson, Angela Lenssen and Scott Mattinson and the rest of the faculty and staff in the School of Food Science for being there for me whenever I needed them. This is a special thank you to the amazing group of people in the Ross lab, first to Karen Weller and Beata Vixie; and to past and current students: Dr. Remedios Villamor, Dr. Luis Castro, Megan Waldrop, Dr. Allison Baker, Emily Fleischman, Kenny McMahon, Daniel Dycus, Ben Bernhard, Megan Schumaker and Emily Walsh for their assistance in my research.

I would also want to thank my friends in the Washington State University International Research and Agricultural Development: Oumarou Badini, Colleen Taugher, Chris Pannkuk and Mary Weitz whose work with the USDA gave me the first opportunity to come to Washington

State University as a visiting scholar before I got accepted into the Ph.D. program. I will always remember you for making this visit fun and for all the resulting networking.

Life during this program wouldn't have been what it was if I had not met some special families in Pullman and Genesee: Dr. Nii and Sherry Ankrah, Robert and Susan Riggs, Emeritus Professor Walt and Elinor Butcher, Clay and Kristi Mallet, 'granny' Elinor Huber as well as Mike and Suzie Chad. Meeting and getting to know you was life-enriching in many ways and I am grateful for your support and for all the fun that knowing you and your families brought me during this program. I would also like to acknowledge the camaraderie of past and present Ghanaian students in Washington State University and the University of Idaho: Dr. Fafanyo Asisse, Dr. Francis Boateng, Dr. Deborah Tamakloe, Dr. Isaiah Gyan, Dr. Ebenezer Jones-Mensah, Yvonne Nyavor, Linda Agyen, Moses Luri, Pearl Kwantwi-Barima and Roberta Koku. We've shared our struggles and our happy moments which made the journey a lot easier.

I have made many good friends along the way thus far. Special thanks to Dr. Tesfaye Girma Deboch for being a brother and a friend before his untimely passing; and to Dr. Nicholas Manuel and Dr. Ahmed Aghalli for being true brothers from different parents. My numerous International student friends have encouraged me in many ways especially Lwanga Nsubuga, Loc Vo, Jaza Alshammari, Cindy Pinhal, Semanur Yildiz, Dirlei Kieling, Gilberto Meideiros de Souza, Taise Toniazzo and Liliana Acurio. Thank you for the moral support!

Last but definitely not the least, I wish to thank my siblings for their prayers and encouragement throughout the program: Ernest, Jane, Julie, Albert, Clemence, Elizabeth, Isaac, Francisca and Daniel. We have come a long way as a family and I always thank God for your lives. To Him who makes all things beautiful in His time be all the praise and glory.

INFLUENCE OF WINE COMPONENTS ON THE CHEMICAL AND

SENSORY QUALITY OF WINES


## Abstract

by Charles Diako, Ph.D.
Washington State University
May 2016

Chair: Carolyn F. Ross

Wine is an alcoholic beverage containing numerous compounds that contribute to its overall quality. The overall objective of this dissertation was to examine the influence of matrix interactions in commercial red wines on the sensory and chemical properties of wines, and explore advanced methods of mathematical analyses of these data. In the first study, the influence of these matrix components and their interactions on wine quality was examined. Commercial Merlot wines (n=61) were evaluated for wine chemistry parameters, with the matrix components of interest identified as alcohol, tannin and mannoproteins. Sensory evaluation results showed complex interactions among tannins, alcohol and mannoproteins on the perception of most aromas, flavors, tastes and mouthfeel attributes ($p<0.05$). Since human subjects vary in their sensory perceptions, panel reproducibility and precision was addressed. Panelists' variation in attribute evaluations was conceptualized as a linear operator called a bias matrix for feedback calibration and correction of panelists' evaluations. Results showed that the bias matrix corrected the panelist ratings of the samples, leading to higher reproducibility and precision. No significant differences ($p>0.05$) were found between the original and corrected

means. Predictive filtering showed that the panelists' corrected means for the attributes were closer to the predicted panel mean compared to their unfiltered means. The application of the electronic tongue for the assessment of wine quality was further explored. Strong correlations ($r^2$>0.930) were reported between the electronic tongue and the sensory perceptions of sweet, sour, bitter, burning, astringent and metallic. Further research on the application of the electronic tongue to discriminate among wines and building predictive models was performed. Non-linear methods showed high discrimination among the commercial wines (90.1%), with high prediction accuracy of the electronic tongue output using the chemical parameters (≥90.0%). These results showed the dependence of the intensity of the electronic tongue signal on the chemical components of the wines. This dissertation demonstrated how wine matrix components influenced perception through suppression and enhancement of various sensory attributes. In addition, advanced methods of chemical and sensory data analysis were developed and validated. Results from this study will be useful for winemakers for wine quality optimization.

## TABLE OF CONTENTS

## LIST OF TABLES

Page

## LIST OF FIGURES

Page

## DEDICATION

*This dissertation is dedicated to my parents, Kofi Kumah and Yaa Marbeley, of blessed memory*

*for teaching me to give off my best wherever life takes me*

# CHAPTER I

# INTRODUCTION

Wine consumption in the United States has increased by 228 million gallons between 2004 and 2014 (wineinstitute.org, 2015). This pattern, a trend starting in the 1970's, has been attributed to the strides made in the science and technology of winemaking (Zraly, 2011). Sustainable growth of the wine industry will require continuous improvement of wine quality by taking advantage of advances in wine science for both objective and rapid evaluation of wine quality.

Central to the substantial growth of the United States wine industry are the consumer expectations of quality. Generally, wine quality is regarded as a multidimensional concept which is judged by criteria such as the absence of detectable defects, visual appeal including color and clarity of the wine, richness of the aromatics, balance among components of the wine and overall finish of the wine (Jackson, 2014). These sensory criteria by which consumer expectation of a wine's conformity to quality is defined are a manifestation of the chemical composition of the wine selected from the shelf.

Indeed, the chemical and sensory quality of wines depends on many factors. Viticultural and enological activities contribute to wine composition, and the subsequent interactions among the matrix components of the wine influence the composition, and thus perceived quality, of the wine. Viticultural activities including harvest date, harvest method and grape maturity have been found to influence both chemical composition and sensory properties of wines, with a subsequent effect on consumer acceptability (Gil *et al.*, 2012; Tian *et al.*, 2013; Bindon *et al.*, 2013; Bindon *et al.*, 2014). Similarly, the winemaking process from crushing to ageing has a profound effect on the wine chemical composition, and as a function of this composition, the interactions that are

present among the matrix components. Specifically in Washington wines, ethanol as a major product of fermentation and its impact on headspace volatile concentration and perception (Villamor *et al.*, 2013a; Villamor *et al.*, 2013b; Villamor and Ross, 2013), phenolic compounds and their relationship with perceived astringency (Harbertson *et al.*, 2007; Landon *et al.*, 2008; Villamor *et al.*, 2009), influence of some processing steps and storage on chemical composition of wines (Harbertson *et al.*, 2009; Villamor *et al.*, 2009; Casassa *et al.*, 2013) have been investigated.

The influence of interactions among matrix components on sensory perception and/or instrumental detection of key wine attributes has gained interest in the research community. The logic behind this shift of attention from compositional analysis to the study of interactions among matrix and volatile components is the dependence of sensory perception upon those compounds available in wine to engage the human senses. Indeed, any discourse on wine matrix components and their relationship to sensory perception is incomplete without highlighting the role of some key components such as ethanol, phenolic compounds and polysaccharides. Notably, ethanol influences the head space partitioning of volatile compounds, leading to reduced volatility of some compounds at high ethanol levels and hence limiting their sensory perception (Robinson *et al.*, 2009; Pozo-Bayon and Reineccius, 2009; Villamor *et al.*, 2013a). In addition to ethanol, phenolic compounds play a significant role in the volatility of volatile compounds (Lorrain *et al.*, 2013) and hence, the associated perception of the aroma and flavors (Sáenz-Navajas *et al.*, 2012). Other wine matrix components include mannoproteins which are hydrocolloids derived from yeast autolysis. Wine sensory properties which are influenced by mannoproteins include an increased perception in the mouthfeel sensation of fullness in wines through increased viscosity

of the wines (Vidal *et al.*, 2004), and retention of some aroma compounds through matrix interactions (Comuzzo *et al.*, 2011; Juega *et al.*, 2012).

Most of the studies that have been conducted exploring matrix interactions in wines have been performed in model wines (Vidal *et al.*, 2004; Lorrain *et al.*, 2013; Villamor *et al.*, 2013a; Villamor *et al.*, 2013b). These model wines are generally prepared with few odorants and a limited number of matrix components, only allowing for the evaluation of low order matrix interactions and their effect on sensory properties. Exploring these interactions in model systems should be accompanied by studies in more complicated systems to evaluate the validity of these relationships. Compared to model wines, commercial wines are complex in terms of their composition (Pola´sˇkova´ *et al.*, 2008), and the change in complexity between these two systems may influence the observed relationships.

Continuous improvement of wine quality will also require the development and application of novel research approaches for more rapid and objective evaluations to keep pace with the quality measurement of the millions of gallons of wine produced in the United States each year. As multisensory instruments, electronic tongues (e-tongues) fulfill the criteria of objectivity and rapid evaluation (Escuder-Gilabert and Peris, 2010; Cosio *et al.*, 2012). The e-tongue is designed to mimic the human sense of taste. It typically consists of an array of sensors and chemometrics software for pattern recognition, thus generating a fingerprint of the taste of the product; this "fingerprint" allows the qualitative and quantitative determination of relevant tastes attributes of a product in solution (Riul *et al.*, 2003; Cabral *et al.*, 2009). These electronic tongues have found relevant application in wine research for the discrimination of wines from different grape varieties and from different geographic area (Gutiérrez *et al.*, 2011), deterioration (Gil-Sánchez *et al.*, 2011) and prediction of the sensory properties of wine (Buratti *et al.*, 2007)

In order to better explore the relationship of wine matrix components and potential application of the e-tongue, the overall objective of this study was to examine the influence of matrix interactions in commercial red wines on the sensory and chemical properties of wines, and explore advanced methods of mathematical analysis in the analysis of both the chemical and sensory data. The specific objectives of the studies were:

i. To evaluate the influence of the interactions among alcohol, tannin and mannoproteins on the aroma, flavor, taste and mouthfeel attributes of selected commercial merlot wines. We hypothesized that the differences in concentration of these matrix components will lead to varying enhancement or suppression of the perception of aroma, flavor, taste and mouthfeel attributes of the selected Merlot wines.

ii. To apply an electronic tongue to discriminate among wines, predict taste attributes and correlate wine sensory attributes to the electronic tongue measurement as an objective and rapid method. We hypothesized that the electronic tongue could discriminate among samples based on their taste profiles and that the response from the electronic tongue would be dependent on the wine matrix components

iii. To estimate panelist variation in a red wine trained panel for panel performance monitoring. We hypothesized that individual variation in a sensory panel could be abstracted as a linear operator known as a bias matrix which could then be used for feedback calibration and adjustment of panelists' intensity ratings of attributes.

**Literature Cited**


Bindon, K., Holt, H., Williamson, P. O., Valera, C., Herderich, M., & Francis, I. L. (2014). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry, 154*, 90-101.

Bindon, K., Valera, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry, 138*, 1696-1705.

Buratti, S., Ballabio, D., Benedetti, S., & Cosio, M. S. (2007). Prediction of Italian red wine sensorial descriptors from electronic nose, electronic tongue and spectrophotometric measurements by means of Genetic Algorithm regression models. *Food Chemistry, 100*, 211-218.

Cabral, F. P. A., Bergamo, B. B., Dantas, C. A. R., Riul, A., & Giancometti, J. A. (2009). Impedance e-tongue instrument for rapid liquid assessment. *Review of Scientific Instruments, 80*, 026107.

Casassa, L. F., Beaver, C. W., Mireles, M. S., & Harbertson, J. F. (2013). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Australian Journal of Grape and Wine Research, 19*, 25-39.

Comuzzo, P., Tat, L., Fenzi, D., Brotto, L., Battistutta, F., & Zironi, R. (2011). Interactions between yeast autolysates and volatile compounds in wine and model solution. *Food Chemistry, 127*, 473–480.

Cosio, M. S., Scampicchio, M., & Benedetti, S. (2012). Electronic noses and tongues. In P. Yolanda (Ed.), *Chemical Analysis of Food: Techniques and Applications* (pp. 219-247): Academic Press, USA.

Escuder-Gilabert, L., & Peris, M. (2010). Review: Highlights in recent applications of electronic tongues in food analysis. *Analytica Chimica Acta, 665*, 15-25.

Gil-Sánchez, L., Soto, J., Martínez-Mánez, R., Garcia-Breijo, E., Ibánez, J., & Llobet, E. (2011). A novel humid electronic nose combined with an electronic tongue for assessing deterioration of wine. *Sensors and Actuators A., 171*, 152-158.

Gil, M., Kontoudakis, N., González, E., Estereulas, M., Fort, F., Canals, J. M., & Zamora, F. (2012). Influence of grape maturity and maceration length on color, polyphenolic composition, and polysaccharide content of Cabernet Sauvignon and Tempranillo wines. *Journal of Agricultural and Food Chemistry, 60*, 7988-8001.

Gutiérrez, M., Llobera, A., Ipatov, A., Vila-Planas, J., Mínguez, S., Demming, S., Büttgenbach, S., Capdevila, F., Domingo, C., & Jiménez-Jorquera, C. (2011). Application of an e-tongue to the analysis of monovarietal and blends of white wines. *Sensors, 11*, 4840-4857.

Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K., & Ross, C. F. (2009). Chemical and sensory effects of Saignee, water addition, and extended maceration on high brix must. *American Journal of Enology and Viticulture, 60*(4), 450-460.

Harbertson, J. F., Zimmerman, B., & Baldwin, L. (2007). Survey of phenolic constituents of Merlot and Cabernet Sauvignon wines produced in Washington. *American Journal of Enology and Viticulture, 58*(3), 410a-419a.

Jackson, R. S. (2014). *Wine Science, Principles and Applications* (4 ed.). USA: Academic Press.

Juega, M., Nunez, Y. P., Carrascosa, A. V., & Martinez-Rodriguez, A. J. (2012). Influence of yeast mannoproteins in the aroma improvement of white wines. *Journal of Food Science, 77*(8), M499-504.

Landon, J. L., Weller, K., Harbertson, J. F., & Ross, C. F. (2008). Chemical and sensory evaluation of astringency in Washington State red wines. *American Journal of Enology and Viticulture, 59*(2), 153-158.

Lorrain, B., Tempere, S., Iturmendi, N., Moine, V., de Revel, G., & Teissedre, P. (2013). Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level. *Food Chemistry, 140*, 76-82.

Pola´sˇkova´ , P., Herszage, J., & Ebeler, S. E. (2008). Wine flavor: chemistry in a glass. *Chemical Society Reviews, 37*, 2478-2489.

Pozo-Bayon, M. A., & Reineccius, G. (2009). Interactions between wine matrix macro-components and aroma compounds. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 417-436). New York: Springer Science+Business Media.

Riul, A., Malmegrim, R. R., Fonseca, F. J., & Mattoso, L. H. C. (2003). An artificial taste sensor based on conducting polymers. *Biosensors and Bioelectronics, 18*, 1365-1369.

Robinson, A. L., Ebeler, S. E., Heymann, H., Boss, P. K., Solomon, P. S., & Trengove, R. D. (2009). Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *Journal of Agricultural and Food Chemistry, 57*, 10313–10322.

Sáenz-Navajas, M., Campo, E., Avizcuri, J. M., Valentin, D., Fernández-Zurbanoa, P., & Ferreira, V. (2012). Contribution of non-volatile and aroma fractions to in-mouth sensory

properties of red wines: Wine reconstitution strategies and sensory sorting task. *Analytica Chimica Acta, 732*, 64-72.

Tian, B., Morton, J., Jaspers, M., Grose, C., & Trought, M. (2013). Effects of mechanical harvesting and grape processing on the composition of 'Sauvignon Blanc' juice and wine. *Acta Horticulturae, 987*, 187-190.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry, 85*, 519–525.

Villamor, R. R., Evans, M. A., Mattinson, D. S., & Ross, C. F. (2013a). Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. *Food Research International, 50*, 38–45.

Villamor, R. R., Evans, M. A., & Ross, C. F. (2013b). Effect of ethanol, tannin, and fructose concentrations on sensory properties of model red wines. *American Journal of Enology and Viticulture, 64*(3), 342-348.

Villamor, R. R., Harbertson, J. F., & Ross, C. F. (2009). Influence of tannin concentration, storage temperature, and time on chemical and sensory properties of Cabernet Sauvignon and Merlot wines. *American Journal of Enology and Viticulture, 60*(4), 422-449.

Villamor, R. R., & Ross, C. F. (2013). Wine matrix compounds affect the perception of wine aromas. *Annual Review of Food Science and Technology, 4*, 1-20.

wineinstitute.org. (2015). Wine consumption in the U.S. Retrieved Jan, 19, 2016, from http://www.wineinstitute.org/resources/statistics/article86

# CHAPTER II

# LITERATURE REVIEW

## Introduction

Wine is defined by Robinson (2006) as the "alcoholic beverage obtained from the fermentation of the juice of freshly gathered grapes, the fermentation taking place in the district of origin according to local tradition and practice". As also pointed out by this author, wine can be made from fruits other than grapes but most of these fruits have less fermentable sugars and hence the need to add sugar from other sources. Wines made from grapes are either made from single grapes or a blends (Gutiérrez *et al.*, 2011). Regardless of whether wine is made from a single or a blend of grapes, the end product of grape juice fermentation is a complex mixture of many compounds with water being the predominant component (Jackson, 2014).

Wine quality is usually discussed in terms of the relationships among its components and its sensory properties (Gawel *et al.*, 2007; Landon *et al.*, 2008; Lund *et al.*, 2009; Gawel *et al.*, 2013). Previous research has shown that the sensory properties of the wine are not only related to the individual concentrations of wine components, but also the interactions among these constituents (Jones *et al.*, 2008; Pozo-Bayon and Reineccius, 2009; Villamor *et al.*, 2013b).

The sensory and chemical qualities of wines have been studied using both sensory and instrumental approaches (Villamor *et al.*, 2013a; Baker and Ross, 2014). Besides the use of both consumer and trained sensory evaluation panels to characterize wine quality, the use of novel instrumentation to evaluate wine quality has also been explored. The use of electronic tongues and electronic noses has been documented in the literature as methods to enhance understanding of wine quality (Buratti *et al.*, 2007; Gil-Sánchez *et al.*, 2011; Cosio *et al.*, 2012).

This review examines the current state of knowledge of the influence of wine components on the sensory and chemical properties of wines as determined by both instrumental and sensory approaches.

**Composition of Wine**

Wine is a complex mixture of volatile and non-volatile components. These components originate from the grapes used in wine production, as well as the chemical and biochemical transformations associated with yeast fermentation and subsequent ageing of wine (Pozo-Bayon and Reineccius, 2009; Robinson *et al.*, 2014). As a result, wine composition varies widely according to grape variety and the winemaking process.

Matrix Components

*Water:* The most abundant constituent of wine is water, composing ~87% by volume (Jackson, 2014). Water functions to provide the flow properties of wine and serves as the medium in which the other constituents are dispersed. Freshly harvested grapes provide the source of water in wines. However, under some processing conditions, water may be added to wines in a process known as watering back (Harbertson *et al*., 2009).

*Alcohols*: Wine is composed of different types of alcohols, including methanol, ethanol, sugar alcohols, fusel alcohols, diols and polyols (Fugelsang and Edwards, 2007; Zamora, 2009; Jackson, 2014). These concentrations of these alcohols range from trace amounts (methanol and sugar alcohols) to substantial quantities (ethanol) and have different functions in wine.

Ethanol is the next most abundant component of wine after water and the major product of alcoholic fermentation. Under standard fermentation conditions, ethanol can accumulate up to 14-15% (Jackson, 2014). Its concentration in wine depends on the initial sugar levels in the grapes at harvest and the extent to which fermentation is allowed to proceed (Margalit, 2004;

Henderson and Rex, 2007). The significance of ethanol to wine is seen in its contribution to the stability, ageing, and extraction of grape constituents, its participation in chemical reactions, and its contribution to the sensory properties of the wine (Robinson, 2006). Ethanol provides microbial stability to wines through its antimicrobial action. The ability of *Saccharomyces cerevisae* to survive the ethanol environment in wines compared to spoilage yeasts and bacteria ensures that ethanol prohibits the growth of most of the microbial populations that would otherwise pose a spoilage risk to wine (Fugelsang and Edwards, 2007). However, while many microorganisms are not ethanol tolerant, some microbes including *Zygosaccharomyces bailii* do display ethanol tolerance up to 18% (v/v) ( Fugelsang and Edwards (2007).

Ageing of wine is characterized by the development of complexity of wine. Ethanol contribute to this process by reacting with organic acids to produce esters and maintaining this equilibrium in favor of ester formation (Jackson, 2014). The sensory impact of ethanol on wine includes a contribution to sweetness (Zamora *et al.*, 2006), burning mouthfeel (Gawel *et al.*, 2007), perception of viscosity (Nurgel and Pickering, 2005) and its influence on the volatility of aroma compounds (Villamor and Ross, 2013).

*Residual Sugar*: The unfermented sugars remaining in a finished wine are called residual sugar. They are present as both fermentable (glucose and fructose) and unfermentable (pentoses like arabinose and rhamnose) sugars (Robinson, 2006; Jackson, 2014). Generally, the concentration of residual sugars in wines influences whether the wine is considered “sweet” or “dry”, even though other matrix components can contribute to the perceived sweetness of wines. Wines with residual sugar concentrations less than 0.2% (w/w) are not detectable as sweet while very sweet wines can have residual sugars more than 10% (Robinson, 2001). Unfermented sugars may remain in wines for several reasons, including differences in the sugar utilization by different

yeast strains, variation in the nutrient composition of grape musts, diversity and competition among microbial populations during fermentation and fermentation temperatures (Robinson, 2001; Fugelsang and Edwards, 2007). The sensory impact of residual sugars is the sweet taste of the wine but also provides a balance with the acidity of the wine (Zraly, 2011).

*Polyphenolic Compounds*: Polyphenolics are a diverse group of compounds which originate mainly from the grapes (skin and seeds), with small concentrations being extracted from the oak cooperage and trace amounts from yeast metabolism (Jackson, 2014). Polyphenolic compounds are cyclic benzene compounds with at least on hydroxyl group attached directly to the carbon ring structure. In wines, they are broadly divided into two major groups: non-flavonoids (benzoic acid, benzaldehyde, cinnamic acid, cinnamaldehyde and tyrosol) and flavonoids (flavonols, anthocyanins and flavan-3-ols). Phenolics influence the color (Brouillard *et al.*, 2003; Marquez *et al.*, 2012), taste (McRae *et al.*, 2013), mouthfeel (Landon *et al.*, 2008; McRae and Kennedy, 2011), and aromas (Villamor *et al.*, 2013b; Villamor and Ross, 2013; Lorrain *et al.*, 2013) of wines.

Proanthocyanidins or condensed tannins are formed as a result of the polymerization of flavan-3-ol monomers and are extracted from the wines from the skins and seeds of grapes (Jackson, 2014). Tannins from oak cooperage that are added to wine during ageing are known as hydrolysable tannins (Moreno and Peinado, 2012).

Variations of polyphenolic profiles in red wines have been attributed largely to winemaking technique and viticultural practices (Harbertson *et al.*, 2008). Regarding viticultural practices, temperature and sunlight exposure, as well as vine water status in different vintages, have been implicated in the differences in phenolic composition observed among some grapes (Lorrain *et al.*, 2011), thus influencing the phenolic composition of their subsequent wines. The

change in phenolic profiles of wines have been documented to change from low levels during crushing of the berries, increasing during the winemaking process and either stabilizing or decreasing during the ageing process (Ginjom *et al.*, 2011). The amount of phenolics extracted into wines depends on many factors, including berry ripeness at harvest and interaction with grape cell wall components (Hanlin *et al.*, 2010), and the use of commercial enzymes (Ortega-Regules *et al.*, 2006). The extraction of phenolic compounds from grapes into the must can occur pre-fermentation or during alcoholic fermentation (Monagas and Bartolomé, 2009). This extraction, as reported by Casassa *et al.* (2013), depends much more on maceration time. Also as noted by Canals *et al.* (2005), alcohol efficiently facilitates the extraction of phenolic compounds in riper grapes

*Acids:* Acids in wines include both organic and inorganic forms. Acids are characterized by their ability to release hydrogen ions ($H^+$) into the wine, resulting in measurable acidity in wines as indicated by pH and titratable acidity. Acidity is divided into two categories: volatile and fixed. Volatile acidity is readily removed by steam distillation while the fixed acidity is not (Jackson, 2014). Acetic acid, with its vinegar-like taste and aroma, characterizes volatile acidity while malic acid and tartaric acid constitute over 90% of wine's fixed acidity, with influences over the pH of the wine.

The microbial and chemical stability of a wine depends on many factors including the pH of the wine. A pH range 3.1 – 3.6 is suitable for most wines (Jackson, 2014). The importance of acids to wines is shown by the long-term stability of the wine and protein haze prevention through the precipitation of proteins due to the relationship between pH and the iso-electric point of wine proteins (Dufrechou *et al.*, 2011). Acids are also important for color stability by favoring the red color of anthocyanins at low pH values (Kontoudakis *et al.*, 2011), and bacterial growth

inhibition (Fugelsang and Edwards, 2007). Sensory attributes are also influenced by the presence of acids, including a "refreshing" taste (Jackson, 2014), perceived acidity (Zraly, 2011) and modification of other tastes and mouthfeel attributes such as the reduction of sweetness (Fischer and Noble, 1994). It has been speculated that acids in wines may be involved in the initiation of acid hydrolysis during crushing of grapes, thus releasing aroma compounds occurring as acid-labile non-volatile glycosides (Jackson, 2014).

*Polysaccharides and Yeast Autolysates:* Grape polysaccharides are one of the main groups of macromolecules released into the wine during the winemaking process. Grape polysaccharides are released after the degradation of cell walls and include arabinogalactan-proteins and rhamnogalacturonan polymers (RG-1 and RG-II respectively). Mannoproteins are hydrocolloids which are released into the wine through yeast autolysis during fermentation. The mannoprotein content of wine have been indicated to be between 100 – 150 ml/l (Perez-Serradilla and de Castro, 2008) and constitute about 35% of wine polysaccharides (Vidal *et al.*, 2003). The importance of mannoproteins in wine includes prevention of tannin aggregation, inhibition of protein precipitation, and promotion of the growth of lactic acid bacteria for malolactic fermentation (Chalier *et al.*, 2007; Perez-Serradilla and de Castro, 2008; Diez *et al.*, 2010). Mannoproteins may also interact with aroma compounds leading to the retention or release of these aromatic compounds, as well as interact with phenolic compounds to reduce astringency and improve color stability.

*Proteins:* The total protein content of wine depends upon both viticultural and enological practices, including cultivar and fining operations (Jackson, 2014). At the end of fermentation, most of these proteins are precipitated with tannins through the formation of insoluble protein-polyphenol complexes, thus making protein hazes less problematic in red wine than whites.

Consequently, protein concentrations in red wines are generally lower than in whites wines (Zoecklein *et al.*, 1999; Moreno and Peinado, 2012). Proteins in wines have been reported to be in low, generally ranging in concentration from15 – 269 mg/l (Monteiro *et al.*, 2001; Ferreira *et al.*, 2002; Lambri *et al.*, 2013; Mainente *et al.*, 2014).

Aroma Compounds

The aroma of wine originates from hundreds of compounds from a myriad of sources, including the grapes (which provide varietal aroma) (Gonzalez-Barreiro *et al.*, 2015), secondary metabolites from microbial action (Herraiz and Ough, 1993), oak cooperage (Cadahia *et al.*, 2009) and chemical transformations occurring during the winemaking process (Ugliano, 2013; Jackson, 2014). The classes of volatile compounds in wine have been reviewed and include terpenes, norisoprenoids, volatile sulfur compounds, phenylpropanoids, higher alcohols, volatile acids, esters, furan derivatives and pyrazines (Robinson *et al.*, 2014). These compounds have broadly been categorized as primary (grape-derived or varietal), secondary (as a result of fermentation) and tertiary (oak and bottle ageing) wine aromas (Robinson, 2006; Villamor and Ross, 2013).

*Varietal Aroma*: These aroma compounds are accumulated in the berries during the second stage of berry development known as the berry ripening stage (Coombe and Mccarthy, 1997, 2000). These compounds usually accumulate as secondary metabolites and include compounds that produce characteristic varietal aromas such as terpenes, pyrazines and norisoprenoids, all of which mostly exist in the glycosylated state (Park *et al.*, 1991; Hashizume and Samuta, 1999; Mateo and Jime´nez, 2000; Zalacain *et al.*, 2007). These groups of compounds impart characteristic aromas to the wines. The terpenes are responsible for floral aromas and include compounds such as linalool, geraniol, nerol, limonene, nerolidol and α-terpineol. Norisoprenoids

are derived from carotenoids, and impart fruity aromas to the wine. Compounds responsible for these fruity aromas include β-damascenone, β-ionone and α-ionone. The pyrazines contribute herbaceous or 'green' aroma to wines. The most important contributors to the characteristic vegetal aroma associated with pyrazines are the 3-isobutyl-2-methoxypyrazine, 3-isopropyl-2-methoxypyrazine, and 3-sec-butyl-2-methoxypyrazine (Sidhu *et al.*, 2015).

*Secondary Aroma:* Wine aromas generated as a result of the fermentation process are called secondary aromas. The fermentation process results in the conversion of sugars in the must to ethanol through the action of yeast. But along with this conversion come the formation of new chemical compounds and the release of aroma compounds from their precursors through the action of acids, enzymes and yeast metabolism (Jarauta *et al.*, 2005). Pola´sˇkova´ *et al.* (2008) indicated that aroma compounds derived from fermentation constitute the highest percentage of the total aroma constituents of wine. According to these authors, formation results in the formation of many alcohols (mostly ethanol and $C_3$-$C_5$ straight and branched chained alcohols) and esters (mainly ethyl acetates and isoamyl acetate).

Apart from these compounds resulting from fermentation, other compounds resulting directly or indirectly from glycolysis are also produced. These compounds include glycerol, acetic acid and acetaldehyde (Styger *et al.*, 2011), which all contribute to the secondary aromas in wine. Beyond being a product of glycolysis, the utilization of amino acids in yeast metabolism also leads to the production of volatile compounds that confer characteristic secondary aromas to wines. These volatile compounds are composed of higher alcohols and their associated volatile acids and esters occurring in trace and quantifiable amounts, such as isovaleraldehyde (fruity, nut-like), isoamyl acetate (banana, pear), ethyl isovalerate (apple, fruity), 2-phenyl acetate (rose, honey, flowery), ethyl-2-methylbutanoate (strawberry, pineapple), amyl alcohol (almond),

isobutanol (fruity, alcohol, solvent-like), isobutyric (sweet, apple-like) and 2-methylbutyraldehyde (green, malty) (Lambrechts and Pretorious, 2000; Styger *et al.*, 2011).

Other volatile components of wine resulting from primary fermentation include esters that are generated from the reaction of ethanol with organic acids. This reaction can either be an enzyme-free or enzyme-mediated reaction. The enzyme-mediated reaction involves an initial activation of the acid by a coenzyme prior to reaction with the alcohol (Styger *et al.*, 2011). Ethyl esters, such as ethyl octanoate, ethyl hexanoate and ethyl butanoate, are the result of the reaction between medium-chained fatty acids which have been pre-activated by an enzyme prior to reacting with ethanol (Saerens *et al.*, 2008).

As a method to reduce high acidity levels, some wines undergo malolactic fermentation as a secondary fermentation. This is a biological conversion of the malic acid to a lactic acid through the use of lactic acid bacteria (Davis *et al.*, 1985). The sensory impact of malolactic fermentation has been reviewed and includes the enhancement and suppression of aroma characteristics and contribution to mouthfeel properties (Liu, 2002). Specifically, fruity and buttery notes are enhanced through malolactic fermentation while vegetal or grassy notes are suppressed (Liu, 2002). Other aroma attributes associated with malolactic fermentation according to this author include floral, nutty, yeasty, oaky, sweaty, spicy, roasted, toasty, vanilla, smoky, earthy, bitter, ropiness and honey. Besides these, this secondary fermentation was linked the sensory perception of body and mouthfeel of wines.

*Tertiary Aroma*: These wine aromas, formed during wine ageing, contribute to the complexity of the wine and are dependent upon yeast autolysis and the extraction of aroma compounds from the barrels in which the wine is aged (Liu, 2002). Jarauta *et al.* (2005) described aroma profile changes and the patterns of these changes as a result of concurrent physical, chemical and

biological phenomena occurring during ageing of wine in oak barrels. These authors observed that the ageing bouquet resulted from the accumulation of some volatile compounds along with the disappearance of other compounds. Compounds that accumulated in the wine were those extracted from wood and its surfaces. Compounds also resulted from oxidation occurring in wood and the release of aroma-active compounds from precursors through chemical and microbiological actions. In addition to the generation of these compounds, condensation and oxidative reactions as well as sorption of aroma compounds to the wooden barrel led to the disappearance of some of compounds. The concentrations of 41 out of the 79 compounds investigated in this study changed either as a result of the ageing process or the type of oak (French or American) used.

A wide range of compounds contribute to the ageing bouquet of wines. Specifically, the contribution of cis-oak lactones, 4-ethyl phenol, acetaldehyde, dimethyl sulfide and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) to the aged bouquet of red and white wines have been reviewed (Villamor and Ross, 2013). Recent studies have also shown the importance of some polyfunctional thiols (2-furanmethanethiol and 3-sulfanylhexanol) and their association with the typicality of aged red Bordeaux wines as well as the contribution of piperitone (a monoterpene ketone) to the mint notes perceived in these wines (Picard *et al.*, 2015; Picard *et al.*, 2016).

Clearly, wine aroma has its origins from the grapes and from the winemaking process. The diversity of these aroma compounds contributes to the distinctiveness of wine as a complex alcoholic beverage.

**Methods for Wine Matrix Interactions Studies**

The wine matrix has an effect on both orthonasal and retronasal perception of aromas and flavors of wine due to its impact on the release of aroma compounds and their subsequent

perception during wine consumption (Druaux and Voilley, 1997; Pozo-Bayon and Reineccius, 2009; Mitropoulou *et al.*, 2011; Sáenz-Navajas *et al.*, 2012). The study of interactions among the volatile and non-volatile components of wine has been conducted using both sensory and analytical techniques which will be discussed in the subsequent sections.

Analytical Techniques for Matrix Interactions Studies

The analytical approaches for studying the matrix interactions with other wine components include the use of dynamic and static methods, as well as nuclear magnetic resonance techniques (Jung *et al.*, 2000; Jung and Ebeler, 2003). Static and dynamic headspace techniques are used to determine levels of odorants in the headspace of a sample, giving an indication of the potential interactive effect of the matrix. This can result in enhancement or suppression of perception of volatiles. Conversely, nuclear magnetic resonance techniques reveal the nature of the interactions among matrix components and odorants leading to the observed headspace concentrations (Dufour and Bayonove, 1999b).

Headspace solid-phase microextraction (HS-SPME) as a dynamic method of volatile compound evaluation has been widely applied to the study of wine aroma in many studies (Hartmann *et al.*, 2002; Jung and Ebeler, 2003; Villamor *et al.*, 2013a). HS-SPME has been cited for being the most suitable for studying the interactions among wine matrix components and wine volatile components (Petrozziello *et al.*, 2014). This method is used to measure volatile compounds in two stages: adsorption of the volatile compounds present in the sample headspace onto a thin silica fiber (SPME fiber) followed by desorption prior to GC–MS analysis (Pawliszyn, 2000). Even though results are very much dependent on the conditions used during the extraction process, the technique is fast, solventless, easy to implement and does not require specific instrumentation on the GC (Fabre *et al.*, 2002). Due to this dependence on extraction

conditions, the extraction time should be as short as possible as equilibrium of the partitioning between the liquid and gaseous phases can affect the adsorption onto the fiber. Jung and Ebeler (2003) reduced the effect of the fiber on redistribution of the molecules between the liquid and gas phases by taking a ‘‘snapshot’’ of the headspace composition. When sampling time is very short (1 min), the technique is known as ‘‘SPME ‘true’ headspace’’(Roberts *et al.*, 2000; Mitropoulou *et al.*, 2011).

Nuclear magnetic resonance (NMR) spectroscopy has been indicated as one of the most powerful tools for determining the structure and conformation of molecules in solution (Bovey, 1988; Lampman *et al.*, 2010). The application of spectroscopic techniques to aroma-matrix interaction research is used to directly identify the nature of molecular interactions (Fares *et al.*, 1998). NMR parameters including changes in chemical shift, line width, and relaxation rate are some of the criteria used for the evaluation of molecular interactions as they are very sensitive to changes in chemical environments and complex formation (Otting, 1993). Measuring these parameters makes NMR a powerful tool for studying intra- and intermolecular interactions. Complexes between odorants and polyphenols, and particularly the interaction mechanism between these compounds, have been investigated using this method (Jung *et al.*, 1998).

Gas chromatography – Olfactometry (GC/O) is used to ascertain the contribution of a compound to the overall aroma of a sample by coupling gas chromatographic effluent to a sniffing port (Mayol and Acree, 2001). A human assessor sniffs the effluent as it emerges from the port and records the time at which a particular aroma is sensed; aroma intensity can also be determined. This method allows the detection of trace volatile compounds which have a major sensory impact, as well as volatile compounds present in high concentrations which may not contribute much to the overall aroma quality of the product being profiled. Aroma Extract

Dilution Analysis (AEDA) is a GC-O method in which aroma extracts are sequentially diluted until the aroma compound cannot be sensed in the effluent of the sniffing port (Pola´sˇkova´ *et al.*, 2008). Plots of the aroma dilution factor versus the retention indices of the compounds are generated and the impact of each aroma, defined as the least dilution factor at which the compound was detected in the effluent, is obtained. Other GC-O methods include time intensity and detection frequency methods.

Reconstitution and omission tests are methods that usually follow GC-O. This is so because GC-O is able to identify important odorants but does not give an indication of interactions or masking effects of these compounds. Using reconstitution and omission tests, the aroma quality of a reconstituted sample of potentially important odorants is compared with the original sample. If the odor quality is not similar to the original sample, the missing aroma compounds need to be identified and added. Omission tests involve the removal of some odorants after reconstitution in order to evaluate their contribution to the overall aroma quality. If the omitted compounds lead to a substantial change in the aroma perception, those aroma compounds are considered important contributors to the aroma of the sample. Using this technique, the masking or enhancing effect of the aroma compounds can be determined (Grosch, 2001). The coupling of instruments with human subjects as performed using GC-O provides data which is more supportive of actual perception. However, sensory evaluation as an independent method has also been used to study interactions.

Sensory Techniques for Matrix Interaction Studies

Sensory evaluation is critical in the evaluation of product quality (Meilgaard *et al.*, 2007) Specifically for wine, regardless of the precision of the flavor composition as determined by instruments, there is still the need for sensory evaluation as flavor composition does not

necessarily translate into a similar human perception (Noble and Ebeler, 2002). Jones *et al.* (2008) is one of the studies in which the authors have used only sensory evaluation for the assessment of matrix interactions. In this study, panelist perception of aroma intensity was influenced by the interaction between ethanol while metallic mouthfeel was influenced by the ethanol-polysaccharide interaction. Furthermore, these authors showed that perceived astringency was influenced by a four-way interaction among ethanol, glycerol, polysaccharides and volatile compound concentration. Studies like this typically use quantitative descriptive tests in which panelists are trained to assess the intensities of specific attributes of a product by assigning a value to the intensity of a product attribute using a predefined scale (Robinson *et al.*, 2011). The ability of the panelists to perceive differences in samples as a result of interactions among matrix components depends upon how well they are trained to recognize the sensory attributes of the product being profiled. Hence, there exists a need to monitor and correct panelist performance during training.

Indeed, monitoring panel performance is important to ensure the panelists agree in the intensity ratings of samples and that their sensory acuity and abilities are not varying beyond acceptable limits as determined by consensus building during training sessions. Panelists vary as a result of physiological and psychological differences. Thus, bringing a sensory panel to the point of reliable and repeatable evaluations requires extensive effort and time. In fact, panel performance is directly related to training time as observed by Chambers *et al.* (2004). In their study, these authors observed that training a panel for 120 hours increased the discrimination capabilities of the panelists and reduced the variability of the results. To this end, several visual and statistical tools have been reported in literature which are used to monitor panel performance (Næs and Solheim, 1991; Rossi, 2001; Brockhoff, 2003; Tomic *et al.*, 2007; Tomic *et al.*, 2013).

To monitor a panel performance in their study, Hirst and Næs (1994) developed a graphical technique to assess the differences in the ranking of attributes in a given set of products. This graph displayed panelists' ranking plots which revealed individual differences in the rankings of the panelists. The closer the panelists' cumulative rank plots were to the 'baseline' plot, the better the agreement among the panelist to the underlying order of the ranking for the characteristic(s) under study. In instances where the underlying ranking was not known, the eigenvector of $\mathbf{XX^T}$ was used, where $\mathbf{X}$ is the $n$ x $m$ matrix rank with columns representing panelists and rows representing the objects being ranked. This matrix was mean-standardized column-wise. By standardizing the means column-wise, the eigenvectors were used to represent the scores of the first principal component of the data while the panelists were regarded as samples. The elements of this vector were ranked and plotted for each panelist.

In a recent study, Tomic *et al.* (2013) computed and demonstrated the use of agreement, repeatability and discrimination performance indices which can be useful to the panel leader. They computed the agreement and reproducibility indices from the RV coefficient and the discrimination index from one- or two-way ANOVA results. These indices addressed the issues of agreement among panelists on product and attribute differences, and reproducibility of assessors on product and attribute evaluations across replicate evaluations. These indices also determined the percentage of attributes for which panelists were able to adequately discriminate among the products at a 5% significance level. In a panel performance monitoring process, when the panel leader presents these performance indices in a table, the panel can have leader a quick overview of panelists who are deviating from the rest of the panel.

**Interaction between Non-Volatile and Volatile Components**

Ethanol, polyphenols and polysaccharides and yeast autolysates are major matrix components whose impacts on volatility and perception of wine aroma have been extensively studied. Ethanol impacts the volatility of aroma compounds by changing the partitioning coefficient of a compound between the liquid and the headspace above the wine (Pozo-Bayon *et al*., 2009). Polyphenols in wines affect the volatile compounds through hydrophobic-driven interactions (Dufour and Bayonove, 1999b). Wine polysaccharides and yeast autolysates have varying impact on volatilities of the aroma compounds depending on the volatile compound being investigated and nature of the polysaccharide or yeast autolysate (Dufour and Bayonove, 1999a; Chalier *et al.*, 2007). The following subsections examine specific studies relating to the impact of these matrix components on the volatility of aroma compounds in wines.

Impact of Ethanol

Whiton and Zoecklein (2000) reported the matrix effect on the detection of aroma compounds of different functionality and volatility in a model wine solution that varied in ethanol concentration. By optimizing the headspace solid-phase microextraction method, changes in the headspace concentrations of selected alcohols, esters, acids, norisoprenoids and phenolics were determined. Results showed a decrease in the headspace concentrations of all compounds, except 3-methyl butanol as ethanol concentration increased from 11-14; indicating a suppressive effect of ethanol on these volatile compounds.

In a related study, Hartmann *et al.* (2002) investigated the recoveries of 3-ethyl-, isopropyl, sec-butyl-, and isobutyl-2-methoxypyrazines as affected by varying ethanol content (0-20% v/v) in a model solution. The authors recovered ~10 times more methoxypyrazines from the non-alcoholic samples compared to the 20% alcohol solution. This phenomenon was

attributed to two factors, with the first factor described as the increase in solubility of the pyrazines in the aqueous phase by ethanol which shifted the equilibrium concentration away from the headspace. The second factor was described as the competition between ethanol with the pyrazines for binding sites on the SPME fiber.

Similarly, Robinson *et al.* (2009) observed that ethanol played an important role in influencing the headspace partitioning of volatile compounds in a model solution. Villamor *et al.* (2013b) observed that the impact of ethanol on wine aroma compounds depended on the aroma compound under investigation. Specifically, increasing ethanol concentration from 8 to 16% (v/v) increased chemical, woody, spicy aroma and flavor, bitter taste and burning sensation while decreasing the perceived fruity, floral, and caramel aroma and flavor.

In a recent study, the effect of non-volatile wine matrix on the temporal aroma release was monitored with an artificial mouth device linked online to a proton transfer reaction-time-of-flight mass spectrometer (Muñoz-González *et al.*, 2015). Five wines (sparkling, aged red, young red, sweet and white) were de-aromatized and reconstituted to the same ethanol level (12% v/v) and then spiked with eight target odorants, including 1-hexanol, (Z)-hex-3-en-1-ol, β-ionone, β-damascenone, β-pinene, furfural, ethyl dodecanoate and eugenol. The red wines which had a high phenolic composition were generally found to have a higher aroma release compared to the sweet wines. The authors also found a higher release of hydrophobic compounds in the aged and young red wines. This result was attributed to the interaction between the glycoproteins in saliva and the phenolic compounds in wines, resulting in a change in the polarity of the wine and enhancing the release of the hydrophobic compounds.

The use of a retronasal aroma trapping device (RATD) to monitor aroma release during consumption of a model derived-wine beverage has been investigated (Muñoz-González *et al.*,

2014). This method involved the use of low alcohol wines which were adjusted to different sugar (0 – 150 mg/l) and ethanol concentrations and then aromatized with isoamyl acetate, ethyl hexanoate and linalool. Using an RATD, the exhaled air of the panelists was trapped after swallowing the wine. The exhaled air was then analyzed for levels of the aroma compounds spiked into the model wines. The results showed no influence of sugar on aroma release, but ethanol enhanced the aroma release. This observation contradicted results obtained from studies involving static conditions (Aznar *et al.*, 2004; Aprea *et al.*, 2007) indicating that monitoring of aroma release *in vivo* does not lead to the same conclusions. This deviation from the expected results was explained by the authors as likely being due to the effect of oro-physiological parameters like breathing and swallowing patterns. Saliva and mucus participate in the *in vivo* delivery of aroma compounds during the drinking process. One weakness of this study was the use of only three panelists to establish these relationships. The research requires more participants to provide an insight into the contribution of individual differences.

Impact of Polyphenols

Using exponential dilution and NMR, Dufour and Bayonove (1999b) studied the interactions among aroma compounds (benzaldehyde, ethyl hexanoate, isoamyle acetate and limonene) and polyphenols (catechin and condensed tannins). From the exponential dilution results, catechin decreased the volatilities of all odorants but limonene. Increasing concentrations of proanthocyanidins slightly suppressed the volatility of benzaldehyde but salted out limonene, thus increasing the headspace concentration of this odorant. No effect on ethyl hexanoate and isoamyl acetate was observed in this study. NMR results revealed a hydrophobic-driven interaction between catechin and all compounds except for limonene. The interactions among

polyphenols and aroma compounds therefore depend upon many factors including the chemical nature of the aroma compound in question.

Mitropoulou *et al.* (2011) studied the volatility of a set of aroma compounds composed of 6 esters, 5 alcohols and 1 acid as influenced by the non-volatile components of a model red wine. Using HS-SPME, the authors showed that polysaccharide extract, skin and seed tannin extract influence the headspace concentrations of the spiked odorants. The impact of the tannin extracts on the volatility of the aroma compounds varied with concentration, with the lower concentrations of tannin extracts resulting in greater volatilities. Both enhancement and suppressive effects of these polyphenols were observed on the headspace concentration of the aroma compounds studied. The interactions among the tannin extracts and polysaccharides lead to increased volatility of octanoic acid and 2-phenylethyl alcohol in the headspace of the model wine. This study showed that the concentrations of tannin extracts, as well as their interaction with polysaccharides in wines have variable impact on the volatilities of aroma compounds.

Interactions among gallic acid and naringin with ethylbenzoate and 2-methyl was studied in a model wine using HS-SPME/GC to determine headspace odorant concentrations (Aronson and Ebeler, 2004). Results of this study revealed a decrease in the volatility of the pyrazine as a result of the presence of gallic acid, with naringin showing less impact on the volatility of this pyrazine. Ethylbenzoate was not affected by these polyphenols as much as the pyrazine was. The authors attributed the differences in the effect to structural differences between the polyphenols and the aroma compounds.

Lorrain *et al.* (2013) investigated the effect of (+)-catechin and gallic acid on the volatility and sensory perception of selected esters using HS-GC-MS and triangle tests, respectively. Catechin significantly reduced the headspace concentration of ethyl octanoate but

the concentrations of ethyl butanoate, isoamyl acetate and ethyl isobuyrate in the headspace of the model wine were not significantly impacted. The sensory results showed increased perception threshold for some esters in the presence catechin, possibly as a result of hydrophobic interactions among catechin and aroma compounds.

Using model wines made with varying levels of naringin and gallic acid, Aronson and Ebeler (2004) demonstrated that the perceived intensity of ethyl benzoate is reduced by these polyphenols and this effect occurred at higher levels of ethyl benzoate. Although both polyphenols influenced the perception of ethyl benzoate, naringin had a greater impact on perception than gallic acid. The lower influence of gallic acid supports the hypothesis that hydrophobic interactions are involved in the binding of phenolic compounds with aroma compounds

In another study of the effect of polyphenols on perception of some aroma compounds in Sauvignon Blanc wines, Lund *et al.* (2009) assessed the effect of catechin, caffeic acid and quercetin on the perception of isobutyl methoxypyrazine, 3-mercaptohexanol, 3-mercaptohexanol acetate and ethyl decanoate using a trained sensory evaluation panel. Generally, the added polyphenols suppressed the sensory perception of these aroma compounds with a few exceptions. Catechin slightly enhanced the perception of 3-mercaptohexanol acetate while the addition of caffeic acid enhanced the perception of 3-mercaptohexanol. In spite of its minor enhancement on 3-mercaptohexanol acetate detection, catechin had the greatest suppressing effect on the aroma compounds compared to the other two polyphenols. The authors explained that the enhancing effect of caffeic acid on 3-mercaptohexanol may be due to the suppression of other compounds in the wine, thus masking perception of the other compounds in the presence of caffeic acid.

Impact of Polysaccharides and Yeast Autolysates

The impact of whole extracts or fractions of mannoproteins on the volatilities of four aroma compounds were studied using static and dynamic headspace techniques (Chalier *et al.*, 2007). The study showed evidence of interactions among mannoproteins and aroma compounds, with differences in the extent of the interactions being dependent upon whether the mannoprotein was whole or fractionated. The authors asserted that this observation has implications for the role of compositional and conformational structure of mannoproteins in their interaction with aroma compounds in wines. Hexanol, ethylhexanoate and β-ionone were suppressed to varying degrees through interaction with mannoproteins thus manifesting as lower headspace concentrations. No effect was noted for isoamyl acetate. Peptidic and glycosidic interactions have been implicated by the authors as a possible explanation for the complexity of the binding of aroma compounds with mannoproteins.

In a similar study of the impact of structural differences in polysaccharides on the headspace concentrations of odorants, Dufour and Bayonove (1999a) used the exponential dilution technique to examine the effect of mannoproteins, arabinogalactans and rhamnogalactans on isoamyl acetate, ethyl hexanoate, hexanol and diacetyl in a model wine. The volatilities of the esters were not affected when polysaccharides present in the range of 5-20 g/l were added to the model wine. Structurally different arabinogalactan proteins (a protein-rich: AGP0 and an acid-rich: AGP4) interacted differently with isoamyl acetate and ethyl hexanoate. While AGP0 retained these esters, AGP4 slightly salted them out. Similarly, rhamnogalactans salted these esters out. These results indicated that the impact of the interactions among polysaccharides and aroma compounds on volatility is variable and depends upon the polysaccharide and the aroma compounds.

Through the use of dextrin and dextran, Duffour and Bayonove (1999a) again demonstrated that structurally different polysaccharides have different impacts on the volatilities of some aroma compounds. Presence of dextran in solution salted out ethyl hexanoate and isoamyl acetate. Hexanol was also affected by dextran but only at concentrations below 4% dextran. However, diacetyl recovery was not impacted by dextran. On the contrary, increasing concentrations of dextrin reduced the volatilities of ethyl hexanoate, isoamyl acetate and hexanol. Of these three volatiles, ethyl hexanoate and hexanol (which had structural similarities due to the presence of a hexyl group) were the most retained as a result of strong complexation with dextrin.

In a model red wine system, Comuzzo *et al.* (2011) studied the retention of aroma compounds by yeast autolysates and interactions of colloids from yeast autolysate with ethyl octanoate, linalool, 2-phenyl ethanol, β-ionone and octanoic acid. Ethyl octanoate was significantly retained by the addition of the yeast autolysates thus illustrating the ability of yeast autolysates to bind some aroma compounds and reduce the perception in model systems. This study also showed that 2-phenyl ethanol was affected by the presence of colloids from yeast autolysates, leading to decreased headspace concentration of this volatile compound. Similar effects were observed in the headspace concentrations of β-ionone and octanoic acid.

Chalier *et al.* (2007) studied the effect of mannoprotein isolated from *Saccharomyces cerevisae* strains on aroma compounds in a model red wine system. The study involved the use of both whole and fractions of mannoproteins and the volatile compounds, isoamyle acetate, hexanol, ethyl hexanoate and β-ionone. The presence of mannoproteins in the model wine reduced the perceived intensities of the aroma compounds except for isoamyl acetate. The perceived intensities were also dependent of the type of mannoprotein in the model wine,

suggesting that the different fractions of mannoproteins interacted differently with the aroma compounds. Results from sensory evaluation confirmed the trends observed in headspace analysis.

Other polysaccharides which have been studied include arabinogalactan and pectins which were generally observed to increase the volatility of some esters, alcohols and octanoic acid (Mitropoulou *et al*., 2011).

The above review of the literature on the individual wine matrix components and how they interact with the volatile components of wine clearly shows complex interactions occurring in wine. Beyond these individual effects, interactions among the matrix components themselves exist, with an influence on sensory perception.

**Higher-Order Interactions**

Higher order interactions among some of the wine macromolecules and their combined influence on odorants have been investigated, mostly conducted using model wines and reconstituted wines which usually have a few odorants (Jones *et al.*, 2008; Robinson *et al.*, 2009; Villamor *et al.*, 2013b), as well as pilot-scale wines (Villamor *et al.*, 2009). For instance, in a five-component study of the interaction among wine components in a model white wine, the perception of sensory attributes were dictated by two, three and four-way interactions among ethanol, polysaccharides, glycerol, protein and volatile compounds (Jones *et al.*, 2008). Specifically, these authors showed that the perceived aroma intensity was influenced by ethanol-glycerol interaction while metallic mouthfeel was impacted by ethanol-polysaccharide interaction. Three-way interactions among protein, glycerol and alcohol affected most of the aroma perception of most of the volatiles, with the greatest impact being observed when the odorants were in low concentrations in the wine. Also, perceived astringency was influenced by

a four-way interaction among ethanol, glycerol, polysaccharides and volatile compound concentration.

Similarly, another study reported the impact of two and three-way interactions among ethanol, tannins and fructose concentrations on the headspace volatiles of a model red wine (Villamor *et al.*, 2013a). Employing HS-SPME and HS-SPME/GC-O methods, the interactions among the model wine matrix components and eight odorants commonly found in red wines and selected to reflect different aroma and physicochemical characteristics was studied. The combined effects of ethanol, tannins and fructose reduced the headspace concentrations of the odorants 2-methoxyphenol (woody aroma), 2-phenylethanol (floral aroma), β-damascenone (fruity aroma), and 1-octen-3-one (earthy aroma). These results indicated the loss of high molecular weight odorants. The authors suggested that these losses may lead to unbalanced aroma perception during wine consumption.

Also, two-way interactions among ethanol and other wine components were found to directly influence the solubility of wine volatile compounds and hence their headspace abundance in model wines (Robinson *et al.*, 2009). Using solid phase microextraction and GC-MS, these authors studied the interaction among ethanol, glycerol, glucose, catechin and proline and their impact on 20 typical aroma compounds. Two-way interactions between ethanol and glucose, ethanol and glycerol; and catechin and glycerol significantly affected the headspace partitioning of the volatiles. The conclusion of this study was that ethanol played an important and significant role among other wine components in influencing the headspace partitioning of volatiles in a model wine solution.

Petrozziello *et al.* (2014) also studied the influence of wine matrix component on the volatility of *Brettanomyces*-related volatile compounds in a model wine. Solid-phase

microextraction and GC-MS was used to evaluate the concentrations of 4-ethylphenol and 4-ethylguaiacol concentrations in the headspace of the samples in the presence of variable levels of ethanol, polyphenols and yeast extracts. In these model wines, results showed a significant impact of ethanol and polyphenols on the partitioning of these *Brettanomyces*-related off-flavors at equilibrium conditions. At higher levels of ethanol and polyphenols, a decrease in the volatility of the ethylphenols was observed in the headspace of the wine. The addition of yeast extract had little effect as observed from the small decrease in the headspace concentrations of the volatile phenols.

Thus far, wine composition, interactions among the components and various sensory and analytical means of determining these interactions have been reviewed. The use of electronic tongues for rapid analysis of samples is desirable. Particularly, the potential application of the electronic tongue technology to the study of wine matrix interactions focusing on how these interactions affects taste and mouthfeel properties will greatly enhance the understanding of the sensory properties of wines.

**Electronic Tongues in Wine Quality Evaluation**

Electronic tongues are multisensory systems used for the analysis of liquid samples based on an array of sensors with a suitable integrated pattern recognition system (Riul *et al.*, 2003; Cabral *et al.*, 2009; Escuder-Gilabert and Peris, 2010). They are usually composed of four components: an autosampler, an array of sensors with different selectivity but cross discriminatory, instrumentation to obtain the response from the interaction between the sensors and the sample and an algorithm to process the signal for results once it is obtained (Escuder-Gilabert and Peris, 2010). Development of the system draws on the underlying principle of the neurophysiology of the sense of taste (Cosio *et al.*, 2012). In a typical operation of the electronic

tongue, the sensors interact with the electrical properties of the analytes in solution and provide responses characteristic of the solution being assessed; this may be considered a fingerprint of the solution similar to the human tongue. The use of multivariate and regression analysis confers separation and prediction capabilities, respectively, to the system.

Electronic tongues are named after the sensor architecture used in their design as electrochemical (potentiometric, voltammetric, amperometric and impedimetric), optical, mass and biosensors. These different sensor architectures have been reviewed (Ciosek and Wro´blewski, 2007). Potentiometric electronic tongues are the most widely used sensor architecture for ion selective electrodes. Their operational principle is the measurement of the potential difference between the reference electrode and the sensor array. Potentiometric electronic tongues also offer the advantages of easy set-up, easy fabrication and the possibility of obtaining sensors that are selective to many chemical species. Voltammetric electronic tongues are limited to redox-active substances but are known for their high selectivity, high signal-to-noise ratio, and selectivity to a wide range of chemical species (Escuder-Gilabert and Peris, 2010). Impedimetric electronic tongues measure impedance using spectroscopy to measure impedance as either fixed frequency or broad spectrum.

The optical and mass electronic tongues operate on principles which are different from electrochemical. Optical electronic tongues are mainly used in biomedical research and their operations are based on optical characteristics such as absorbance, reflectance and fluorescence. Other analytes which are suitable for this method are those that are difficult to be detected through electrochemical means as they are either uncharged or not electroactive. Mass sensors are rarely used in electronic tongues and are instead used mostly used in electronic nose systems. However, some characteristics of this architecture make their use in electronic tongues attractive.

These include their high sensitivity, robustness and their detection principle being based upon weight changes (Ciosek and Wro´blewski, 2007; Escuder-Gilabert and Peris, 2010).

The electronic tongue has found utility in the quality assessment of wines in terms of differentiation among wines based on their taste as affected by many factors. Specifically, Legin *et al.* (2003) developed an electronic tongue consisting of 23 potentiometric cross-sensitive sensors to discriminate among wines and predict some chemical parameters and human sensory evaluation scores. Results from the study showed that the electronic tongue was able to discriminate among the 58 wines from three varietals and different geographical areas using 19 sensory attributes. Error rate for the prediction of chemical parameters did not exceed 12% while the error rates for the prediction of the human sensory evaluation was between 8-13%. Wines from different origins, grape varieties and vintages have been successfully differentiated using the electronic tongue (Riul *et al.*, 2003; Gutiérrez *et al.*, 2011). Wines from different vineyards have also been studied and distinguished among using the electric tongue (Riul *et al.*, 2004). A combination of the electronic tongue and electronic nose have been used in the study of wine spoilage, specifically oxidation through a period of 48 days (Gil-Sánchez *et al.*, 2011). The electronic tongue was also used for the prediction of the sensorial parameters and overall quality of dry wines (Buratti *et al.*, 2007).

In a related study, a voltammetric electronic tongue was used to detect adulteration in wines (Parra *et al.*, 2006). The authors adulterated a wine by changing its levels of alcohol, acidity, astringency, $SO_2$, volatile acidity and reducing sugar through the addition of extra ethanol, tartaric acid, tannic acid, $SO_2$, acetic acid and sucrose, respectively. Principal component analysis biplot of the electronic tongue results and wine samples showed clustering of wines with

their respective adulterants in a similar region in two-dimensional space, indicating that samples were associated with the adulterant that was used to alter their chemical composition.

The electronic tongue is therefore becoming increasingly popular in wine quality evaluation, not only as a means of discriminating among wines, but also as a tool for predicting the sensory attributes of wines.

**Literature Cited**


Aprea, E., Biasioli, F., Maerk, T. D. and Gasperi, F. (2007). PTR-MS study of esters in water and water/ethanol solutions: fragmentation patterns and partition coefficients. *International Journal of Mass Spectrometry,* 262:114–121.

Aronson, J., & Ebeler, S. E. (2004). Effect of polyphenol compounds on the headspace volatility of flavors. *American Journal of Enology and Viticulture, 55*(1), 13-21.

Aznar, M., Tsachaki, M., Linforth, R. S. T., Ferreira, V. and Taylor, A.J. (2004). Headspace analysis of volatile organic compounds from ethanolic systems by direct APCI-MS. *International Journal of Mass Spectrometry,* 239:17–25.

Baker, A. K., & Ross, C. F. (2014). Sensory evaluation of impact of wine matrix on red wine finish: a preliminary study. *Journal of Sensory Studies, 29*(2), 139-148.

Bovey, F. A. (1988). *Nuclear Magnetic Resonance Spectroscopy* (2 ed.). San Diego Academic Press.

Brockhoff, P. B. (2003). Statistical testing of individual differences in sensory profiling. *Food Quality and Preference, 14*, 425–434.

Brouillard, R., Chassaing, S., & Fougerousse, A. (2003). Why are grape/fresh wine anthocyanins so simple and why is it that red wine color lasts so long? *Phytochemistry, 64*, 1179-1186.

Buratti, S., Ballabio, D., Benedetti, S., & Cosio, M. S. (2007). Prediction of Italian red wine sensorial descriptors from electronic nose, electronic tongue and spectrophotometric measurements by means of Genetic Algorithm regression models. *Food Chemistry, 100*, 211-218.

Cabral, F. P. A., Bergamo, B. B., Dantas, C. A. R., Riul, A., & Giancometti, J. A. (2009). Impedance e-tongue instrument for rapid liquid assessment. *Review of Scientific Instruments, 80*, 026107.

Cadahia, E., de Simon, B. F., Sans, M., Poveda, P., & Colio, J. (2009). Chemical and chromatic characteristics of Tempranillo, Cabernet Sauvignon and Merlot wines from DO Navarra aged in Spanish and French oak barrels. *Food Chemistry, 115*, 639-649.

Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry, 53*, 4019-4025.

Casassa, L. F., Beaver, C. W., Mireles, M. S., & Harbertson, J. F. (2013). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Australian Journal of Grape and Wine Research, 19*, 25-39.

Chalier, P., Angot, B., Delteil, D., Doco, T., & Gunata, Z. (2007). Interactions between aroma compounds and whole mannoprotein isolated from *Saccharomyces cerevisiae* strains. *Food Chemistry, 100*(1), 22-30.

Chambers, D. H., Allison, A. A., & Chambers, E. I. V. (2004). Training effects on performance of descriptive panelists. *Journal of Sensory Studies, 19*, 486-499.

Ciosek, P., & Wro´blewski, W. (2007). Sensor arrays for liquid sensing – electronic tongue systems. *Analyst, 132* 963-978.

Comuzzo, P., Tat, L., Fenzi, D., Brotto, L., Battistutta, F., & Zironi, R. (2011). Interactions between yeast autolysates and volatile compounds in wine and model solution. *Food Chemistry, 127*, 473–480.

Coombe, B. G., & Mccarthy, M. G. (1997). Identification and naming of the inception of aroma development in ripening grape berries. *Australian Journal of Grape and Wine Research, 3*, 18-20.

Coombe, B. G., & Mccarthy, M. G. (2000). Dynamics of grape berry growth and physiology of ripening. *Australian Journal of Grape and Wine Research, 6*, 131-135.

Cosio, M. S., Scampicchio, M., & Benedetti, S. (2012). Electronic noses and tongues. In P. Yolanda (Ed.), *Chemical Analysis of Food: Techniques and Applications* (pp. 219-247): Academic Press, USA.

Davis, C. R., Wibowo, D., Eschenbruch, R., Lee, T. H., & Fleet, G. H. (1985). Practical implications of malolactic fermentation: A review. *American Journal of Enology and Viticulture, 36*(4), 290-301.

Diez, L., Guadalupe, Z., Ayestaran, B., & Ruiz-Larrea, F. (2010). Effect of yeast mannoproteins and grape polysaccharides on the growth of wine lactic acid and acetic acid bacteria. *Journal of Agricultural and Food Chemistry, 58*, 7731-7739.

Druaux, C., & Voilley, A. (1997). Effect of food composition and microstructure on volatile flavour release *Trends in Food Science & Technology, 8*, 364-368.

Dufour, C., & Bayonove, C. L. (1999a). Influence of wine structurally different polysaccharides on the volatility of aroma substances in a model system. *Journal of Agricultural and Food Chemistry, 47*, 671–677.

Dufour, C., & Bayonove, C. L. (1999b). Interactions between wine polyphenols and aroma substances. An insight at the molecular level. *Journal of Agricultural and Food Chemistry, 47*, 678-684.

Dufrechou, M., Poncet-Legrand, C., Sauvage, F., & Vernhet, A. (2011). Stability of white wine proteins: combined effect of pH, ionic strenght and temperature on their aggregation. *Journal of Agricultural and Food Chemistry, 60*, 1308-1319.

Escuder-Gilabert, L., & Peris, M. (2010). Review: Highlights in recent applications of electronic tongues in food analysis. *Analytica Chimica Acta, 665*, 15-25.

Fabre, M., Aubry, V., & Guichard, E. (2002). Comparison of different methods: Static and dynamic headspace and solid-phase microextraction for the measurement of interactions between milk proteins and flavor compounds with an application to emulsions. *Journal of Agricultural and Food Chemistry, 50* 1497-1501.

Fares, K., Landy, P., Guilard, R., & Voilley, A. (1998). Physicochemical interactions between aroma compounds and milk proteins: effect of water and protein modification. *Journal of Dairy Science, 81*, 82-91.

Ferreira, R. B., Picarra-Pereira, M. A., Monteiro, S., Loureiro, V. B., & Teixeira, A. R. (2002). The wine proteins. *Trends in Food Science & Technology, 12*, 230-239.

Fischer, U., & Noble, A. C. (1994). The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine. *American Journal of Enology and Viticulture, 45*(1), 6-10.

Fugelsang, K. C., & Edwards, C. G. (2007). *Wine Microbiology. Practical Applications and Procedures* (Second ed.). USA: Springer Science+Business Media.

Gawel, R., Sluyter, S. V., Smith, P. A., & Waters, E. J. (2013). Effect of pH and alcohol on perception of phenolic character in white wine. *American Journal of Enology and Viticulture, 64*(4), 425-429.

Gawel, R., Sluyter, S. V., & Waters, E. J. (2007). The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines. *Australian Journal of Grape and Wine Research, 13*, 38–45.

Gil-Sánchez, L., Soto, J., Martínez-Mánez, R., Garcia-Breijo, E., Ibánez, J., & Llobet, E. (2011). A novel humid electronic nose combined with an electronic tongue for assessing deterioration of wine. *Sensors and Actuators A., 171*, 152-158.

Ginjom, I., D'Arcy, B., Caffin, N., & Gidley, M. (2011). Phenolic compound profiles in selected Queensland red wines at all stages of the wine-making process. *Food Chemistry, 125*, 823-834.

Gonzalez-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., & Simal-Gandara, J. (2015). Wine aroma compounds in grapes: A critical review. *Critical Reviews in Food Science and Nutrition, 55*, 202-218.

Grosch, W. (2001). Evaluation of key odorants of foods by dilution experiments, aroma model and omission. *Chemical Senses, 26*, 533-545.

Gutiérrez, M., Llobera, A., Ipatov, A., Vila-Planas, J., Mínguez, S., Demming, S., Büttgenbach, S., Capdevila, F., Domingo, C., & Jiménez-Jorquera, C. (2011). Application of an e-tongue to the analysis of monovarietal and blends of white wines. *Sensors, 11*, 4840-4857.

Hanlin, R. L., Hrmova, M., Harbertson, J. F., & Downey, M. O. (2010). Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. *Australian Journal of Grape and Wine Research, 16*, 173-188.

Harbertson, J. F., Hodgins, R. E., Thurston, L. N., Schaffer, L. J., Reid, M. S., Landon, J. L., Ross, C. F., & Adams, D. O. (2008). Variability of tannin concentration in red wines. *American Journal of Enology and Viticulture, 59*(2), 210-214.

Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K., & Ross, C. F. (2009). Chemical and sensory effects of Saignee, water addition, and extended maceration on high brix must. *American Journal of Enology and Viticulture, 60*(4), 450-460.

Hartmann, P. J., McNair, H. M., & Zoecklein, B. W. (2002). Measurement of 3-alkyl-2-methoxypyrazine by headspace solid-phase microextraction in spiked model wines. *American Journal of Enology and Viticulture, 53*(4), 285-288.

Hashizume , K., & Samuta, T. (1999). Grape maturity and light exposure affect berry methoxypyrazine concentration. *American Journal of Enology and Viticulture, 50*(2), 194-198.

Henderson, J. P., & Rex, D. (2007). *About Wine*. New York: Thompson Delmar Learning.

Herraiz, T., & Ough, C. S. (1993). Formation of ethyl esters of amino acids by yeasts during the alcoholic fermentation of grape juice. *American Journal of Enology and Viticulture, 44*(1), 41-48.

Hirst, D., & Næs, T. (1994). A graphical technique for assessing differences among a set of rankings. *Journal of Chemometrics, 8*, 81-93.

Jackson, R. S. (2014). *Wine Science, Principles and Applications* (4 ed.). USA: Academic Press.

Jarauta, I., Cacho, J., & Ferreira, V. (2005). Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: An analytical study. *Journal of Agricultural and Food Chemistry, 53*, 4166-4177.

Jones, P. R., Gawel, R., Francis, I. L., & Waters, E. J. (2008). The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Quality and Preference, 19*, 596–607.

Jung, D., de Ropp, J. S., & Ebeler, S. E. (1998). Study of interaction between polyphenols and aromatic flavors by $^{1}$H NMR spectroscopy. *American Journal of Enology and Viticulture, 49*, 455.

Jung, D., de Ropp, J. S., & Ebeler, S. E. (2000). Study of interactions between food phenolics and aromatic flavors using one- and two-dimensional $^{1}$H NMR spectroscopy. *Journal of Agricultural and Food Chemistry, 48*, 407-412.

Jung, D., & Ebeler, S. E. (2003). Headspace Solid-Phase Microextraction method for the study of the volatility of selected flavor compounds. *Journal of Agricultural and Food Chemistry, 51*, 200-205.

Kontoudakis, N., Gonzalez, E., Gil, M., Estereulas, M., Fort, F., & Canals, J. M. (2011). Influence of wine pH on changes in color and polyphenol composition induced by micro-oxygenation. . *Journal of Agricultural and Food Chemistry, 59*, 1974-1984.

Lambrechts, M. G., & Pretorious, I. S. (2000). Yeast and its importance to wine aroma. *South African Journal of Enology and Viticulture, 21*, 97-129.

Lambri, M., Dordoni, R., Giribaldi, M., Violetta, M. R., & Guiffrida, M. G. (2013). Effect of pH on the protein profile and heat stability of an Italian white wine. *Food Research International, 54*, 1781-1786.

Lampman, G. M., Pavia, D. L., Kriz, G. S., & Vyvyan, J. R. (2010). *Spectroscopy* (4 ed.). USA: Brooks/Cole.

Landon, J. L., Weller, K., Harbertson, J. F., & Ross, C. F. (2008). Chemical and sensory evaluation of astringency in Washington State red wines. *American Journal of Enology and Viticulture, 59*(2), 153-158.

Legin, A., Rudnitskaya, A., Lvova, L., Vlasov, Y., Natale, C. D., & D'Amico, A. (2003). Evaluation of Italian wine by the electronic tongue: recognition, quantitative analysis and correlation with human sensory perception. *Analytica Chimica Acta, 484*, 33–44.

Liu, S.-Q. (2002). Malolactic fermentation in wine – beyond deacidification. *Journal of Applied Microbiology, 92*, 589-601.

Lorrain, B., Chira, K., & Teissedre, P. (2011). Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: comparison to 2006, 2007 and 2008 vintages. *Food Chemistry, 126*, 1991-1999.

Lorrain, B., Tempere, S., Iturmendi, N., Moine, V., de Revel, G., & Teissedre, P. (2013). Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level. *Food Chemistry, 140*, 76-82.

Lund, C. M., Nicolau, L., Gardner, R. C., & Kilmartin, P. A. (2009). Effect of polyphenols on the perception of key aroma compounds from Sauvignon Blanc wine. *Australian Journal of Grape and Wine Research, 15*, 18-26.

Mainente, F., Zoccatelli, G., Lorenzini, M., Cecconi, D., Vincenzi, S., Rizzi, C., & Simonato, B. (2014). Red wine proteins: Two dimensional (2-D) electrophoresis and mass spectrometry analysis. *Food Chemistry, 164*, 413-417.

Margalit, Y. (2004). *Concepts in Wine Chemistry* (2nd ed.). CA, USA: The Wine Appreciation Guild.

Marquez, A., Serratosa, M. P., Lopez-Toledano, A., & Merida, J. (2012). Colour and phenolic compounds in sweet red wines from Merlot and Tempranillo grapes chamber-dried under controlled conditions. *Food Chemistry, 130*, 111-120.

Mateo, J. J., & Jime´nez, M. (2000). Monoterpenes in grape juice and wines. *Journal of Chromatography A, 881*, 557-567.

Mayol, A. R., & Acree, T. E. (2001). Advances in gas chromatography-olfactometry. In J. V. Leland, P. Schieberle, A. Buettner & T. E. Acree (Eds.), *Gas Chromatography-Olfactometery, the State of the Art* (pp. 1-10). USA: American Chemical Society.

McRae, J. M., & Kennedy, J. A. (2011). Wine and grape tannin interactions with salivary proteins and their impact on astringency: a review of current research. *Molecules, 16*, 2348-2364.

McRae, J. M., Schulkin, A., Kassara, S., Holt, H. E., & Smith, P. A. (2013). Sensory properties of wine tannin fractions: implications for in-mouth sensory properties. *Journal of Agricultural and Food Chemistry, 61*, 719-727.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory Evaluation Techniques* (4th ed.). Boca Raton: CRC Press, Taylor and Francis Group.

Mitropoulou, A., Hatzidimitriou, E., & Paraskevopoulou, A. (2011). Aroma release of a model wine solution as influenced by the presence of non-volatile components. Effect of commercial tannin extracts, polysaccharides and artificial saliva. *Food Research International, 44*(5), 1561-1570.

Monagas, M., & Bartolomé, B. (2009). Anthocyanins and anthocyanin-derived compounds. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry.* (pp. 439-462). New York, USA.: Springer Science+Business Media.

Monteiro, S., Picarra-Pereira, M. A., Mesquita, P. R., Loureiro, V. B., Teixeira, A., & Ferreira, R. B. (2001). The diversity of structurally similar wine proteins. *Journal of Agricultural and Food Chemistry, 49*, 3999-4010.

Moreno, J., & Peinado, R. (2012). *Enological Chemistry*. CA, USA: Academy Press, Elsevier.

Muñoz-González, C., Rodriguez-Bencomo, J. J., Moreno-Arribas, M. V., & Pozo-Bayon, M. A. (2014). Feasibility and application of a retronasal aroma-trapping device to study in vivo aroma release during the consumption of model wine-derived beverages. *Food Science and Nutrition, 2*(4), 361-370.

Muñoz-González, C., Sémon, E., Martín-Álvarez, P. J., Guichard, E., Moreno-Arribas, M. V., Feron, G., & Pozo-Bayón, M. Á. (2015). Wine matrix composition affects temporal aroma release as measured by proton transfer reaction – time-of-flight –mass spectrometry. *Australian Journal of Grape and Wine Research, 21*, 367–375.

Næs, T., & Solheim, R. (1991). Detection and interpretation of variation within and between assessors in sensory profiling. . *Journal of Sensory Studies, 6*, 159-177.

Noble, A. C., & Ebeler, S. E. (2002). Use of multivariate statistics in understanding wine flavor. *Food Reviews International, 18*(1), 1–21.

Nurgel, C., & Pickering, G. (2005). Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model wines. *Journal of Texture Studies, 36*, 303–323.

Ortega-Regules, A., Romero-Cascales, I., Ros-Garcia, J. M., Lopez-Roca, J. M., & Gomez-Plaza, E. (2006). A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. *Analytica Chimica Acta, 563*, 26-32.

Otting, G. (1993). Experimental NMR techniques for studies of protein-ligand interaction. *Current Opinion in Structural Biology, 3*, 760-768.

Park, S. K., Morrison, J. C., Adams, D. O., & Noble, A. C. (1991). Distribution of free and glycosidically bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. *Journal of Agricultural and Food Chemistry, 39*, 514-518.

Parra, V., Arrieta, A. A., Fernandez-Escudero, J. A., Rodrıguez-Mendez, M. L., & De Saja, J. A. (2006). Electronic tongue based on chemically modified electrodes and voltammetry for the detection of adulterations in wines. *Sensors and Actuators B, 118*, 448–453.

Pawliszyn, J. (2000). Theory of solid-phase microextraction. *Journal of Chromatographic Science, 38*, 270-278.

Perez-Serradilla, J. A., & de Castro, M. D. L. (2008). Role of lees in wine production: A review. *Food Chemistry, 111*, 447-456.

Petrozziello, M., Asproudi, A., Guaita, M., Borsa, D., Motta, S., Panero, L., & Bosso, A. (2014). Influence of the matrix composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. *Food Chemistry, 149*, 197-202.

Picard, M., Lytra, G., Tempere, S., Barbe, J., de Revel, G., & Marchand, S. (2016). Identification of piperitone as an aroma compound contributing to the positive mint nuances perceived in aged red Bordeaux wines. *Journal of Agricultural and Food Chemistry, 64*, 451−460.

Picard, M., Thibon, C., Redon, P., Darriet, P., de Revel, G., & Marchand, S. (2015). Involvement of dimethyl sulfide and several polyfunctional thiols in the aromatic expression of the aging bouquet of red Bordeaux wines. *Journal of Agricultural and Food Chemistry, 63*, 8879−8889.

Pola´sˇkova´ , P., Herszage, J., & Ebeler, S. E. (2008). Wine flavor: chemistry in a glass. *Chemical Society Reviews, 37*, 2478-2489.

Pozo-Bayon, M. A., & Reineccius, G. (2009). Interactions between wine matrix macro-components and aroma compounds. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 417-436). New York: Springer Science+Business Media.

Riul, A., de Sousa, H. C., Malmegrim, R. R., dos Santos, D. S., André Carvalho, A. C. P. L. F., Fonseca, F. J., Oliveira, O. N., & Mattoso, L. H. C. (2004). Wine classification by taste sensors made from ultra-thin films and using neural networks. *Sensors and Actuators B, 98*, 77–82.

Riul, A., Malmegrim, R. R., Fonseca, F. J., & Mattoso, L. H. C. (2003). An artificial taste sensor based on conducting polymers. *Biosensors and Bioelectronics, 18*, 1365-1369.

Roberts, D. D., Pollien, P., & Milo, C. (2000). Solid-phase microextraction method development for headspace analysis of volatile flavor compounds. *Journal of Agricultural and Food Chemistry, 48*, 430-2437.

Robinson, A. L., Adams, D. O., Boss, P. K., Heymann, H., Solomon, P. S., & Trengove, R. D. (2011). The relationship between sensory attributes and wine composition for Australian Cabernet Sauvignon wines. *Australian Journal of Grape and Wine Research, 17*, 327–340.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., & Ebeler, S. E. (2014). Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture, 65*, 1-24.

Robinson, A. L., Ebeler, S. E., Heymann, H., Boss, P. K., Solomon, P. S., & Trengove, R. D. (2009). Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *Journal of Agricultural and Food Chemistry, 57*, 10313–10322.

Robinson, J. (2001). *Concise Wine Companion*. New York: Oxford University Press, Inc.

Robinson, J. (2006). *The Oxford Companion to Wine* (3 ed.). New York: Oxford University Press.

Rossi, F. (2001). Assessing sensory panelist performance using repeatability and reproducibility measures. *Food Quality and Preference, 12*, 467–479.

Sáenz-Navajas, M., Campo, E., Avizcuri, J. M., Valentin, D., Fernández-Zurbanoa, P., & Ferreira, V. (2012). Contribution of non-volatile and aroma fractions to in-mouth sensory properties of red wines: Wine reconstitution strategies and sensory sorting task. *Analytica Chimica Acta, 732*, 64-72.

Saerens, S. M. G., Delvaux, F., Verstrepen, K. J., Van Dijck, P., Thevelein, J. M., & Delvaux, F. R. (2008). Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Applied and Environmental Microbiology, 74*(2), 454–461.

Sidhu, D., Lund, J., Kotseridis, Y., & Saucier, C. (2015). Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Critical Reviews in Food Science and Nutrition*(55), 485–502.

Styger, G., Prior, B., & Bauer, F. F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology & Biotechnology, 38*, 1145–1159.

Tomic, O., Forde, C., Delahunty, C., & Næs, T. (2013). Performance indices in descriptive sensory analysis – A complimentary screening tool for assessor and panel performance. *Food Quality and Preference, 28*, 122–133.

Tomic, O., Nilsen, A., Martens, M., & Næs, T. (2007). Visualization of sensory profiling data for performance monitoring. *LWT, 40*, 262–269.

Ugliano, M. (2013). Oxygen contribution to wine aroma evolution during bottle aging. *Journal of Agricultural and Food Chemistry, 61*, 6125-6136.

Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The polysaccharides of red wine: total fractionation and characterization. *Carbohydrate Polymers, 54*(4), 439-447.

Villamor, R. R., Evans, M. A., Mattinson, D. S., & Ross, C. F. (2013a). Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. *Food Research International, 50*, 38–45.

Villamor, R. R., Evans, M. A., & Ross, C. F. (2013b). Effect of ethanol, tannin, and fructose concentrations on sensory properties of model red wines. *American Journal of Enology and Viticulture, 64*(3), 342-348.

Villamor, R. R., Harbertson, J. F., & Ross, C. F. (2009). Influence of tannin concentration, storage temperature, and time on chemical and sensory properties of Cabernet Sauvignon and Merlot wines. *American Journal of Enology and Viticulture, 60*(4), 422-449.

Villamor, R. R., & Ross, C. F. (2013). Wine matrix compounds affect the perception of wine aromas. *Annual Review of Food Science and Technology, 4*, 1-20.

Whiton, R. S., & Zoecklein, B. W. (2000). Optimization of headspace solid-phase microextraction for analysis of wine aroma compounds. *American Journal of Enology and Viticulture, 51*(4), 379-382.

Zalacain, A., Mar´ın, J., Alonso, G. L., & Salinas, M. R. (2007). Analysis of wine primary aroma compounds by stir bar sorptive extraction. *Talanta, 71*, 1610-1615.

Zamora, F. (2009). Biochemistry of Alcoholic Fermentation. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 3-25). New York: Springer Science+Business Media.

Zamora, M. C., Goldner, M. C., & Galmarini, M. V. (2006). Sourness-sweetness interactions in different media: white wine, ethanol and water. *Journal of Sensory Studies, 21*, 601–611.

Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., & Nury, F. S. (1999). *Wine Analysis and Production*. New York: Kluwer Academic/Plenum Publishers.

Zraly, K. (2011). *Complete Wine Course* (2012 ed.). New York: Sterling Publishing.

# CHAPTER III

# ALCOHOL, TANNINS AND MANNOPROTEIN AND THEIR INTERACTIONS INFLUENCE THE SENSORY PROPERTIES OF SELECTED COMMERCIAL MERLOT WINES: A PRELIMINARY STUDY


## Abstract

The objective of this study was to assess the influence of the interaction among alcohol, tannins and mannoproteins on the aroma, flavor, taste and mouthfeel characteristics of selected commercial Merlot wines. Merlot wines (n=61) were characterized for wine chemistry parameters, including pH, titratable acidity, alcohol, soluble solids, tannin profile, total proteins and mannoprotein content. Agglomerative clustering of these physicochemical characteristics revealed six groups of wines. Two wines were selected from each group (n=12) and profiled by a trained sensory evaluation panel. One wine from each group was evaluated using the electronic tongue. Sensory evaluation results showed complex effects among tannins, alcohol and mannoproteins on the perception of most aromas, flavors, tastes and mouthfeel attributes ($p<0.05$). The e-tongue showed distinct differences among the taste attributes of the six groups of wines as indicated by a high discrimination index (D.I=95). Strong correlations ($r^2>0.930$) were reported between the electronic tongue and sensory perception of sweet, sour, bitter, burning, astringent and metallic. This study showed that interactions among wine matrix components influence the resulting sensory perceptions. The strong correlation between the e-tongue and trained panel evaluations indicated the electronic tongue can complement sensory evaluations to improve wine quality assessment.

## Introduction

Wine is a complex alcoholic beverage that is composed of both volatile and non-volatile components. These components include water, ethanol, phenolic compounds, polysaccharides and numerous aroma and flavor compounds. The interactions among these components influence sensory perception, as demonstrated by previous studies (Villamor *et al.*, 2009; Villamor *et al.*, 2013b; Baker and Ross, 2014).

Ethanol is the major component in wine beside water (Pozo-Bayon and Reineccius, 2009). The influence of ethanol on the sensory profile of wines has been extensively studied. In wines, ethanol contributes to a burning sensation or palate warming (Gawel *et al.*, 2007), physical and perceived viscosity (Nurgel and Pickering, 2005; Gawel *et al.*, 2007), sourness and sweetness balance (Zamora *et al.*, 2006) and the head space partitioning of volatile compounds, leading to reduced volatility of some compounds at high ethanol levels (Robinson *et al.*, 2009; Pozo-Bayon and Reineccius, 2009; Villamor *et al.*, 2013a). Beyond ethanol, the contribution of other wine matrix components to the sensory properties of wines has been investigated. Proanthocyanidins constitute a significant portion of the wine non-volatile component and include a wide range of phenolic compounds made up of flavan-3-ol monomers. These phenolic compounds play a significant role in perception of astringency and bitterness, particularly in red wines (Gawel, 1998; McRae and Kennedy, 2011; McRae *et al.*, 2013), volatility of volatile compounds (Lorrain *et al.*, 2013) and hence, the associated perception of the aroma and flavors (Sáenz-Navajas *et al.*, 2012).

Other wine matrix components include mannoproteins which are yeast-derived polysaccharides. Mannoproteins from yeast constitute 35% of the total polysaccharide content and are indicated to be the second most abundant polysaccharide fraction in red wine (Vidal *et*

*al.*, 2003). Mannoproteins are secreted into wines during yeast growth and autolysis and have been found to increase in concentration during ageing on lees (Dupin *et al.*, 2000; Giovani and Rosi, 2007; Rowe *et al.*, 2010). Wine sensory properties which are influenced by mannoproteins include an increased perception in the mouthfeel sensation of fullness in wines (Vidal *et al.*, 2004), delay or prevention of tannin polymerization, leading to a reduction of astringency (Rodrigues *et al.*, 2012), retention of aroma compounds (Comuzzo *et al.*, 2011; Juega *et al.*, 2012) and prevention of protein hazes (Dupin *et al.*, 2000).

Besides these findings about the main effects of tannins, ethanol and mannoproteins, fewer studies have determined the interactions among these matrix components on the sensory perception of wines. Although higher order interactions among some of these components have been investigated, studies were mostly conducted using model wines and reconstituted wines which usually have a few odorants (Jones *et al.*, 2008; Robinson *et al.*, 2009; Villamor *et al.*, 2013b), as well as pilot-scale wines (Villamor *et al.*, 2009). For instance, in a five-component study of the interaction among wine components in white wine, the perception of sensory attributes were dictated by two, three and four-way interactions (Jones *et al.*, 2008), illustrating the complexity of these interactions. Specifically, perceived aroma intensity was influenced by ethanol-glycerol interaction while metallic mouthfeel was impacted by ethanol-polysaccharide interaction. Also, perceived astringency was influenced by a four-way interaction among ethanol, glycerol, polysaccharides and volatile compound concentration. Another study reported the impact of two and three-way interactions among ethanol, tannins and fructose concentrations on the headspace volatiles of a model red wine (Villamor *et al.*, 2013a). High concentrations of ethanol, tannins and fructose led to greater losses of high molecular weight odorants, with speculation that this phenomenon may lead to unbalanced aroma perception during wine

consumption. Similarly, also in model wines, two-way interactions between ethanol and other wine components were found to directly impact the solubility of wine volatile compounds and hence their headspace abundance (Robinson *et al.*, 2009).

The quest to understand the sensory quality of wine has led to the application of instrumental approaches to augment human sensory evaluation. Electronic tongues are novel instrumental techniques recently applied to better understand the gustatory response to non-volatile components of wines. Wines from different origins, grape varieties, vintages and vineyards have been characterized (Riul *et al.*, 2004; Gutiérrez *et al.*, 2011) and correlation ($r^2$=0.78-0.91) of electronic tongue response with data from an expert panel (Buratti *et al.*, 2007) has been performed using electronic tongues.

While these studies, mostly in model wines, suggest the interactions that may be occurring among volatile and wine macro components, conclusions about commercial wines based on these finding may be limited and will continue to remain speculative due to the complexity of the composition of commercial wines. Specifically, questions about the major determinants of physicochemical differences among commercial wines, correlations between human and instrumental sensory evaluations and the significance of the interaction among key matrix component on sensory perception need to be answered. The objective of this study, therefore, was to characterize commercial Merlot wines from different wineries using both instrumental and sensory methods in order to enhance understanding of the interactions among key wine components selected based on their importance in studies of model wines (ethanol, tannins and mannoproteins) and their implications on the sensory perception of commercial wines.

## Materials and Methods

### Materials

Bovine serum albumin (BSA, Fraction V powder), sodium dodecyl sulfate (SDS, lauryl sulfate, sodium salt), triethanolamine (TEA), ferric chloride hexahydrate, potassium metabisulfite, and (+)-catechin, citric acid, 6-propyl-2-thiouracil, tartaric acid, tannic acid, alum, quinine sulfate, yeast invertase and mannan standard were purchased from Sigma (St. Louis, MO, USA). Hydrochloric acid, sodium chloride, sodium hydroxide, 100% ethanol, and glacial acetic acid, acetone and trichloroacetic acid were obtained from J.T. Baker (Phillipsburg, NJ). For the electronic tongue analysis, hydrochloric acid, sodium chloride and sodium-L-glutamate standards were obtained from Alpha MOS (Tolouse, France). Chemiluminescent alkaline phosphatase substrate, Tris, biotinylated Narcissus pseudonarcissus lectin, Tween 20 and streptavidin-conjugated alkaline phosphatase were obtained from Vector Laboratories Inc. (Burlingame, CA). Materials for standard recipe preparation for aroma and flavor evaluation included pure cane sugar (C&H sugar company, CA, USA), Kool-Aid (cherry artificial flavor, Kraft Foods Group Inc., Northfield, IL, USA), whole berries (black berries, raspberries and strawberries), cloves (McCormick & Co., Inc. Hunt Valley, MD, USA), iron tablets (65mg, Pharmavite LLC, Mission Hills, CA, USA) and olive brine (Safeway black olives, large, pitted, ripe, Pleasanton CA, USA), violet aroma (Wine Awakening Inc., Canada) and oak chips (Gusmer Enterprises, Inc., CA, USA). MilliQ water was obtained through purification (Millipore Corporation, Billerica, MA, USA).

### Wine Samples

Sixty one (61) commercial Merlot wines with vintages varying as follows: 2011 (n=13), 2010 (n=21), 2009 (n=12) and 2008-2006 (n=9) were purchased from retail outlets and characterized

using physicochemical and sensory methods. Sample selection was random. All samples were commercial wines from either California (n=24) or Washington State wineries (n=37). All wine samples met the state requirement for use of the grape variety indicated on the label.

Chemical Analyses

°Brix, pH and titratable acidity determinations were all made following the procedures previously described (Iland *et al.*, 2004). Ethanol concentrations were determined using the Ebulliometer (ALLA, France) following the previously described procedure (Iland *et al.*, 2004). The trichloroacetic acid (TCA)/Acetone and Bradford assay methods were used to precipitate and quantify, respectively, total protein as previously described (Smith *et al.*, 2011). All measurements of wine chemistry parameters were made in triplicate. Small polymeric pigments (SPP) and large polymeric pigments (LPP) (absorbance units at 520 nm), tannins (mg/l catechin equivalents) and total phenolics (mg/l catechin equivalents) were determined as previously described (Hagerman and Butler, 1978) and modified (Harbertson *et al.*, 2003).

*Mannan Analysis*: Mannan was quantified in the TCA/acetone solubilized wine precipitates by lectin blotting, essentially as previously described (Rowe *et al.*, 2010) with modifications. Specifically, 4 µg/ ml of the mannose-specific, biotinylated *Narcissus pseudonarcissus* lectin was used and in place of X-ray detection, a Bio-Dot SF 48 well slot blot apparatus (BioRad Laboratories, Hercules, CA) was used. Briefly, a nitrocellulose membrane (0.45 µm Bio-Rad Laboratories Hercules, CA) was soaked in a Tris buffer solution (TBS, 20 mM Tris, pH 7.5, 500 mM NaCl). To this membrane, 100 µl TBS was added per well. The flow valve was adjusted so that the vacuum chamber was open to air. A volume of 200 µl of sample/antigen was added to each well and measured in duplicate. Mannan standards (400, 200, 100, and 50 ppm) and a positive control (yeast Invertase – 400 ppm) were blotted along with the wine samples. The

samples were filtered through the membrane using a gentle vacuum. Once the samples were loaded, each well was washed with 250 µl of TBS under vacuum. While maintaining the vacuum, the membrane was removed from the apparatus, placed in a plastic box, and rinsed twice with a blocking/wash solution of Tris-Buffered Saline containing Tween 20 (TBST) (TBS + 0.1% Tween 20) for five min per rinse and decanted after each rinse. The membrane was incubated for 30 min at room temperature with continuous mixing with 4 µg/ml of the mannose-specific, biotinylated *Narcissus pseudonarcissus* lectin in TBST. The membrane was washed twice with TBST and then placed in TBST containing 1 µl/ml of streptavidin-conjugated alkaline phosphatase for 30 min at room temperature with continuous mixing and decanted after each rinse. Afterwards, the membrane was washed twice in TBST and rinsed once in TBS for five min to remove residual TBST. Following this, 100 mM Tris (pH 9.5) solution was added to the membrane for five min and decanted. After equilibration, the membrane was removed and excess liquid was drained. It was then placed blotted side up in a plastic box wrap within a dry aluminum foil to prevent UV light exposure before imaging. Once the membrane was placed within the Versadoc CCD Imager (Versadoc 4000MP, Bio-Rad Laboratories, Hercules, CA), the chemiluminescent alkaline phosphatase substrate was added to the membrane and covered with a plastic wrap to uniformly spread the substrate. This was incubated for 5 min at room temperature in the imager before imaging. Concentrations were determined based on band density using the imaging software (ImageJ). The mannan content of the samples was quantified in mannan equivalents from a standard curve generated using the mannan standards.

Sample Selection for Sensory Evaluation and Electronic Tongue Analysis

Results from the chemical analyses were used in hierarchical cluster analysis and six groups were generated (described in the Statistical Analyses section). The six groups were labeled A

through F and two samples from each group were randomly selected for sensory evaluation. One sample from each group used in the sensory evaluation was further selected and used for electronic tongue analysis to allow for instrumental and trained panel correlations.

*Electronic Tongue Analysis:* Wine *samples* (n=6) selected based on cluster analysis (described below) were equilibrated to room temperature and filtered through a P8 Fisher brand filter paper (Fisher Scientific, Suwanee, GA. USA). Taste attributes of wine samples (saltiness, sourness, sweetness, umami, metallic, bitterness and spiciness) were analyzed using a potentiometric electronic tongue (Astree® II electronic tongue unit Alpha MOS) equipped with a liquid auto sampler (LS48) and seven set #5 sensors (sour, sweet, bitter, salty, umami, spicy and metallic). A pre-run system preparation comprising of conditioning, calibration and diagnostics were performed according to manufacturer's instruction using 25 ml of 0.01M standard solutions prepared from 0.1M each of hydrochloric acid, sodium chloride and sodium-L-glutamate. This was followed by an overnight hydration of the set #5 sensors (saltiness, sourness, sweetness, umami, metallic, bitterness and spiciness) in 25 ml reagent grade MilliQ filtered water. A confirmatory diagnostic run was performed prior to sample analysis. A programmed auto sampler method consisting of the following parameters was used: delay = 0 sec; acquisition time = 120 sec; stirring rate = 1 and acquisition period = 1. A six-looped sequence consisting of a 10 sec sensor cleaning in 25 ml reagent grade MilliQ filtered water between samples was used during data acquisition.

*Trained Panel:* Panelists (n=13) were recruited from the Washington State University community through electronic advertisement. Previous training in wine or sensory evaluation was not a requirement for participation. The panel was composed of 54% males and 46% females with ages between 21 and 60. Most of the panelists (77%) were between 21-30 years

while the rest were between 41 and 60 years. The wine consumption patterns of the panelists varied with most panelists consuming wine once to a few times a month. The panelists received minimum background information about the study to reduce potential bias and were simply informed they would be evaluating red wines over 12 training sessions followed by two formal evaluation sessions. The project was approved by the Washington State University Institutional Review Board for human subject participation. On the first day of training, all panelists signed an informed consent form and received nonmonetary incentive after each training and formal evaluation sessions.

Inconsistency in the evaluation of taste and mouthfeel compared to flavor evaluation of model solutions has been documented (Ott and Palmer, 1990). Because genetic and individual differences could account for this, the saliva flow rate and taster status as dictated by individual sensitivity to *6-n*-propylthiouracil (PROP of each panelist were determined on the first day of training. PROP taster status was determined as previously described (Tepper *et al.*, 2001), as was saliva flow rate (Mialon and Ebeler, 1997).

The panelists were instructed on the techniques to use for the evaluation of color, aroma, flavor and taste of wines. The first three training sessions were devoted to building consensus and defining appropriate standards that defined the Merlot wines. Training was conducted through presentation of standard solutions prepared in base wine (Livingston Red Rosé, Gallo, Modesto, CA USA). The recipes for the standards are presented in **Table 1**. In subsequent sessions, panelists were presented with these standard solutions to illustrate attributes, followed by the evaluation and subsequent discussion of commercial red wine samples. Panelists were gradually introduced to the different taste and mouthfeel attributes. For both training and formal evaluation, wine samples (25 ml) were pre-poured into ISO/INAO (International Standards

Organization) tasting glasses and covered with Petri dishes for one hour before tasting to allow for equilibration. The samples were labeled with three-digit codes and presented to panelists one at a time in a randomized serving order. Panelist performance was evaluated using the PROP status, saliva flow rate and score distribution. Scores for each attribute were summarized using boxplots to provide a graphical representation of outliers. Both individual and panel means and standard deviations were obtained after each training session. Panelists who scored attributes much lower or higher than the overall panel mean were provided with additional training. Data from the taster status and saliva flow rate helped to better understand the rating trends of panelist for tailored feedback on performance.

Panelists rated the perception of intensity of six aroma and flavor attributes (artificial fruit, herbaceous, earthy, fruity, floral, woody and spicy), three taste attributes (sweet, sour, bitter) and three mouthfeel attributes (astringent, burning, metallic) along a 15-cm structured line scale anchored at 1.5 (low) and 13.5 cm (high). Samples were assigned three-digit codes and presented to the panelist one after the other in individual booths. Twelve samples were evaluated in two replicates over the three formal evaluation sessions, with 8 samples evaluated per session. Panelists were required to pause for 1 min between samples, with a 10 min forced break after the fourth sample to refresh their palate and minimize fatigue. Panelists were provided with crackers and distilled water for palate cleansing. All instructions, scale presentations, and data collection were carried out using Compusense *five*, release 5.2 (Guelph, Canada).

Statistical Analyses

Analysis of variance (ANOVA) was conducted to determine significant differences among the samples for each of the attributes and significance reported at $\alpha = 0.05$. Factor analysis was used to determine the latent variables for these wines.

**Table 1**. Recipes and standards used in panel training for the sensory evaluation of samples

| Category | Attribute | Preparation |
|---|---|---|
| Aroma | Artificial fruit | 1 ml Kool-Aid liquid (red fruit) in 20 ml base wine[a] |
| and flavor | Fruity | 2 ml of mixed fruit juice in 20 ml base wine [Mixed fruit juice: Blackberries(~ 25g), strawberries (~ 13.5g) and raspberries (~ 18.5g) crushed and strained through a cheese cloth] |
| | Woody | Three oak chips (~2.0g) in 10 ml deionized water + 5 ml base wine. Kept overnight at room temperature. 15 ml of base wine added prior to training |
| | Spicy | 3 whole cloves (~. 0.2g) soaked in 20 ml deionized water for 30 min. Ground black pepper (~ 0.1g) added. After 10 min, 5 ml of this solution added to 20 ml base wine and allowed to sit overnight at room temperature. |
| | Herbaceous | 3 ml of olive brine added to 15 ml base wine prior to training |
| | Floral | 2 drops of violet aroma (Wine Awakening) to 100 ml deionized water. One ml of this solution was added to 50 ml of base wine |
| | Earthy | Freshly uprooted roots of backyard weed |
| Taste | Sweet | 3.3% (w/v) cane sugar in base wine |
| | Sour | 0.3% tartaric acid in base wine |
| | Bitter | 0.001% (w/v) quinine sulfate in base wine |
| Mouthfeel | Astringent | 0.78g tannic acid + 0.35g alum in 300 ml base wine |
| | Burning | 60 ml 100 proof ethanol in 240 ml base wine |
| | Metallic | 8 iron tablets (~ 3.0 g) dissolved in 300 ml base wine and filtered |

[a]Red Rosé, Livingstone Cellars, Modesto, CA.

From these latent variables, alcohol, tannins and mannoprotein were selected as chemical characteristics of interest for these wines. Linear regression was used to assess the main and interaction effects of alcohol, tannins and mannoproteins on the perception of aroma, flavor, taste and mouthfeel attributes of the samples. Model building started with correlation analysis between the predictors (alcohol, tannins and mannoprotein) and the responses to determine the nature of correlations and their significances. The predictors were then centered to reduce multicollinearity. The model for each response was built using the stepwise selection technique and the AIC criterion, ensuring that the sign and the significances of the parameter estimates reflected the existing trends in the data as previously determined using correlation analysis. The models selected through this procedure were further diagnosed for outliers and influential points. ANOVA and linear regression were performed using R Studio (ver. 3.0.2).

Factor analysis (principal components method) was conducted using SAS software (ver. 9.2; SAS Institute, Cary, NC). This was to explore the latent factors responsible for the covariations among the variables measured for the wines using three factors with Varimax rotation. The three factors were selected using the scree plot based on Kaiser criterion (Bryant and Yarnold, 1995). Hierarchical agglomerative clustering employing Euclidean distance with Ward linkage and a stopping criterion of 6 to explore similarities and differences among the samples based on their physicochemical characteristics. A stopping value of 6 was chosen based on percentage of variance criterion (Bryant and Yarnold, 1995). Cluster analysis was performed using R Studio (ver. 3.0.2). The data used from the e-tongue were those from the three most consistent loops with a relative standard deviation not exceeding 15%. Correlations between this electronic tongue data and sensory panel evaluation as well as principal component analysis were performed using the Astree® Alpha Soft (ver. 12) (Alpha MOS).

## Results and Discussion

Chemical Characteristics

Variations were observed among the chemical parameters of the wines even though these wines were of the same grape variety (**Table 2**). The pH of the wines in this study ranged from 3.21 to 3.90, with a mean of 3.63. The pH range observed in these wines agreed with those previously reported in the literature for red wines (Cliff *et al.*, 2002; Harbertson *et al.*, 2009; Kontoudakis *et al.*, 2011; Casassa *et al.*, 2013). Titratable acidity (TA) in wine is a measure of free non-neutralized acids which are consumed in titration with a strong base (Moreno and Peinado, 2012). Wine acidity help in wine ageing, especially in whites but if the acidity is excessively high, wine becomes characterized by a harsh and biting taste while less acidity makes wines 'flat' (Zraly, 2011). The wines in the present study had a mean titratable acidity value of 0.57 g/100 ml. These results were within the range reported the in literature for red wine (Darias-Martin *et al.*, 2003; Moreno and Peinado, 2012), although some higher titratable acidity levels have been found in some red wines (Cliff *et al.*, 2002).

The range of residual sugar observed in this study (6.9-9.5 $^{o}$Brix.) was consistent with semi-dry red wines (Moreno and Peinado, 2012). Alcohol concentrations ranged from 11.3 to 16.0, with a mean of 13.4% (v/v). The alcohol levels observed in these samples established these wines in the category of table wines (Robinson, 2001; Henderson and Rex, 2007). Several of these wines had alcohol levels up to 16% (v/v), attesting to the current trend of producing table wines with high alcohol (Alston *et al.*, 2011). The total protein concentration reported in this study ranged from 35 to 163 mg/l, agreeing with levels reported in literature (Monteiro *et al.*, 2001; Ferreira *et al.*, 2002; Lambri *et al.*, 2013; Mainente *et al.*, 2014).

**Table 2**. Summary statistics (mean, standard deviation (sd) and range), coefficient of variation (CV) and F-ratios of chemical characteristics of commercial Merlot wines (n=61). Asterisk (*) indicates significant F-ratio for the characteristic at $p \leq 0.05$ using Fisher LSD

| **Parameter** | **Mean ± sd** | **Range** | **CV** | **F-ratio** |
|---|---|---|---|---|
| pH | 3.627 ± 0.12 | 3.21 – 3.90 | 3.31 | 1379.5* |
| Titratable Acidity (g/100 ml) | 0.574 ± 0.067 | 0.42 – 0.81 | 11.67 | 38.9* |
| Soluble Solids ($^{o}$Brix) | 8.4 ± 0.44 | 6.9 – 9.5 | 5.26 | 337.2* |
| Alcohol (%) | 13.36 ± 0.897 | 11.30 – 16.0 | 6.71 | 59.6* |
| LPP[a] (AU) | 0.9055 ± 0.454 | 0.024 – 1.986 | 50.14 | 213.2* |
| SPP[b] (AU) | 1.066 ± 0.247 | 0.548 – 1.800 | 23.17 | 92.9* |
| Tannins (mg/l CE[c]) | 410.6 ± 183.2 | 37.0 – 935.0 | 44.62 | 939.3* |
| Total Phenolics (mg/l CE) | 162 ± 47.61 | 69 -306 | 29.39 | 150.2* |
| Total Proteins (mg/l) | 78.51 ± 24.14 | 35 – 163 | 30.75 | 16.3* |
| Mannoproteins (mg/l) | 143.2 ± 103.44 | nd – 601.3 | 72.23 | 6.3* |

[a]LPP = Large polymeric pigments measured in Absorbance Units (AU)

[b]SPP= Small polymeric pigments measured in Absorbance Units (AU)

[c]CE = Catechin Equivalents

The mannoprotein concentration of the wines surveyed were between 100 to 150 ml/l, a range that has previously been reported in red wines (Perez-Serradilla and de Castro, 2008).

To characterize the phenolic composition of the wines used in this experiment, the large polymeric pigments (LPP), small polymeric pigments (SPP), tannins and total phenolic (iron reactive phenolics) content were determined. The concentrations of SPP and LPP in the present study were found to be within the range previously reported in some Washington State Merlot wines (Landon *et al.*, 2008). The iron reactive phenolic content of the samples was low compared to some experimental Merlot made from high brix musts (Harbertson *et al.*, 2009). The tannin levels of these wines ranged from 37 to 935 mg/l CE which is comparable to findings from previous authors (Harbertson *et al.*, 2008; Landon *et al.*, 2008). Variations of tannin concentrations in red wines have been attributed largely to winemaking technique and viticultural practices (Harbertson *et al.*, 2008). The observed physicochemical variations in these wines have possible implications for differences in the perception of the sensory properties of these wines.

Factor and Hierarchical Cluster Analysis

Principal components factor analysis revealed three factors with eigenvalues greater than 1 (**Figure 1A**) as required by the Kaiser's stopping rule and six principal components which explained a proportion of 0.9 of the variance (**Figure 1B**) per the percentage of variance criterion (Bryant and Yarnold, 1995). Information from these figures was used in the extraction of important factors to explore interaction effects and also as basis for agglomerative hierarchical clustering. The first factor consisted of alcohol, large polymeric pigments, tannins and total phenolics and explained ~50% of the common variance (**Figure 2**). Alcohol acts as a co-solvent in the extraction of grape constituents during winemaking (Jackson, 2014). This first factor is

therefore related to wine components that tend to accumulate during the vinification as a result of their extraction by alcohol.

Previous studies on model and pilot scale wines have established the importance of ethanol and tannin concentration in influencing the headspace odorant concentration and sensory perception of astringency, bitterness, alcohol burn as well as woody, spicy, fruity and floral aromas and flavors in these wines (Landon *et al.*, 2008; Villamor *et al.*, 2009; Villamor *et al.*, 2013b; Villamor *et al.*, 2013a). These previous studies on model and pilot scale wines reported that astringency and bitterness in wines were positively correlated with tannins as well as small and large polymeric pigments. Also, depending on the concentration of ethanol, tannins tended to enhance the release of some volatiles while alcohol increased the perception of woody, spicy and chemical aromas and flavors as well as bitter taste and burning sensation but reduced fruity and floral aromas and flavors. Results from the current research highlighted the role of ethanol and tannin profiles in determining physicochemical differences, with these differences possibly influencing the sensory properties of the commercial Merlot wines under study.

The second factor accounted for 29% of the common variance while the third explained the remaining common variance (21%). The second factor was defined by small polymeric pigments (SPP), protein and soluble solids. The SPPs are made up of anthocyanin-tannin dimers which are not precipitable by proteins (Adams *et al.*, 2004). This may explain the correlation between protein and SPPs and hence their clustering in the same factor. Factor two, made up of SPP, protein and soluble solids, is related to the characteristics which tend to decrease with vinification. The utilization of sugars by yeast for the production of ethanol will reduce the soluble solids; acids in wines contribute to protein precipitation and the polymerization of SPPs to large polymeric pigments (LPPs) which will decrease the SPP content (Jackson, 2014).

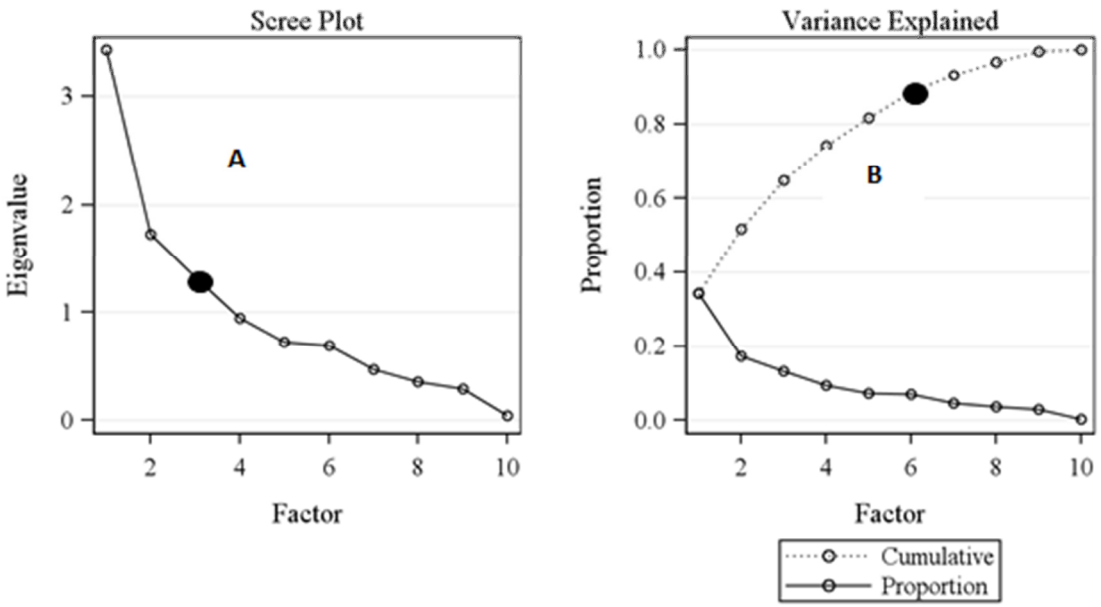


**Figure 1**. Scree plot (**A**) and variance explained by principal components (**B**) for the physicochemical characteristics of commercial Merlot wines (n=61). The physicochemical characteristics included were: soluble solids, pH, titratable acidity, alcohol, small polymeric pigments, large polymeric pigments, tannins, total phenolics, total protein and mannoprotein. Figure 1(A) shows three factors (big dark spot) having eigenvalues greater than 1; Figure 1 (B) shows 6 factors (big dark spot) accounting for 0.9 proportion of explained variance.

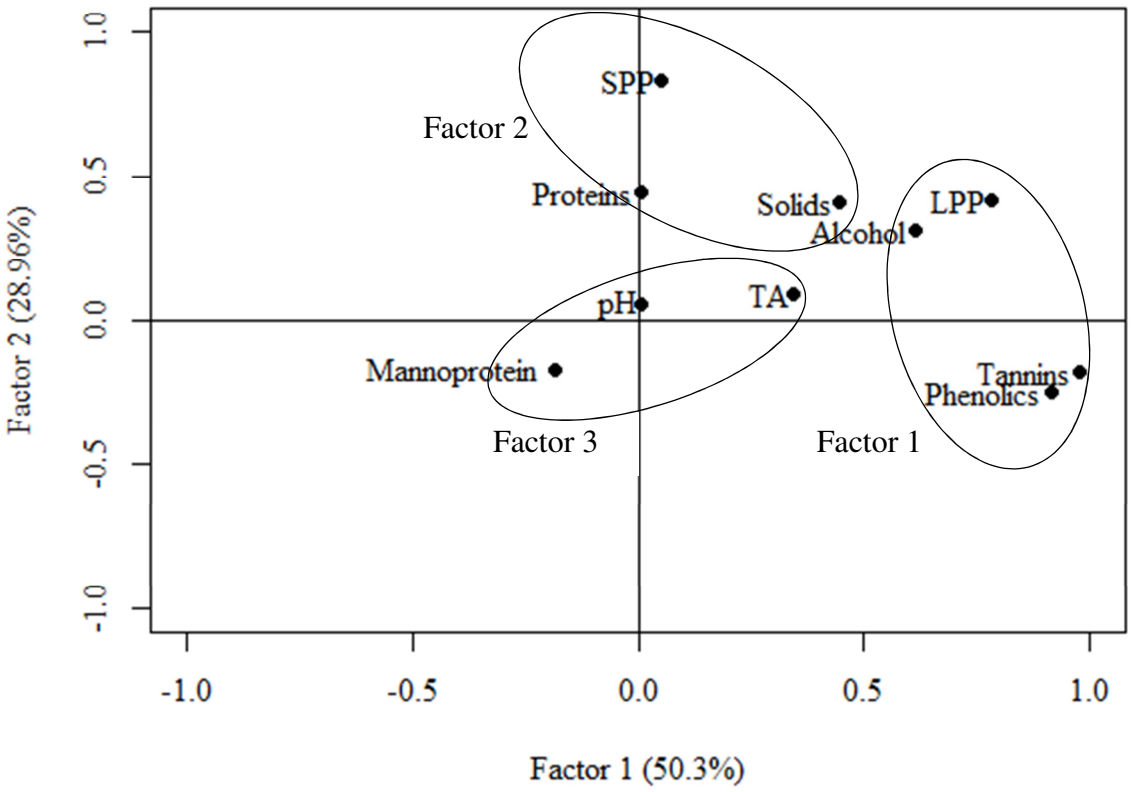


**Figure 2**. Principal component factor analysis plot of chemical characteristics of commercial Merlot wines for factor 1 and 2 with VARIMAX rotation (n=61). The physicochemical characteristics included were: soluble solids, pH, titratable acidity, alcohol, small polymeric pigments, large polymeric pigments, tannins, total phenolics, total protein and mannoprotein. Ellipses correspond to characteristics with high standardized score coefficients in each factor.

Factor three was represented by mannoprotein, titratable acidity and pH. This factor is made up of those chemical parameters that impact the mouthfeel characteristics in wines. Acids in wines (represented by titratable acidity and pH) account for acidity and also modify other tastes and mouthfeel sensations. In model wines, earlier studies have demonstrated the retentive effect of mannoproteins on aroma compounds, such as ethyl hexanoate (Chalier *et al.*, 2007), linalool and geraniol (Juega *et al.*, 2012), beta-ionone and 2-phenylethanol (Comuzzo *et al.*, 2011). Mannoproteins were also shown to influence tannin aggregation by delaying tannin polymerization (Rodrigues *et al.*, 2012). While these previous studies showed that mannoprotein influences both mouthfeel and aroma properties of model wines, further examination of the interactions of mannoprotein with other components in the wines was of interest.

Agglomerative hierarchical clustering of the samples based on their physicochemical properties using the number of principal components that explained 90% of the variance identified six clusters of wines, of the 61 wines studied, based on similarities within groups and differences among groups (**Figure 3**).These six clusters of wines were identified as Clusters A to F, with Cluster A (n= 3 wines), Cluster B (n=7 wines), Cluster C (n=9 wines), Cluster D, was the largest (n=21 wines), Cluster E (n=8 wines) and Cluster F (n=13 wines) (**Table 3**). Two wines from each of these groups were randomly selected for sensory profiling and subsequent evaluation of the impact of ethanol, tannins, mannoproteins and their interaction on the sensory properties of these wines.

Main Effects and Interactions

Interactions among wine components affect the sensory properties of wines and their subsequent perception (Villamor *et al.*, 2009; Villamor *et al.*, 2013b; Baker and Ross, 2014). Having profiled the selected wines for aroma, flavor, taste and mouthfeel properties based on results

from cluster analysis, the impact of the wines' alcohol, tannin and mannoprotein contents on panelists' perception of aroma, flavor, taste and mouthfeel characteristics were explored. Each of the columns in **Tables 4, 5** and **6** represents the coefficients of regression for a model describing the perception of the specified attribute by the panelists. For instance, the model selected by stepwise regression using AIC for estimating the perception of herbaceous aroma of the wines is presented in Table 4 as follows:

$$\hat{y}_{herbaceous}=3.62-0.423(Alcohol)-0.001(Tannins)+0.002(Mannoprotein)+0.002(Alcohol*Tannins)$$

From the signs of the regression coefficients, alcohol, tannins and their interaction suppressed the perception of herbaceous aroma while mannoproteins enhanced it. Results indicated significant main effects of ethanol, tannins and mannoproteins and their interaction on aroma (Table 4), flavor (Table 5) and taste and mouthfeel (Table 6) properties of the wines.

Ethanol significantly enhanced the perception of spicy, floral and fruity flavors, artificial fruit aromas, bitter taste and burning mouthfeel while suppressing the perception of herbaceous and earthy aromas and metallic mouthfeel of the wines. Overall, ethanol showed more significant suppressive effect on the aroma perception than flavor perception as observed by the negative coefficients for herbaceous and earthy aromas versus the positive coefficients for fruity, floral and spicy flavors. Also, the positive relationship between the perception of alcohol and woody aroma and its negative relationship with the perception of fruity aroma were not statistically significant; nevertheless, the trends were in agreement with previous studies. The enhancing effect of ethanol on the perception of woody aromas and its suppressing effect on the perception of floral flavors and fruity notes have been previously reported in model wines (Escudero *et al.*, 2007; Villamor *et al.*, 2013b; King *et al.*, 2013). .

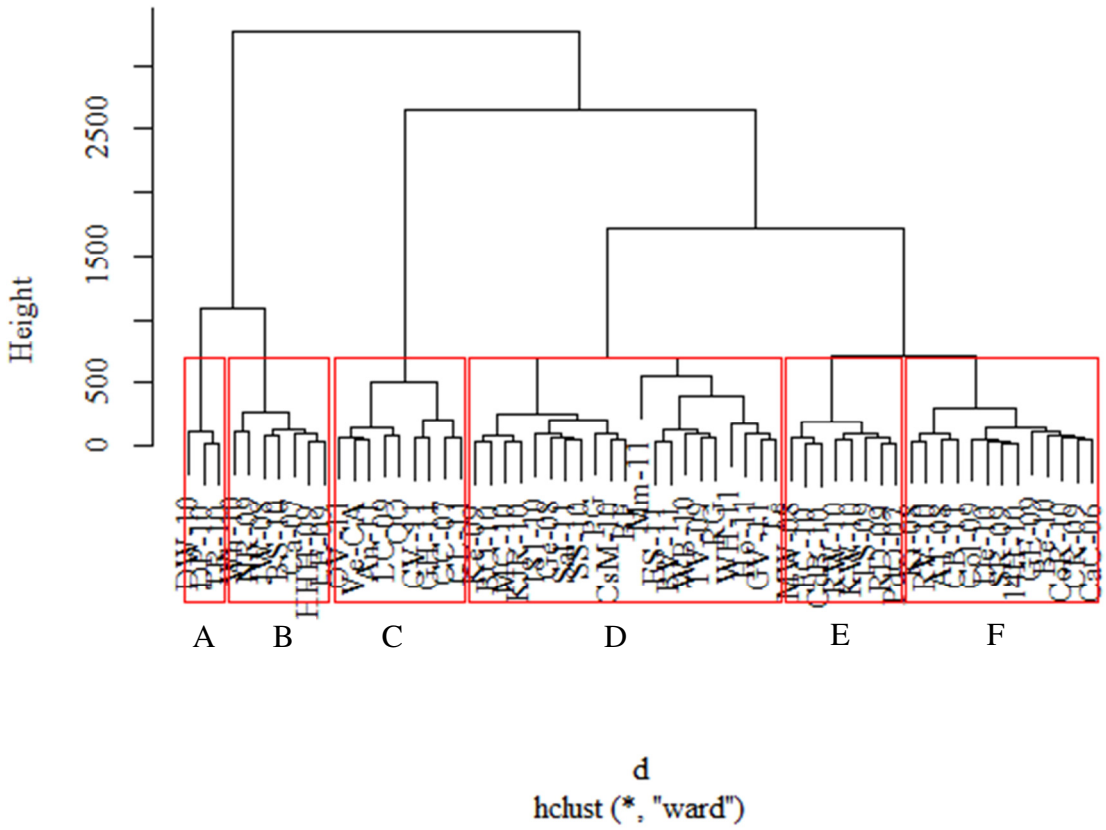


**Figure 3**. Cluster dendogram for commercial Merlot wines (n=61) based on the chemical parameters (soluble solids, pH, titratable acidity, alcohol, small polymeric pigments, large polymeric pigments, tannins, total phenolics, total protein and mannoprotein) and Euclidean distances with ward linkage showing clusters A(n=3), B(n=7), C(n=9), D(n=21), E(n=8) and F(n=13).

**Table 3**. Chemical characteristics and sizes of wine clusters obtained from hierarchical clustering using Ward linkage. Means in columns with different superscripts are statistically significant ($p \leq 0.05$).

| Group name (Size) | Solids ($^{o}$Brix) | pH | TA[a] (g/100 ml) | Alcohol (%) | LPP[b] (AU) | SPP[c] (AU) | Tannins (mg/l CE) | TP[d] (mg/l CE) | Proteins (mg/l) | Mannoprotein (mg/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| A (n=3) | 8.6$^{a}$ | 3.67$^{a}$ | 0.572$^{b,c}$ | 13.7$^{a,b}$ | 1.333$^{a,b}$ | 0.858$^{b}$ | 892.1$^{a}$ | 287.0$^{a}$ | 60.2$^{a}$ | 81.4$^{c,d}$ |
| B (n=7) | 8.5$^{a}$ | 3.56$^{a,b}$ | 0.629$^{a}$ | 14.4$^{a}$ | 1.378$^{a}$ | 0.993$^{a,b}$ | 618.4$^{b}$ | 198.3$^{b}$ | 78.7$^{a}$ | 184.1$^{a,b}$ |
| C (n=9) | 8.0$^{b}$ | 3.64$^{a,b}$ | 0.535$^{c}$ | 12.4$^{c}$ | 0.343$^{d}$ | 1.059$^{a,b}$ | 131.9$^{f}$ | 93.9$^{e}$ | 75.5$^{a}$ | 121.3$^{b,c}$ |
| D (n=21) | 8.1$^{b}$ | 3.63$^{a,b}$ | 0.561$^{b,c}$ | 13.2$^{b}$ | 0.713$^{c}$ | 1.039$^{a,b}$ | 341.1$^{e}$ | 147.4$^{d}$ | 78.2$^{a}$ | 199.1$^{a}$ |
| E (n=8) | 8.5$^{a}$ | 3.56$^{a,b}$ | 0.602$^{a,b}$ | 13.5$^{b}$ | 1.072$^{b}$ | 1.174$^{a}$ | 421.02$^{d}$ | 164.6$^{c}$ | 78.0$^{a}$ | 36.7$^{d}$ |
| F (n=13) | 8.4$^{a}$ | 3.67$^{a}$ | 0.576$^{a,b,c}$ | 13.6$^{b}$ | 1.151$^{a,b}$ | 1.134$^{a}$ | 486.6$^{c}$ | 182.5$^{b}$ | 85.5$^{a}$ | 123.62$^{b,c}$ |

[a]TA=Titratable acidity

[b]LPP = Large polymeric pigments measured in Absorbance Units (AU)

[c]SPP = Small polymeric pigments measured in Absorbance Units (AU)

[d]TP = Total phenolics measured in Catechin Equivalents (CE)

**Table 4**. Regression coefficients for main and interaction effects of ethanol, tannins and mannoprotein on the trained panel evaluation of aroma attributes intensity in commercial Merlot wines.

| Predictor | Aroma Attributes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Artificial Fruit** | **Herbaceous** | **Earthy** | **Fruity** | **Floral** | **Woody** | **Spicy** |
| (Intercept) | 2.6146*** | 3.6173*** | 2.8993*** | 3.6065** | 3.0247*** | 2.954*** | 2.472*** |
| Alcohol (A) | 0.117** | -0.4229*** | -0.1081** | -0.0128 | 0.0765 | 1.5e-02 | 6.2e-02 |
| Tannins (T) | 0.0004* | -0.0012** | -0.0003* | 0.0007** | 0.0003 | -2.0e-04 | 5.0e-05 |
| Mannoprotein (M) | -0.0011** | 2.1e-03** | 0.0004 | -0.0014** | -0.0004 | 5.0e-04 | -2.0e-04 |
| Interactions | None | A*T(-0.0022*) | None | A*M(0.001*) | None | A*T(-0.0006*) A*T*M(6.0e-06*) | None |

***, **, * indicate significance at $p \leq 0.001$; $p \leq 0.01$; and $p \leq 0.05$ respectively

**Table 5**. Regression coefficients for main and interaction effects of ethanol, tannins and mannoprotein on the trained panel evaluation of flavor attributes intensity in commercial Merlot wines.

| Predictor | Flavor Attributes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Artificial Fruit** | **Herbaceous** | **Earthy** | **Fruity** | **Floral** | **Woody** | **Spicy** |
| (Intercept) | 2.628*** | 3.131*** | 2.708*** | 3.480*** | 2.739*** | 2.998*** | 2.458*** |
| Alcohol (A) | 0.0366 | -0.1682 | -0.0327 | 0.1185* | 0.0830* | -0.0500 | 1.15e-01* |
| Tannins (T) | 0.0006** | -0.0011*** | -0.0004* | 00003 | 0.0004* | -0.0001 | 5.0e-06 |
| Mannoprotein (M) | -0.0015** | 0.0006 | 0.0002 | -0.0013* | -0.0006 | 0.0012* | -5.1e-04 |
| Interactions | None | A*T (-0.001**) | None | None | None | None | A*T (-0.0009**) |
| | | T*M (-7.486e-06*) | | | | | A*M (0.0012) |
| | | | | | | | T*M (1.135e-06) |
| | | | | | | | A*T*M (1.051e-05*) |

***, **, * indicate significance at $p \leq 0.001$; $p \leq 0.01$; and $p \leq 0.05$ respectively

**Table 6**. Regression coefficients for main and interaction effects of ethanol, tannins and mannoprotein on the trained panel evaluation of taste and mouthfeel attributes intensity in commercial Merlot wines.

| Predictor | Taste and Mouthfeel Attributes | | | | | |
|---|---|---|---|---|---|---|
| | **Sweet** | **Sour** | **Bitter** | **Astringent** | **Burning** | **Metallic** |
| (Intercept) | 2.849*** | 3.323*** | 3.326*** | 4.015*** | 3.354*** | 2.015*** |
| Alcohol (A) | 6.27e-02 | -0.0086 | 0.1140* | 0.1526 | 3.23e-01*** | -0.0793** |
| Tannins (T) | -1.0e-04 | -0.0001 | 0.0002 | 0.0028*** | 5.0e-05 | -2.4e-04* |
| Mannoprotein (M) | 4.5e-04 | -0.0004 | 0.0003 | -0.0004 | -3.7e-04 | -3.2e-04 |
| Interactions | T*M (6.0e-06*) | None | A*M (-0.0006) | A*T (0.0012**) | A*M (-0.0007**) | None |

***, **, * indicate significance at $p \leq 0.001$; $p \leq 0.01$; and $p \leq 0.05$ respectively

Ethanol concentration also significantly and positively influenced the burning perception and bitter taste of the wines. This agrees with previous findings in which the burning mouthfeel and bitter taste were enhanced by increasing levels of ethanol while simultaneously suppressing the perception of fruity and floral flavors and aromas (Villamor *et al.*, 2013b). Results also showed that ethanol was positively related to sweetness and significantly enhanced astringency while suppressing sourness and metallic mouthfeel. Ethanol in wines has been observed to affect the sourness and sweetness balance by enhancing sweetness perception at high ethanol concentrations (Zamora *et al.*, 2006).

Tannins had both suppressive and enhancing effect on the perception of aroma and flavors as already shown in Table 6. Tannins significantly enhanced the perception of artificial fruit and fruity aromas, floral flavors as well as astringency of the wines. However, these phenolic compounds suppressed the perception of herbaceous and earthy aromas and flavors. Phenolic compounds exert varying effects on the volatility and subsequent sensory perception of aroma compounds (Lorrain *et al.*, 2013). These effects have been attributed to the polarities and spatial conformation of aroma compounds which affect the strength of the interaction among polyphenolic compounds and aroma compounds (Lorrain *et al.*, 2013). Smaller and more hydrophobic tannins have been found to be more bitter than the larger hydrophilic tannins (McRae *et al.*, 2013). The difference in bitterness of tannin fractions imparted bitter taste to wines and contributed to the positive relationship between tannins and the perception of bitterness in the wines studied. It has been found in a study of astringency perception in Cabernet-Sauvignon and Merlot wines that astringency is positively correlated with tannin concentration (Landon *et al.*, 2008).

The mannoprotein concentration generally had a suppressive effect on the aroma and flavor, with a significant main effect on the suppression of artificial fruit and fruity aroma and flavor as previously shown in Table 6. The retention of aroma compounds by mannoproteins has been previously documented (Comuzzo *et al.*, 2011; Juega *et al.*, 2012). Mannoproteins may delay or prevent tannin polymerization, thereby reducing astringency (Rodrigues *et al.*, 2012). The impact of mannoprotein on taste and mouthfeel perception was due to its interaction with other components of the wines as can be seen from its non-significance as a main effect (Table 5). In the present study, mannoprotein was negatively related to astringency, indicating a reduction of perceived astringency with increasing mannoprotein concentration.

Several two-way interactions between alcohol, tannins and mannoproteins were observed in this study. These two-way interactions affected the perception of herbaceous aromas and flavors, fruity and woody aroma, spicy flavor, sweet taste, astringent and burning mouthfeel of the wines. The interaction between alcohol and mannoproteins significantly enhanced the perception of fruity aromas. Studies have shown that ethanol changes the binding capacity of proteins with aroma compounds (Druaux *et al.*, 1995). The enhanced perception of fruity aroma in the presence of increased alcohol and mannoprotein may be as a result of the modification of the protein portion of the mannoprotein molecule. This change in molecular conformation may lead to decreased binding of aroma compounds by mannoproteins with a consequent decrease in binding of mannoproteins with fruity aromas of the samples. The interaction of alcohol and mannoproteins also significantly reduced the burning sensation of alcohol and displayed a negative relationship with bitter taste. This is consistent with findings regarding the modification of mouthfeel properties of wines by mannoproteins (Vidal *et al.*, 2004; Rodrigues *et al.*, 2012).

The significant increase in the perception of sweet taste as a function of the interaction between tannins and mannoproteins may be due to binding of tannins by mannoproteins. This binding may lead to a decrease in the contribution of tannins to the bitter taste of wine and a subsequent enhancement of sweet taste. Tannin and mannoprotein interaction also decreased the herbaceous flavor perception. Tannins are known to interact non-covalently and through mutual hydrophobicity with aroma compounds in wines. The impact of tannins on the herbaceous character of these samples is therefore conditional on the presence of mannoproteins due to the observed interaction. The interaction between alcohol and tannin generally reduced the perception of aromas and flavors but enhanced astringency. This may be due to the combined effect of the solubility of aroma compounds in ethanol and the hydrophobic interaction of the tannins with aroma compounds (Robinson *et al.*, 2009; Pozo-Bayon and Reineccius, 2009).

Beyond the two-way interactions already discussed, some three-way interactions were observed in this study as shown in Tables 4-6. These three-way interactions show that perceptions of certain attributes in the wines by the panelists were conditional on the presence of ethanol, tannins and mannoproteins. The three-way interactions significantly enhanced woody aroma and spicy flavor. These findings show the importance of matrix interaction on the perception of aromas, flavors, taste and mouthfeel properties of commercial wines.

Electronic Tongue Discrimination of Wines and Sensory Correlation

The electronic tongue was designed to mimic the neurophysiology of the sense of taste (Cosio *et al.*, 2012). It is, therefore, important to explore the relationships between human sensory evaluation and the electronic tongue analysis. From the hierarchical cluster analysis results already discussed in Figure 3, one wine was selected from each group to explore sensory and electronic tongue correlations. From the trained panel evaluation, significant differences

($p<0.05$) were perceived in the perception of sweet, bitter, burning, astringency and metallic attributes of the wines (**Table 7**). Samples were found to be low in the attributes evaluated. The metallic mouthfeel in the wines was the least intense. While astringency was the most variable and intense attribute perceived, no differences were perceived in the sourness of the samples. Since the samples evaluated by the panelists were selected from groups obtained using the hierarchical clustering algorithm, sample differences in the sensory attributes evaluated can be related to the group differences based on the clustering algorithm used. This clustering algorithm maximizes the between-group differences and within-group similarity.

Based on the electronic tongue's response for sweet, sour, bitter, salty, spicy, umami and metallic, the samples were reported as being different, as witnessed by a high discrimination index (DI = 95) based on principal component analysis (**Figure 4**). A total of 88% of the variation in the electronic tongue data was explained by the first two principal components. Bitterness, spiciness and sourness were loaded on PC 1 and hence explained most of the variation (68%) seen in the data. The remaining 21% was attributable to the rest of the sensors.

From principal component 1, samples from groups A, B and E were different from the rest based on the response from the bitter, sweet and sour sensors of the electronic tongue. These groups of samples had high tannin (groups A and B), high small polymeric pigments (group E) and high soluble solids (groups A, B and E) as seen previously in Table 3. This shows that the responses from the electronic tongue were dependent on the physicochemical components of the samples. Results further showed a high correlation between the overall electronic tongue sensor signal and the human sensory evaluation of the taste and mouthfeel attributes (**Table 8**). All correlation values were high, above 0.93.

**Table 7**. Mean values of taste and mouthfeel attributes of commercial Merlot wines evaluated by trained panelists (n=13). Evaluations were made in replicate using a 15-cm unstructured line scale. Different letters in the same column indicate significant differences as analyzed by Fisher's LSD ($p \leq 0.05$).

| Wine [a] Sample (Group) | Attribute | | | | | |
|---|---|---|---|---|---|---|
| | **Sweet** | **Sour** | **Bitter** | **Burning** | **Astringent** | **Metallic** |
| DP-10 (A) | $2.9^{b}$ | $3.2^{a}$ | $3.4^{a}$ | $3.4^{b}$ | $5.0^{a}$ | $1.9^{b,c}$ |
| BS-10 (B) | $3.1^{b}$ | $3.2^{a}$ | $3.4^{a}$ | $3.2^{b}$ | $3.5^{c,d}$ | $2.0^{a,b}$ |
| GH-11 (C) | $3.4^{a}$ | $3.3^{a}$ | $2.9^{b}$ | $3.1^{b}$ | $3.5^{d}$ | $1.9^{b,c}$ |
| MC-10 (D) | $3.1^{b}$ | $3.2^{a}$ | $3.5^{a}$ | $4.0^{a}$ | $3.6^{c,d}$ | $1.\ 9^{c}$ |
| RW-10 (E) | $2.5^{c}$ | $3.4^{a}$ | $3.3^{a}$ | $3.3^{b}$ | $3.8^{c}$ | $2.2^{a}$ |
| CoR-10 (F) | $2.5^{c}$ | $3.3^{a}$ | $3.5^{a}$ | $3.3^{b}$ | $4.2^{b}$ | $2.0^{b,c}$ |

[a]Six groups of wines were generated using hierarchical clustering. Samples were randomly selected these pre-established groups (A – F) for sensory evaluation (n=6)

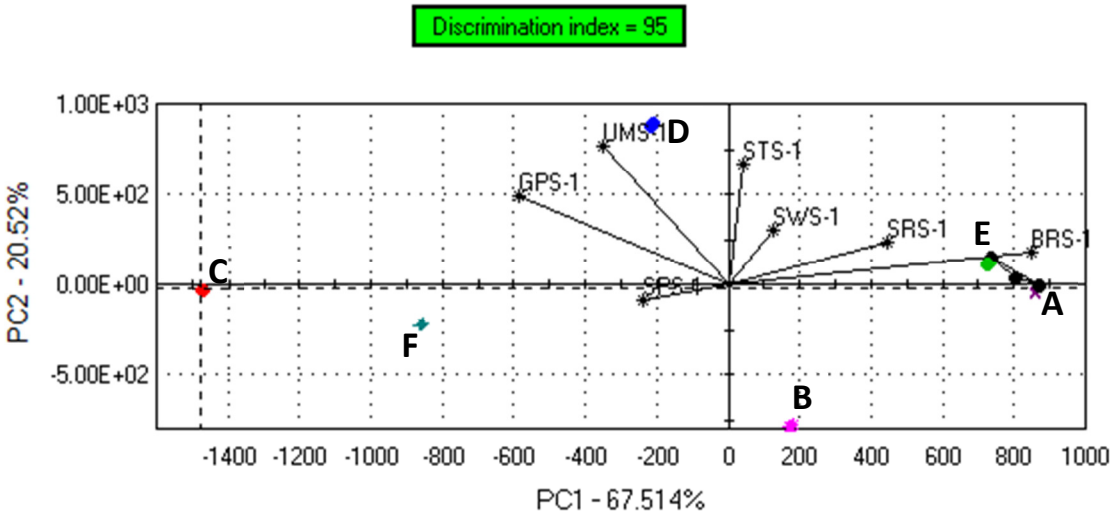


**Figure 4**. Electronic tongue discrimination of samples used for sensory profiling (n=6) showing high discrimination of the samples based on the Astree® set #5 sensors. The sensors are indicated by: UMS (umami), GPS (metallic), BRS (bitter), SWS (sweet), SPS (spicy), SRS (sour) and STS (salty). Groups A – F represent the groups from which each sample was randomly selected.

**Table 8**. Partial least squares correlation between sensory evaluation and electronic tongue analysis for taste and mouthfeel attributes of commercial Merlot wines (n=6).

| **Attribute** | **Electronic tongue and trained panel correlation ($r^2$)** |
|---|---|
| Sweet | 0.9300 |
| Sour | 0.9840 |
| Bitter | 0.9655 |
| Burning | 0.9974 |
| Astringent | 0.9980 |
| Metallic | 0.9825 |

The lowest correlation observed was for sweetness ($r^2 = 0.930$) while the highest correlation was for astringent ($r^2 = 0.998$). These results demonstrated a high agreement between the human and instrumental sensory evaluation as determined by the physicochemical composition of the wines.

**Conclusions**

In conclusion, this study documented the major physicochemical characteristics which explained the greatest differences among commercial Washington State and California Merlot, selected to reflect broad differences in chemical characteristics. Ethanol content and tannin profiles accounted for the major variations among the wines. The influence of these variations on the sensory properties of the wines was detected both through trained panel and the electronic tongue analysis. Significant main effects and some interaction effects were observed among alcohol, tannins and mannoproteins on their sensory perception of aroma, flavor, taste and mouthfeel. The high discrimination index and correlation coefficients observed between the electronic tongue and the trained panel is an indication of the potential for the use of the electronic tongue in wine research.

**Literature Cited**

Adams, D. O., Harbertson, J. F., & Picciotto, E. A. (2004). Fractionation of red wine polymeric pigments by protein precipitation and bisulfite bleaching. In A. L. Waterhouse & J. A. Kennedy (Eds.), *Red Wine Color: Revealing the Mysteries* (pp. 275-288). Washington DC: Am. Chem. Soc.

Alston, J. M., Fuller, K. B., Lapsley, J. T., & Soleas, G. (2011). Too much of a good thing? Causes and consequences of increases in sugar content of California wine grapes. *Journal of Wine Economics, 6*(2), 135-159.

Baker, A. K., & Ross, C. F. (2014). Sensory evaluation of impact of wine matrix on red wine finish: a preliminary study. *Journal of Sensory Studies, 29*(2), 139-148.

Bryant, F. B., & Yarnold, P. R. (1995). Principal Components Analysis and Exploratory and Confirmatory Factor Analysis. In L. G. Grimm & P. R. Yarnold (Eds.), *Reading and Understanding Multivariate Statistics* (pp. 99-136). Washington, DC: American Psychological Association.

Buratti, S., Ballabio, D., Benedetti, S., & Cosio, M. S. (2007). Prediction of Italian red wine sensorial descriptors from electronic nose, electronic tongue and spectrophotometric measurements by means of Genetic Algorithm regression models. *Food Chemistry, 100*, 211-218.

Casassa, L. F., Beaver, C. W., Mireles, M. S., & Harbertson, J. F. (2013). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Australian Journal of Grape and Wine Research, 19*, 25-39.

Chalier, P., Angot, B., Delteil, D., Doco, T., & Gunata, Z. (2007). Interactions between aroma compounds and whole mannoprotein isolated from *Saccharomyces cerevisiae* strains. *Food Chemistry, 100*(1), 22-30.

Cliff, M., Yuksel, D., Girard, B., & King, M. (2002). Characterization of Canadian ice wine by sensory and compositional analyses. *American Journal of Enology and Viticulture, 53*(1), 46-53.

Comuzzo, P., Tat, L., Fenzi, D., Brotto, L., Battistutta, F., & Zironi, R. (2011). Interactions between yeast autolysates and volatile compounds in wine and model solution. *Food Chemistry, 127*, 473–480.

Cosio, M. S., Scampicchio, M., & Benedetti, S. (2012). Electronic noses and tongues. In P. Yolanda (Ed.), *Chemical Analysis of Food: Techniques and Applications* (pp. 219-247): Academic Press, USA.

Darias-Martin, J., Socas-Hernandez, A., Diaz-Romero, C., & Diaz-Diaz, E. (2003). Comparative study of methods for determination of titratable acidity in wine. *Journal of Food Composition and Analysis, 16*, 555-562.

Druaux, C., Lubbers, S., Charpentier, C., & Voilley, A. (1995). Effects of physico-chemical parameters of a model wine on the binding of ɣ-decalactone on bovine serum albumin. *Food Chemistry, 53*, 203-207.

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

Escudero, A., Campo, E., Farina, L., Cacho, J., & Ferreira, V. (2007). Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry, 55*, 4501-4510.

Ferreira, R. B., Picarra-Pereira, M. A., Monteiro, S., Loureiro, V. B., & Teixeira, A. R. (2002). The wine proteins. *Trends in Food Science & Technology, 12*, 230-239.

Gawel, R. (1998). Red wine astringency, a review. *Australian Journal of Grape and Wine Research, 4*, 74-95.

Gawel, R., Sluyter, S. V., & Waters, E. J. (2007). The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines. *Australian Journal of Grape and Wine Research, 13*, 38–45.

Giovani, G., & Rosi, I. (2007). Release of cell wall polysaccharides from *Saccharomyces cerevisiae* thermosensitive autolytic mutants during alcoholic fermentation. *International Journal of Food Microbiology, 116*, 19–24.

Gutiérrez, M., Llobera, A., Ipatov, A., Vila-Planas, J., Mínguez, S., Demming, S., Büttgenbach, S., Capdevila, F., Domingo, C., & Jiménez-Jorquera, C. (2011). Application of an e-tongue to the analysis of monovarietal and blends of white wines. *Sensors, 11*, 4840-4857.

Hagerman, A. E., & Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry, 26*, 809-812.

Harbertson, J. F., Hodgins, R. E., Thurston, L. N., Schaffer, L. J., Reid, M. S., Landon, J. L., Ross, C. F., & Adams, D. O. (2008). Variability of tannin concentration in red wines. *American Journal of Enology and Viticulture, 59*(2), 210-214.

Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K., & Ross, C. F. (2009). Chemical and sensory effects of Saignee, water addition, and extended maceration on high brix must. *American Journal of Enology and Viticulture, 60*(4), 450-460.

Harbertson, J. F., Picciotto, E. A., & Adams, D. O. (2003). Measurement of polymeric pigments in grape berry extracts and wines using protein precipitation assay combined with bisulfite bleaching. *American Journal of Enology and Viticulture, 54*(4), 301-306.

Henderson, J. P., & Rex, D. (2007). *About Wine*. New York: Thompson Delmar Learning.

Iland, P., Bruer, N., Edwards, G., Weeks, S., & Wilkes, E. (2004). *Chemical Analysis of Grapes and Wine: Techniques and Concepts*. Campbelltown, South Australia: Patric Iland Wine Promotions.

Jackson, R. S. (2014). *Wine Science, Principles and Applications* (4 ed.). USA: Academic Press.

Jones, P. R., Gawel, R., Francis, I. L., & Waters, E. J. (2008). The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Quality and Preference, 19*, 596–607.

Juega, M., Nunez, Y. P., Carrascosa, A. V., & Martinez-Rodriguez, A. J. (2012). Influence of yeast mannoproteins in the aroma improvement of white wines. *Journal of Food Science, 77*(8), M499-504.

King, E. S., Dunn, R. L., & Heymann, H. (2013). The influence of alcohol on the sensory perception of red wines. *Food Quality and Preference, 28*, 235–243.

Kontoudakis, N., Gonzalez, E., Gil, M., Estereulas, M., Fort, F., & Canals, J. M. (2011). Influence of wine pH on changes in color and polyphenol composition induced by micro-oxygenation. . *Journal of Agricultural and Food Chemistry, 59*, 1974-1984.

Lambri, M., Dordoni, R., Giribaldi, M., Violetta, M. R., & Guiffrida, M. G. (2013). Effect of pH on the protein profile and heat stability of an Italian white wine. *Food Research International, 54*, 1781-1786.

Landon, J. L., Weller, K., Harbertson, J. F., & Ross, C. F. (2008). Chemical and sensory evaluation of astringency in Washington State red wines. *American Journal of Enology and Viticulture, 59*(2), 153-158.

Lorrain, B., Tempere, S., Iturmendi, N., Moine, V., de Revel, G., & Teissedre, P. (2013). Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level. *Food Chemistry, 140*, 76-82.

Mainente, F., Zoccatelli, G., Lorenzini, M., Cecconi, D., Vincenzi, S., Rizzi, C., & Simonato, B. (2014). Red wine proteins: Two dimensional (2-D) electrophoresis and mass spectrometry analysis. *Food Chemistry, 164*, 413-417.

McRae, J. M., & Kennedy, J. A. (2011). Wine and grape tannin interactions with salivary proteins and their impact on astringency: a review of current research. *Molecules, 16*, 2348-2364.

McRae, J. M., Schulkin, A., Kassara, S., Holt, H. E., & Smith, P. A. (2013). Sensory properties of wine tannin fractions: implications for in-mouth sensory properties. *Journal of Agricultural and Food Chemistry, 61*, 719-727.

Mialon, V. S., & Ebeler, S. E. (1997). Time-intensity measurement of matrix effects on retronasal aroma perception. *Journal of Sensory Studies, 12*(4), 303–316.

Monteiro, S., Picarra-Pereira, M. A., Mesquita, P. R., Loureiro, V. B., Teixeira, A., & Ferreira, R. B. (2001). The diversity of structurally similar wine proteins. *Journal of Agricultural and Food Chemistry, 49*, 3999-4010.

Moreno, J., & Peinado, R. (2012). *Enological Chemistry*. CA, USA: Academy Press, Elsevier.

Nurgel, C., & Pickering, G. (2005). Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model wines. *Journal of Texture Studies, 36*, 303–323.

Ott, D. B., & Palmer, U. J. (1990). Ingestion and expectoration sampling methods of four tastes in a model system using time-intensity evaluations. *Journal of Sensory Studies, 5*(1), 53–70.

Perez-Serradilla, J. A., & de Castro, M. D. L. (2008). Role of lees in wine production: A review. *Food Chemistry, 111*, 447-456.

Pozo-Bayon, M. A., & Reineccius, G. (2009). Interactions between wine matrix macro-components and aroma compounds. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 417-436). New York: Springer Science+Business Media.

Riul, A., de Sousa, H. C., Malmegrim, R. R., dos Santos, D. S., André Carvalho, A. C. P. L. F., Fonseca, F. J., Oliveira, O. N., & Mattoso, L. H. C. (2004). Wine classification by taste sensors made from ultra-thin films and using neural networks. *Sensors and Actuators B, 98*, 77–82.

Robinson, A. L., Ebeler, S. E., Heymann, H., Boss, P. K., Solomon, P. S., & Trengove, R. D. (2009). Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *Journal of Agricultural and Food Chemistry, 57*, 10313–10322.

Robinson, J. (2001). *Concise Wine Companion*. New York: Oxford University Press, Inc.

Rodrigues, A., Ricardo-Da-Silva, J. M., Lucas, C., & Laureano, O. (2012). Effect of commercial mannoproteins on wine colour and tannins stability. *Food Chemistry, 131*, 907–914.

Rowe, J. D., Harbertson, J. F., Osborne, J. P., Freitag, M., Lim, J., & Bakalinsky, A. T. (2010). Systematic identification of yeast proteins extracted into model wine during ageing on lees. *Journal of Agricultural and Food Chemistry, 58*, 2337-2346.

Sáenz-Navajas, M., Campo, E., Avizcuri, J. M., Valentin, D., Fernández-Zurbanoa, P., & Ferreira, V. (2012). Contribution of non-volatile and aroma fractions to in-mouth sensory properties of red wines: Wine reconstitution strategies and sensory sorting task. *Analytica Chimica Acta, 732*, 64-72.

Smith, M. R., Penner, M. H., Bennett, S. E., & Bakalinsky, A. T. (2011). Quantitative colorimetric assay for total protein applied to the red wine Pinot Noir. *Journal of Agricultural and Food Chemistry, 59*, 6871-6876.

Tepper, B. J., Christensen, C. M., & Cao, J. (2001). Development of brief methods to classify individuals by PROP taster status. *Physiology & Behavior, 73*(4), 571–577.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry, 85*, 519–525.

Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The polysaccharides of red wine: total fractionation and characterization. *Carbohydrate Polymers, 54*(4), 439-447.

Villamor, R. R., Evans, M. A., Mattinson, D. S., & Ross, C. F. (2013a). Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. *Food Research International, 50*, 38–45.

Villamor, R. R., Evans, M. A., & Ross, C. F. (2013b). Effect of ethanol, tannin, and fructose concentrations on sensory properties of model red wines. *American Journal of Enology and Viticulture, 64*(3), 342-348.

Villamor, R. R., Harbertson, J. F., & Ross, C. F. (2009). Influence of tannin concentration, storage temperature, and time on chemical and sensory properties of Cabernet Sauvignon and Merlot wines. *American Journal of Enology and Viticulture, 60*(4), 422-449.

Zamora, M. C., Goldner, M. C., & Galmarini, M. V. (2006). Sourness-sweetness interactions in different media: white wine, ethanol and water. *Journal of Sensory Studies, 21*, 601–611.

Zraly, K. (2011). *Complete Wine Course* (2012 ed.). New York: Sterling Publishing.

# CHAPTER IV

# EVALUATION OF COMMERCIAL MERLOT WINES USING SENSORY EVALUATION AND A POTENTIOMETRIC ELECTRONIC TONGUE


## Abstract

Electronic tongues have been used to study discrimination and prediction of wines based on differences in some criteria such as vintage or geographic origin. However, studies on the discrimination of wines using physicochemical parameters are limited. The objective of this study was to evaluate selected commercial wines with an electronic tongue in order to discriminate among samples, predict wine chemical characteristics with the e-tongue and correlate human sensory evaluation with e-tongue signal intensity. Merlot wines (n=61) were profiled for eight chemical parameters and analyzed using the electronic tongue for seven non-volatile taste attributes. A subset of eight representative wines was selected using K-Means clustering and profiled by a sensory trained panel for taste and mouthfeel attributes. Support vector machines, artificial neural networks, multiple regression and partial least squares regression were used to explore classification and prediction of the electronic tongue data. Results from the support vector machines discrimination of samples based on their electronic tongue response for sour, metallic, spicy, salty, umami, sweet and bitter showed that individual samples were correctly identified at an accuracy rate of 90.1%. Prediction of each of the seven electronic tongue responses from the chemical parameters resulted in the following accuracy rates: sour (88%), metallic (92%), salty (93%), umami (90%), spicy (49%), sweet (90%) and bitter (92%). Results of the prediction of the electronic tongue sensor responses from the chemical parameters using multiple regression showed some linear relationships between electronic tongue response and chemical data ($p<0.05$) but the highest R-squared obtained was

0.24 between umami sensor response and three predictors (alcohol, tannins and large polymeric pigments). A comparison of artificial neural networks and multiple regression results indicated that electronic tongue responses for the 61 wines were more related to chemical parameters in a nonlinear manner. For the eight samples used for sensory evaluation, PLS showed high correlation between the electronic tongue data and the following sensory attributes rated by the trained panel: bitter ($r^2$=0.99), sour ($r^2$=0.97), sweet ($r^2$=0.89), ethanol burn ($r^2$=0.95), astringent ($r^2$=0.89) and metallic ($r^2$=0.92). This study demonstrated a non-linear relationship between electronic tongue output and chemical analyses of selected wines, with strong correlations found between some sensory attributes and electronic tongue data.

.

## Introduction

Wine sensory evaluation plays a critical role in the quality assessment of wines (Zraly, 2011; Jackson, 2014). Particularly of interest is the use of human beings to assign intensity values to selected sensory attributes of the wine, thus describing the sensory properties of the wines under study. One way to accomplish this is through the use of a trained sensory evaluation panel composed of panelists trained over a given period of time (Meilgaard *et al.*, 2007) to reliably evaluate the visual, aroma, flavor, taste and mouthfeel attributes of wines. However, this process is time-consuming and expensive as reproducible results require extensive training time and only a limited number of samples can be evaluated per day (Moscowitz, 2008; Munoz, 2008). Therefore, a need exists for the development of rapid methods for sensory evaluation of wines.

The electronic tongue multisensory systems are analytical techniques that combine rapid analysis and minimal sample preparation with cross-sensitivity to components in liquid samples, thus providing a profile of these samples. The application of electronic tongue systems in the analysis of liquid samples is increasingly reported in literature, with previous reviews describing its applications in food products including wine (Parra *et al.*, 2006a; Ciosek and Wro´blewski, 2007; Rodriguez-Mendez *et al.*, 2008; Escuder-Gilabert and Peris, 2010). Electronic tongues are typically sensor array instruments equipped with integrated pattern recognition systems that provide qualitative and quantitative information about multicomponent solutions (Riul *et al.*, 2003; Cabral *et al.*, 2009). Development of the system draws on the underlying principle of the neurophysiology of the sense of taste (Cosio *et al.*, 2012). As a result of the ionic, redox or molecular interaction of the sensor array with the multicomponent liquid sample, the electronic tongue gives a multidimensional output which captures the complexity of the sample being

analyzed. The output thus reflects a characteristic response that can be correlated with the human taste response (Ciosek and Wro´blewski, 2007).

As electronic tongues meet the requirement for rapid, objective and high throughput analytical instruments (Ciosek and Wro´blewski, 2007), they have found utility in the qualitative and quantitative assessment of wine quality as influenced by viticultural and enological factors (Riul *et al.*, 2004; Gil-Sánchez *et al.*, 2011). Among the applications of electronic tongues in wine quality evaluation are the verification of wine authenticity, process monitoring and examination of wine quality. Regarding authenticity studies, wines from different origins, grape varieties, vineyards and vintages have been successfully classified and discriminated using the electronic tongue (Riul *et al.*, 2003; Riul *et al.*, 2004; Rodriguez-Mendez *et al.*, 2008; Gil-Sánchez *et al.*, 2011). Wine adulteration has also been studied using the electronic tongue (Parra *et al.*, 2006b). Process monitoring studies in which the electronic tongue has been used include the assessment of wines aged in different oak barrels with different toasting levels (Parra *et al.*, 2006a), effect of micro-oxygenation and maceration with oak chips on phenolic profile (Rudnitskaya *et al.*, 2009) and wine spoilage using a combination of the electronic tongue and electronic nose (Gil-Sánchez *et al.*, 2011). Quantitative analysis of wine includes the quantification of some chemical parameters of red and white wines and the prediction of the sensory parameters and overall quality (Legin *et al.*, 2003; Buratti *et al.*, 2007).

The multidimensional nature of the response from the electronic tongue makes use of multivariate analysis and machine learning tools appropriate for data handling and interpretation. Specifically, principal component analysis, partial least square regression/discriminant analysis, and artificial neural networks are some of the tools that have been used to handle classification

and prediction questions regarding the use of sensor arrays for wine analysis (Vlassides *et al.*, 2001; Riul *et al.*, 2004; Puech *et al.*, 2007; Siivonen *et al.*, 2014).

Some studies using electronic tongues in the study of wines explored wines from different geographic areas, vintages, varieties and different treatments (Riul *et al.*, 2003; Riul *et al.*, 2004). Studies involving the use of the electronic tongue to discriminate among wines to ascertain the extent to which the electronic tongue identifies differences among these wines are lacking. Thus the objectives of the study were to evaluate the ability of the electronic tongue to quantitatively predict the taste attributes of the wines from the routine chemical parameters, discriminate among wines of the same variety and correlate sensory and electronic tongue evaluations using statistical and machine learning tools.

## Materials and Methods

### Materials

Bovine serum albumin (BSA, Fraction V powder), sodium dodecyl sulfate (SDS, lauryl sulfate, sodium salt), triethanolamine (TEA), ferric chloride hexahydrate, potassium metabisulfite, and (+)-catechin, citric acid, 6-propyl-2-thiouracil, tartaric acid, tannic acid, alum and quinine sulfate (St. Louis, MO, USA). Hydrochloric acid, sodium chloride, sodium hydroxide, 100% ethanol, and glacial acetic acid, acetone and trichloroacetic acid were obtained from J.T. Baker (Phillipsburg, NJ). For the electronic tongue analysis, hydrochloric acid, sodium chloride and sodium-L-glutamate standards were obtained from Alpha MOS (Tolouse, France). The rest of the materials for standard recipe preparation for evaluation of taste and mouthfeel properties of the wines were pure cane sugar (C&H sugar company, CA USA) and iron tablets (65mg, Pharmavite LLC, Mission Hills, CA. USA). MilliQ water was obtained through purification (Millipore Corporation, Billerica, MA, USA).

Red Wine Samples

Commercial Merlot wines (n=61), from 2006 to 2011, were selected based on their availability and representation of different physicochemical characteristics such as alcohol, tannins, acidity, residual sugars and pH. Samples were produced in California (n=24) and Washington State (n= 37). These were considered monovarietal based on wine labeling requirement: 75% or more of wine made from one variety grown in the labelled appellation of origin (TTB.Gov).

Electronic Tongue Analysis

Wine samples were equilibrated to room temperature and filtered through a coarse P8 Fisher brand filter paper (Fisher Scientific, Suwanee, GA. USA) to remove any sediments in the wine. The taste profile of wine the samples was analyzed using a potentiometric electronic tongue (Astree® II Electronic Tongue Unit, Alpha MOS) equipped with a liquid auto sampler (LS48) and seven cross-discriminatory food grade sensor array (known as set #5 sensors): sour (SRS), salty (STS), sweet (SWS), umami (UMS), metallic (GPS), bitter (BRS) and spicy (SPS). The sensors are based on the Chemically Modified Field Effect Transistor (CHEMFET) technology (Alpha MOS, 2011). The sensor response was the voltage difference between the Ag/AgCl reference electrode and each of the sensors.

A pre-run system preparation comprised of conditioning, calibration and diagnostics were performed according to manufacturer's instruction using 25 ml of 0.01M standard solutions prepared from 0.1M each of hydrochloric acid, sodium chloride and sodium-L-glutamate. This pre-run preparation was followed by an overnight hydration of the sensors (saltiness, sourness, sweetness, umami, metallic, bitterness and spiciness) in 25 ml reagent grade MilliQ filtered water. Another diagnostic run was performed prior to sample analysis. A programmed auto-

sampler method consisting of the following parameters was used: delay = 0 sec; acquisition time = 120 sec; stirring rate = 1 and acquisition = 1. A six-looped sequence consisting of a 10 sec sensor cleaning in 25 ml reagent grade MilliQ filtered water between samples was used during data acquisition.

Chemical Analyses

All chemicals analyses were conducted as described previously (Diako *et al.*, in review). $^{o}$Brix, pH and titratable acidity determinations were all made following the procedures previously described (Iland *et al.*, 2004). Ethanol concentrations were determined using an ebulliometer (ALLA, France) following the previously described procedure (Iland *et al.*, 2004). Small polymeric pigments (SPP) and large polymeric pigments (LPP) (absorbance units at 520 nm), tannins (mg/l catechin equivalents) and total phenolics (mg/l catechin equivalents) were determined as previously described (Hagerman and Butler, 1978) and modified (Harbertson *et al.*, 2003). All measurements of wine chemistry parameters were conducted in triplicate.

Trained Panel

The trained panel was conducted as described previously (Diako *et al*., in review). Panelists (n=13) were recruited from the Washington State community through electronic advertisement. The panel was composed of 54% males and 46% females with ages between 21 and 60. Most of the panelists (77%) were between 21-30 years while the rest were between 41 and 60 years. The wine consumption patterns of the panelists varied, with most panelists consuming wine once to a few times a month. The panelists received minimum background information about the study to reduce potential bias and were simply informed they would be evaluating red wines over 12 training sessions followed by two formal evaluation sessions. The project was approved by the Washington State University Institutional Review Board for human

subject participation. On the first day of training; all panelists signed an informed consent form and received nonmonetary incentive after each training and formal evaluation sessions.

Inconsistency in the evaluation of taste and mouthfeel compared to flavor evaluation of model solutions has been documented (Ott and Palmer, 1990). Because genetic and individual differences could account for this, the saliva flow rate and taster status as dictated by individual sensitivity to 6-n-propylthiouracil (PROP). The taster status of each panelist, as determined using PROP, was determined as previously described (Tepper *et al.*, 2001), as was saliva flow rate (Mialon and Ebeler, 1997).

The panelists were instructed on the techniques to use in the evaluation of taste and mouthfeel attributes of wines. The first three training sessions were devoted to building consensus and defining appropriate standards that defined the Merlot wines. Training was conducted through presentation of standard solutions prepared in base wine (Livingston Red Rosé, Gallo, Modesto, CA USA). The recipes for the standards are presented in **Table 9**. In subsequent sessions, panelists were presented with these standard solutions to illustrate attributes, followed by the evaluation and subsequent discussion of commercial red wine samples. Panelists were gradually introduced to the different taste and mouthfeel attributes. For both training and formal evaluation, wine samples (25 ml) were pre-poured into ISO/INAO (International Standards Organization) tasting glasses and covered with petri dishes for one hour before tasting to allow for equilibration. The samples were labeled with three-digit codes and presented to panelists one at a time using a randomized serving order. The PROP status and saliva flow rate were used to characterize the panelists and helped with understanding their individual rating of the intensities of the attributes of the samples. Scores for each attribute were summarized using boxplots to provide a graphical representation of outliers. Both individual and

**Table 9.** Recipes and standards used in panel training for the sensory evaluation of samples.

| Category | Attribute | Preparation |
|---|---|---|
| Taste | Sweet | 3.3% (w/v) cane sugar in base wine[a] |
| | Sour | 0.3% tartaric acid in base wine |
| | Bitter | 0.001% (w/v) quinine sulfate in base wine |
| Mouthfeel | Astringent | 0.78 g tannic acid + 0.35g alum in 300 ml base wine |
| | Burning | 60 ml ethanol (95%) in 240 ml base wine |
| | Metallic | 8 iron tablets (~ 3.0 g) dissolved in 300 ml base wine and filtered |

[a]Base wine was a Red Rosé, Livingstone Cellars, Modesto, CA.

panel means and standard deviations were obtained after each training session. Panelists who scored attributes much lower or higher than the overall panel mean were provided with additional training. Data from the taster status and saliva flow rate helped to better understand the rating trends of panelist for tailored feedback on performance.

Panelists rated the perception of intensity of three taste attributes (sweet, sour, and bitter) and three mouthfeel attributes (astringent, burning, and metallic) along a 15-cm structured line scale anchored at 1.5 (low) and 13.5 cm (high). Samples were assigned three-digit codes and monadically presented to the panelist in individual tasting booths. Eight samples were evaluated per session replicates over the three formal evaluation sessions. Panelists were required to pause for 1 min between samples, with a 10-min forced break after the fourth sample to refresh their palate and minimize fatigue. Panelists were provided with crackers and distilled water for palate cleansing. All instructions, scale presentations, and data collection were carried out using Compusense *five*, release 5.2 (Guelph, Canada).

**Data Analysis**

Support Vector Machine (SVM) classification of wines from the different wineries was performed using a 10-fold cross validation for tuning the support vector machine parameters. The best parameters obtained were cost=100 and gamma=1. To use SVM to explore the separation capability of the electronic tongue for the samples analyzed, the triplicate determination for each sample was considered as a group thus generating 61 groups for the 61 samples analyzed. An R program was written which used two-thirds of the data points to train the SVM and used the remaining one-third of the data points as test data. Using this method, two data points from each sample were used to train the SVM and the third data point used to test to assess if they were correctly classified into their respective groups.

Prediction of the electronic tongue response from the chemical parameters was performed using linear multiple regression and artificial neural networks. With multiple regression analysis, the model for each response was built using the stepwise selection technique and the Akaike Information Criterion (AIC), ensuring that the sign and the significance of the parameter estimates reflected the existing trends in the data as previously determined using correlation analysis. The models selected through this procedure were further diagnosed for outliers and influential points. Artificial Neural Network (ANN) analysis was performed using resilient backpropagation with weight backtracking using the logistic function as the activation function. A sigmoidal transfer function was used for the hidden nodes while a linear transfer function was used for the output nodes to permit the artificial neural network regression. For ANN, the data were divided into training data and test data as previously done for SVM

Selection of the subset of the samples for sensory profiling was performed using K-Means clustering. The samples were partitioned into four groups based on their chemical and electronic tongue parameters. This allowed for the selection of 8 wine samples for sensory profiling according to group sizes. Sensory correlation with electronic tongue measurements was done using the Astree® chemometrics software (AlphaSoft ver. 12)

**Results and Discussion**

Discrimination of Wine Samples

A characteristic signal of the Astree® electronic tongue is shown in **Figure 5.** This signal gives average values for sour, metallic, salty, umami, spicy, sweet and bitter sensors describing the taste profile of the sample. Electronic tongues operate on the principle of redox, ionic and molecular interaction between components of a liquid sample and a sensor array coated with various polymeric membranes. The non-specificity and cross-sensitivity of the sensors lead to

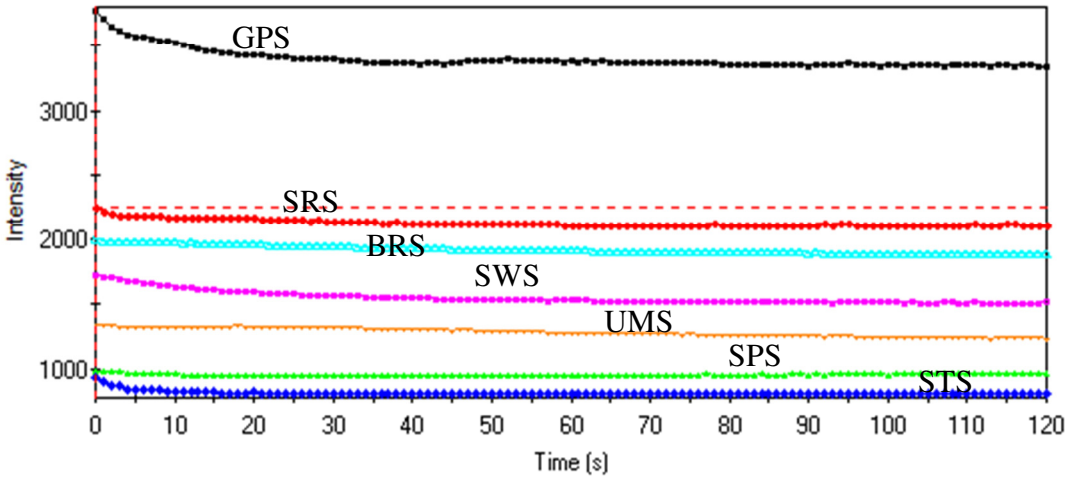


**Figure 5.** Typical Astree® electronic tongue signal for wines evaluated showing intensity (mV) of electronic tongue response and time (s) of data acquisition. The sensors are GPS (Metallic), SRS (Sour), BRS (Bitter), SWS (Sweet), UMS (Umami), SPS (Spicy) and STS (Salty)

the acquisition of multidimensional information from a sample in solution and when information is coupled with multivariate and machine learning techniques, electronic tongues become powerful tools for qualitative and quantitative evaluation of food products in solution. The electronic tongue used in this study was a potentiometric electronic tongue programmed to acquire data over a 120 seconds period. Data reported from the signals represent the average of the 20 data points acquired in the last 20 seconds of the run hence a stable signal is required for the last 20 seconds of data acquisition as drifting sensor signals indicates issues with the sensors..

Using two-thirds of the data as training data and one-third as test data, the support vector machine classification showed a 90.1% correct classification rate for the test data (**Figure 6**). Support vector machines are kernel-based algorithms that use a boundary known as a hyperplane to partition data into groups of similar input classes by maximizing the distance between the nearest data points of the input classes known as support vectors. Originally implemented as a binary classifier, support vector machines were extended to multiclass classification by reducing the multiclass to several binary classes and performing several binary classifications.

Classification rates greater than 90% as observed in this study are common with support vector machines. For instance, Jurado *et al.* (2012) reported a 100% accuracy rate for the classification of white wines from different appellations. Beers from different geographical regions have also seen high classification accuracy rates (99.3%) using support vector machines and chemical data (Alcázar *et al.*, 2012). The high classification rate observed in this study implies that the electronic tongue profiles of the samples, to a large extent, were characteristic of the samples analyzed. The electronic tongue was therefore able to discriminate among the samples and indicated differences among the samples. Many of the previous studies in wines using the electronic tongue were aimed at distinguishing among wines based on some

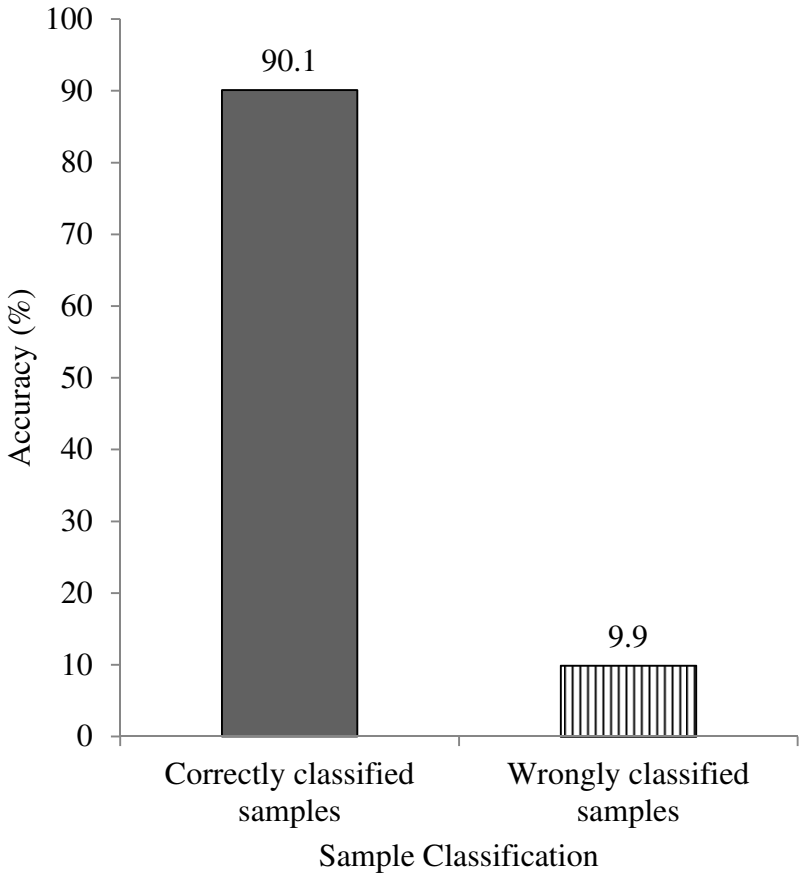


**Figure 6.** Support vector classification of wines samples by winery of origin using the following parameters: SVM-Kernel = polynomial, cost=10, gamma=1, degree=3. Two data points from each of the 61 samples were used to train the support vector machine while the third data point was classified to test for group membership.

criteria including vintage and origin of sample (Legin *et al.*, 2003; Gutiérrez *et al.*, 2011). The results from this study indicate that the wines can be classified to a high degree of accuracy using support vector machines.

Prediction of Electronic Tongue Taste Profiles from Wine Chemical Parameters

The prediction of electronic tongue taste profiles from chemical analysis was performed using both a linear method (multiple regression) and non-linear method (artificial neural networks). Multiple regression is based on ordinary least squares and assumes a linear relationship between response and predictor variables (Kutner *et al.*, 2005).

Statistically significant linear relationships exist between the electronic tongue output from the chemical parameters ($p<0.05$), with the exception of spicy (**Table 10**). For each model, these results show that at least one predictor was significantly related to the sensor output of the electronic tongue. However, the $R^2$ values were low, with the reported maximum being $R^2 =0.24$. These results indicated that a substantial amount of variation in the response variable was not being explained by the model. Furthermore, results suggested that either important predictors of the electronic tongue output were not determined as part of the chemical parameters evaluated or the relationship between the electronic tongue output and the chemical parameters was non-linear.

Artificial neural networks are inspired by biological systems. In these systems, one neuron acts as a computational unit and when many neurons come together, they form a huge network that is capable of learning trends and patterns, thus leading to powerful non-linear modeling capability (Forte, 2015). The artificial neural network consists of multiple layers, an input layer made up the number of predictors in the model, a hidden layer and an output layer. A

**Table 10.** Predictors, $R^2$ and p-values for the prediction of electronic tongue output from commercial Merlot wine chemical parameters using multiple regression with stepwise selection (n=61).

| E-Tongue Response | Significant Predictors [a] | $R^2$ | P-value |
|---|---|---|---|
| SRS (Sour) | pH, ALC | 0.14 | 0.014 |
| GPS (Metallic) | SOL, ALC, PHEN | 0.21 | 0.003 |
| STS (Salty) | SOL , ALC | 0.16 | 0.007 |
| UMS (Umami) | TA, ALC, LPP | 0.24 | 0.002 |
| SPS (Spicy) | None | N/A | N/A |
| SWS (Sweet) | ALC, TAN | 0.11 | 0.032 |
| BRS (Bitter) | SPP | 0.07 | 0.033 |

[a]Definition of predictor names are as follows:, ALC= alcohol, SOL= soluble solids, PHEN=phenolics, TA=Titratable acidity, LPP=large polymeric pigments, TAN= tannins, SPP = small polymeric pigments

neural network's architecture is determined by the number of nodes and layers in the hidden layer. In Table 11, the sour sensor response was predicted by a 10, 5 architecture. This meant the first layer had 10 nodes and the second layer had 5. Spicy had 6,10,4 indicating 6, 10 and 4 nodes in layers 1, 2 and 3, respectively. Prediction of the electronic tongue profiles from the chemical analysis using artificial neural networks showed better results than the multiple regression method. The results showed that each of the electronic tongue output is predicted by different network architecture (**Table 11**). The different sensors representing different tastes were predicted by different architectures is comparable to the perception of tastes by the human tongue. The human tongue has different mechanisms of perceiving basic tastes. Sour and salty are perceived through ion channels while sweet, bitter and umami are perceived through the gastric protein-coupled receptors.

Spicy showed the most complex architecture and it was also the sensor with the least prediction accuracy of 48%. This low prediction accuracy implied that additional layers and/or more training are needed to increase the prediction accuracy of this sensor. Besides spicy, the high accuracy rate of the remaining sensors (88-93%) indicates that the electronic tongue output can be predicted using nonlinear methods from the determined physico-chemical parameters. Although most used in wine studies for classification purposes (Pérez-Magariño *et al.*, 2004; Penza and Cassano, 2004; Kruzlicova *et al.*, 2009), the use of artificial neural networks for predictive purposes, such as done in this study, has been used by other authors in wine optimization studies (Vlassides *et al.*, 2001; Ferrier and Block, 2001). Results from these studies show the robustness of artificial neural network in modeling non-linear responses with high prediction accuracy rates.

**Table 11.** Neural network architecture and prediction accuracy for the prediction of electronic tongue response from chemical parameters of commercial merlot wines (n=61).

| E-Tongue Sensor Response | Network Architecture (hidden nodes [a]) | Prediction (%) |
|---|---|---|
| Sour | 10,5 | 88 |
| Metallic | 6,8 | 92 |
| Salty | 8,6 | 93 |
| Umami | 8,8 | 90 |
| Spicy | 6,10,4 | 49 |
| Sweet | 7,7 | 90 |
| Bitter | 10,10 | 92 |

[a] Hidden nodes represent the number of layers between the input node and the output node in an artificial neural network. Each of the sensor response prediction was made using two layers represented as (a,b) number of nodes, except for spicy which had three layers (a,b,c).

Correlation between Sensory Evaluation and Electronic Tongue Analysis

To obtain a trained sensory panel profile of the wines and correlate with instrumental measurements, a number of wines with chemical and electronic tongue profiles representative of all sixty one wines had to be selected. This was performed using K-means clustering which uses a partition algorithm that minimizes the within cluster sum of squares. K-means clustering is an unsupervised learning algorithm which groups a set of observations around pre-defined number centers, one for each cluster. The goal is to group the observations around the centers in such a way that the distance between an observation and its assigned center is the least. In this manner, the within cluster distances are minimized while distances between the different clusters are maximized. This clustering method resulted in the identification of four clusters (**Figure 7**). Eight samples were randomly and proportionately selected from the four clusters as follows: one from cluster 1, two from cluster 2, four from cluster 3 and one from cluster 4. The samples selected were then profiled for taste and mouthfeel attributes previously indicated in Table 9 by the sensory panel.

Sensor discrimination power ranges between 0 and 1.0. This discrimination power is important for the electronic tongue results used in this study as this value highlights the sensors which are responsible for the differences observed among the samples. The higher the discrimination power, the greater the contribution of the sensor to the differences observed in the electronic tongue profile of the sample. Since a subset of the wines were selected for sensory profiling, it was important to determine whether the samples were distinctly different based on the electronic tongue analysis.

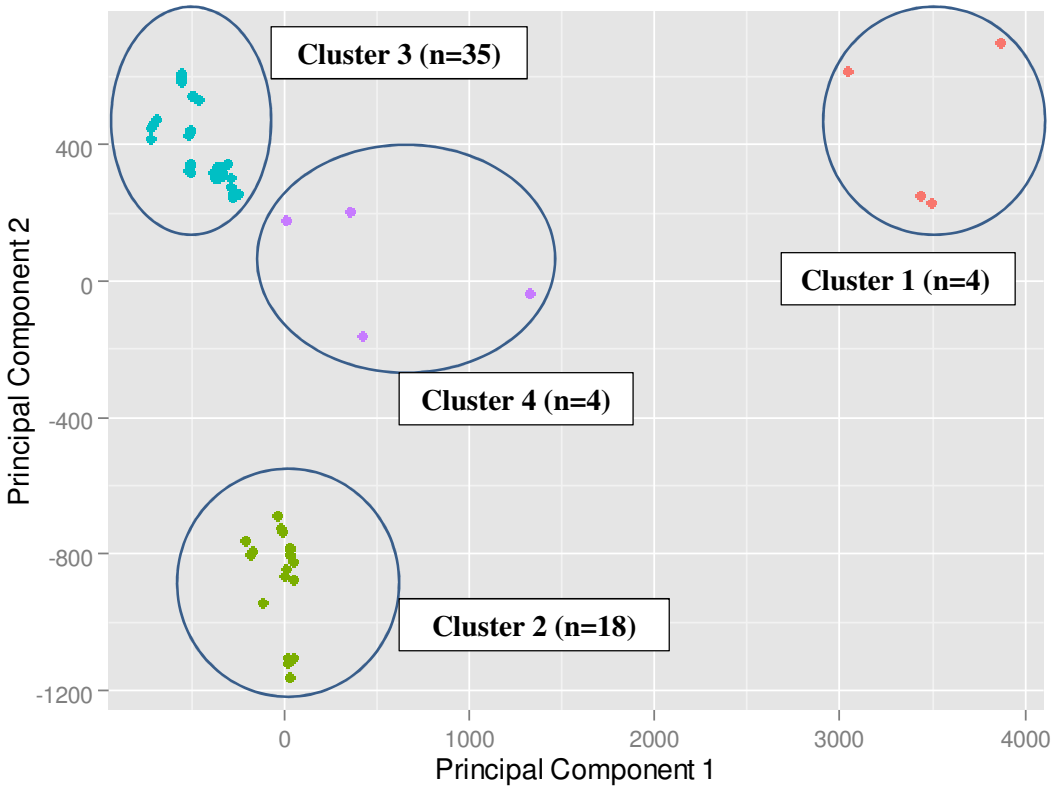


**Figure 7.** K-Means clustering of 61 commercial Merlot wines showing four clusters based on the chemical and electronic tongue profiles.

One way to confirm these sample differences was to check the discrimination power of the sensors for these samples. Clearly, high discrimination power was observed for the seven sensors (**Figure 8**). The spicy sensor registered the lowest discrimination power (0.91) while the rest of the sensors were between 0.99 and 1.0. The high discrimination power of the sensors implied that each of sensors contributed significantly to the resulting taste profile of the wines as determined by the electronic tongue analysis except the spicy sensor. This also meant that all the sensor results were important for modelling the relationship between instrumental and sensory evaluation for the subset of samples selected for sensory evaluation.

Because the electronic tongue was developed to mimic the human sense of taste, it is possible to explore relationships between the human taste profile of a sample and instrumental profiles. Results showed strong correlations between the electronic tongue taste profile and the taste and mouthfeel attributes as determined through the sensory evaluation panel (**Table 12**). The lowest correlations observed were between the electronic tongue and sensory panel evaluation of sweetness ($r^2$=89) as well as the drying mouthfeel as determined by the sensory panel ($r^2$=89). Bitterness perceived by the trained panel had the highest correlation ($r^2$=0.99) with the electronic tongue. This high correlation is indicative of the ability of the electronic tongue to be used to complement sensory evaluation in the assessment of wine quality.

Correlation of sensory and chemical data with electronic tongue data has been studied to determine changes in apricots during storage (Kantor *et al.*, 2008). The authors observed high correlations between the electronic tongue data and refractometer results (0.81 – 0.92), moderate

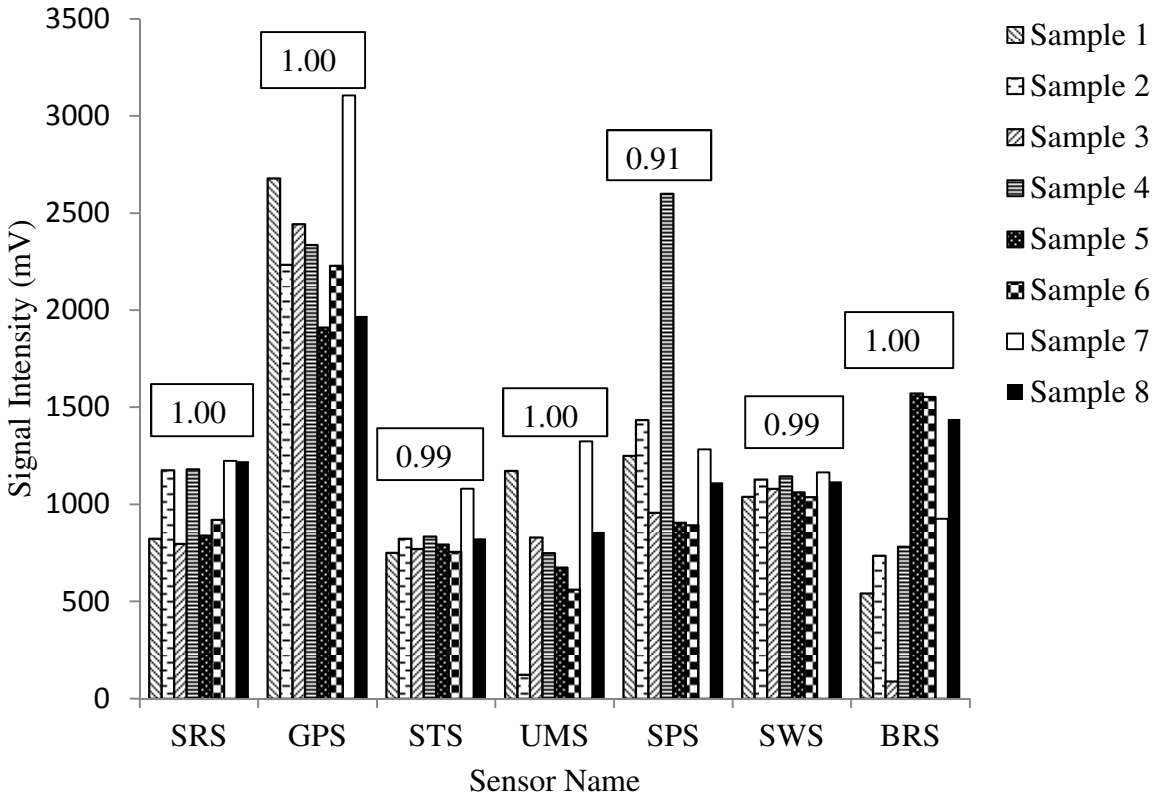


**Figure 8.** Discrimination power of the electronic tongue sensors for the eight commercial Merlot wines used in the trained sensory evaluation. Sensors are represented by sour (SRS), metallic (GPS), salty (STS), umami (UMS), spicy (SPS), sweet (SWS) and bitter (BRS). Maximum discrimination power attainable is 1.0.

**Table 12.** Partial Least Squares correlation between trained panel evaluation of taste and mouthfeel attributes and the overall electronic tongue response (n=8).

| **Sensory Taste and Mouthfeel Attributes** | **Correlation ($r^2$)** |
|---|---|
| Bitter | 0.99 |
| Burning | 0.95 |
| Drying | 0.89 |
| Metallic | 0.92 |
| Sour | 0.97 |
| Sweet | 0.89 |

correlations with flavor attributes evaluated by a trained panel but poor correlation with overall impression as evaluated by a sensory panel. Overall impression is a hedonic response thus caution should be exercised in correlating it with instrumental evaluations (Stone *et al.* (2012). Equating a hedonic response to instrumental evaluations is complicated since the hedonic response and instrumental evaluations may be governed by different functions. While hedonic response may be described by a parabolic function, other measures may be defined by linear, curvilinear or sigmoidal functions. The high correlations observed in this study may be due to the fact that none of the sensory measures were hedonic in nature.

In conclusion, this research showed that the 61 monovarietal wines under study could be discriminated using the electronic tongue. This separation was accomplished based only on their characteristic response from the electronic tongue using support vector machines, without the need to separate them into broad groups. Furthermore, the relationship between the chemical composition and the output of the electronic tongue is complex and while a linear method (multiple linear regression) could predict the relationship, the relationship was more successfully predicted using a nonlinear method (artificial neural networks). Also, the high correlation between trained panel evaluation and the electronic tongue suggests that these two methods could be used synergistically to evaluate wine quality.

**Literature Cited**


Alcázar, A., Jurado, J. M., Palacios-Morillo, A., de Pablos, F., & Martín, M. J. (2012). Recognition of the geographical origin of beer based on support vector machines applied to chemical descriptors. *Food Control, 23*, 258-262.

Alpha MOS. (2011). Hardware User Guide. Toulouse, France: Alpha MOS.

Buratti, S., Ballabio, D., Benedetti, S., & Cosio, M. S. (2007). Prediction of Italian red wine sensorial descriptors from electronic nose, electronic tongue and spectrophotometric measurements by means of Genetic Algorithm regression models. *Food Chemistry, 100*, 211-218.

Cabral, F. P. A., Bergamo, B. B., Dantas, C. A. R., Riul, A., & Giancometti, J. A. (2009). Impedance e-tongue instrument for rapid liquid assessment. *Review of Scientific Instruments, 80*, 026107.

Ciosek, P., & Wro´blewski, W. (2007). Sensor arrays for liquid sensing – electronic tongue systems. *Analyst, 132* 963-978.

Cosio, M. S., Scampicchio, M., & Benedetti, S. (2012). Electronic noses and tongues. In P. Yolanda (Ed.), *Chemical Analysis of Food: Techniques and Applications* (pp. 219-247): Academic Press, USA.

Escuder-Gilabert, L., & Peris, M. (2010). Review: Highlights in recent applications of electronic tongues in food analysis. *Analytica Chimica Acta, 665*, 15-25.

Ferrier, J. G., & Block, D. E. (2001). Neural-network-assisted optimization of wine blending based on sensory analysis. *American Journal of Enology and Viticulture, 52*(4), 386-395.

Forte, R. M. (2015). *Mastering Predictive Analytics with R*. Birmingham, UK: Packt Publishing Ltd.

Gil-Sánchez, L., Soto, J., Martínez-Mánez, R., Garcia-Breijo, E., Ibánez, J., & Llobet, E. (2011). A novel humid electronic nose combined with an electronic tongue for assessing deterioration of wine. *Sensors and Actuators A., 171*, 152-158.

Gutiérrez, M., Llobera, A., Ipatov, A., Vila-Planas, J., Mínguez, S., Demming, S., Büttgenbach, S., Capdevila, F., Domingo, C., & Jiménez-Jorquera, C. (2011). Application of an e-tongue to the analysis of monovarietal and blends of white wines. *Sensors, 11*, 4840-4857.

Hagerman, A. E., & Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry, 26*, 809-812.

Harbertson, J. F., Picciotto, E. A., & Adams, D. O. (2003). Measurement of polymeric pigments in grape berry extracts and wines using protein precipitation assay combined with bisulfite bleaching. *American Journal of Enology and Viticulture, 54*(4), 301-306.

Iland, P., Bruer, N., Edwards, G., Weeks, S., & Wilkes, E. (2004). *Chemical Analysis of Grapes and Wine: Techniques and Concepts*. Campbelltown, South Australia: Patric Iland Wine Promotions.

Jackson, R. S. (2014). *Wine Science, Principles and Applications* (4 ed.). USA: Academic Press.

Jurado, J. M., Alcázar, A., Palacios-Morillo, A., & de Pablos, F. (2012). Classification of Spanish DO white wines according to their elemental profile by means of support vector machines. *Food Chemistry, 135*, 898–903.

Kantor, D. B., Hitka, G., Fekete, A., & Balla, C. (2008). Electronic tongue for sensing taste changes with apricots during storage. *Sensors and Actuators B, 131*, 43-47.

Kruzlicova, D., Mocak, J., Balla, B., Petka, J., Farkova, M., & Havel, J. (2009). Classification of Slovak white wines using artificial neural networks and discriminant techniques. *Food Chemistry, 112*, 1046–1052.

Kutner, M. H., Nachtsheim, C. J., Neter, J., & Li, W. (2005). *Applied Linear Statistical Models* (5 ed.). New York, USA: McGraw-Hill/Irwin.

Legin, A., Rudnitskaya, A., Lvova, L., Vlasov, Y., Natale, C. D., & D'Amico, A. (2003). Evaluation of Italian wine by the electronic tongue: recognition, quantitative analysis and correlation with human sensory perception. *Analytica Chimica Acta, 484*, 33–44.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory Evaluation Techniques* (4th ed.). Boca Raton: CRC Press, Taylor and Francis Group.

Mialon, V. S., & Ebeler, S. E. (1997). Time-intensity measurement of matrix effects on retronasal aroma perception. *Journal of Sensory Studies, 12*(4), 303–316.

Moscowitz, H. R. (2008). Product and panelist variabilty in sensory testing. In H. R. Moscowitz, A. M. Munoz & M. C. Gaculas (Eds.), *Viewpoints and Controversies in Sensory Science and Consumer Product Testing* (pp. 375-378). Hoboken, NJ, USA Wiley-Blackwell.

Munoz, A. M. (2008). Training time in descriptive analysis. In H. R. Moscowitz, A. M. Munoz & M. C. Gaculas (Eds.), *Viewpoints and Controversies in Sensory Science and Consumer Product Testing* (pp. 351-356). Hoboken, NJ, USA: Wiley-Blackwell.

Ott, D. B., & Palmer, U. J. (1990). Ingestion and expectoration sampling methods of four tastes in a model system using time-intensity evaluations. *Journal of Sensory Studies, 5*(1), 53–70.

Parra, V., Arrieta, A. A., Fernandez-Escudero, J. A., Iniguez, M., De Saja, J. A., & Rodriguez-Mendez, M., L. (2006a). Monitoring of the ageing of red wines in oak barrels by means of an hybrid electronic tongue. *Analytica Chimica Acta, 563*, 229–237.

Parra, V., Arrieta, A. A., Fernandez-Escudero, J. A., Rodrıguez-Mendez, M. L., & De Saja, J. A. (2006b). Electronic tongue based on chemically modified electrodes and voltammetry for the detection of adulterations in wines. *Sensors and Actuators B, 118*, 448–453.

Penza, M., & Cassano, G. (2004). Chemometric characterization of Italian wines by thin-film multisensors array and artificial neural networks. *Food Chemistry, 86*, 283–296.

Pérez-Magariño, Ortega-Heras, S. M., González-San José, M. L., & Boger, Z. (2004). Comparative study of artificial neural network and multivariate methods to classify Spanish DO rose wines. *Talanta, 62*, 983-990.

Puech, J.-L., Prida, A., & Isz, S. (2007). Quality assessment of oenological tannins utilising global selectivity chemical sensors array ("Electronic Tongue"). *South African Journal of Enology and Viticulture, 28*(2), 101-106.

Riul, A., de Sousa, H. C., Malmegrim, R. R., dos Santos, D. S., André Carvalho, A. C. P. L. F., Fonseca, F. J., Oliveira, O. N., & Mattoso, L. H. C. (2004). Wine classification by taste sensors made from ultra-thin films and using neural networks. *Sensors and Actuators B, 98*, 77–82.

Riul, A., Malmegrim, R. R., Fonseca, F. J., & Mattoso, L. H. C. (2003). An artificial taste sensor based on conducting polymers. *Biosensors and Bioelectronics, 18*, 1365-1369.

Rodriguez-Mendez, M., L., Parra, V., Apetrei, C., Villanueva, S., Gay, M., Prieto, N., Martinez, J., & de Saja, J. A. (2008). Electronic tongue based on voltammetric electrodes modified with materials showing complementary electroactive properties. Applications. *Microchim Acta, 163*, 23–31.

Rudnitskaya, A., Schmidtkec, L. M., Delgadillo, I., Legin, A., & Scollary, G. (2009). Study of the influence of micro-oxygenation and oak chip maceration on wine composition using an electronic tongue and chemical analysis. *Analytica Chimica Acta, 642*, 235–245.

Siivonen, J. J., Väisänen, P. I., Tiittane, S. A., Lehmusto, M. M., Lehtonen, P., Patrikainen, E., Teimonen, T., Törnkvist, N., Mundill, P., Hänninen, P., & Härmä, H. (2014). Novel non-specific liquid fingerprint technology for wine analysis: a feasibility study. *Australian Journal of Grape and Wine Research, 20*, 172–177.

Stone, H., Bleibaum, R. N., & Thomas, H. A. (2012). *Sensory Evaluation Practices* (4th ed.). USA: Academic Press.

Tepper, B. J., Christensen, C. M., & Cao, J. (2001). Development of brief methods to classify individuals by PROP taster status. *Physiology & Behavior, 73*(4), 571–577.

TTB.Gov. Requirements for using grape variety names on American wine labels. Retrieved March 9, 2016, from http://www.ttb.gov/wine/grape-varieties.shtml#use_of_a_grape_name

Vlassides, S., Ferrier, J. G., & Block, D. E. (2001). Using historical data for bioprocess optimization: Modeling wine characteristics using artificial neural networks and archived process information. *Biotechnology and Bioengineering, 73*(1), 55-68.

Zraly, K. (2011). *Complete Wine Course* (2012 ed.). New York: Sterling Publishing.

# CHAPTER V

# PANELISTS' BIAS ESTIMATION IN A RED WINE SENSORY PANEL


## Abstract

In using trained panelists in sensory evaluation studies, panel performance monitoring is conducted to ensure repeatability and reproducibility of evaluations, thus increasing confidence in the results. Beyond the sample itself, the sensory verdict is influenced by both physiological and psychological differences among panelists. The goal of the panel leader is to use training to reduce the variability in the panelists' ratings contributed by individual differences. Using trained panel data of red wine, the objective of this study was to conceptualize panelists' bias as a linear operator to correct panelists' evaluations and predict attribute ratings of unknown samples. Panelists (n=13) evaluated 12 commercial Merlot wines for 7 aromas and flavors, 3 tastes and 3 mouthfeel attributes. A bias matrix was computed for each panelist and used to adjust their intensity ratings, resulting in consistent sample evaluations. Differences between actual and filtered evaluations were evaluated using a t-test while predictive filtering was performed on a previously unrated sample using the known panelist bias. Results showed that the bias matrix corrected the individual ratings of the samples leading to higher reproducibility among panelists. The t-test indicated no significant differences ($p>0.05$) between original and filtered means indicating that filtering only reduced the dispersion of ratings around the mean without significantly affecting the mean. Predictive filtering showed that the panelists' corrected means for the attributes were closer to the predicted panel mean compared to their predicted unfiltered means. Overall, this study showed that trained panelists' biases influence their ratings during product evaluations, but agreement among their ratings can be improved by abstracting these biases and using them for monitoring and corrective purposes.

## Introduction

The panelist is the analytical instrument used in sensory evaluation (Stone *et al.*, 2012). In quantitative descriptive analysis, the desired capabilities of panelists include the ability to identify and detect differences in the characteristics of the product being profiled, assign intensities to these pertinent characteristics using appropriate scaling method(s) and verbalize and articulate opinions for consensus building during panel training sessions (Meilgaard *et al.*, 2007; Stone *et al.*, 2012). To this end, care is taken to select panelists by using screening tools which include personal interviews and sensory tests such as matching, discrimination, acuity and ranking tests.

However, these steps do not remove the contribution of the individual differences of the panelists participating in the sensory panel. A panelist's sensory response is dependent on some physiological and psychological factors, as well as the physical and chemical factors of the product under investigation. The goal of panel training is to reduce variability in the ratings of panelists by improving repeatability (the ability of a panelist to rate a given attribute of a product consistently) and reproducibility (panelist's ability to rate products in a similar manner as other panelists). Once this is accomplished, product differences can be ascertained. Although panelists may be enthusiastic about taking part in a trained panel, their variabilities (biases) are pervasive and should be dealt with as a matter of necessity.

The objective of performance monitoring is to detect atypical patterns that may require a dialogue with a panelist or further training (Stone *et al.*, 2012) Approaches ranging from univariate to multivariate methods have been used to monitor panel performance to understand panelist variations in a trained panel (Meullenet *et al.*, 2007). Univariate methods of panelists evaluation include visualization of raw data (Meullenet *et al.*, 2007), computation of descriptive

statistics involving deviations of a panelist's evaluations from the average panel evaluation of an attribute to monitor repeatability and reproducibility (Rossi, 2001) and use of one-way ANOVA model with accompanying graphical techniques to visualize and monitor panel performance and individual differences (Tomic *et al.*, 2007)

The one-way ANOVA approach uses the F-value, Mean Square Error (MSE) and the p-value to judge performance. An F-plot gives information on the discrimination performance of each panelist, with a higher F-value showing better discrimination ability of the assessor. If there is a difference among samples, the F-value of the assessor is generally higher than the F-value corresponding to the level of significance chosen for the experiment. The MSE plot can be used as a measure of the repeatability of each assessor. The closer the MSE is to zero, the better the repeatability of that assessor. It is important that MSE values are interpreted in conjunction with F-values as the panelists' desire to achieve a low MSE can lead to the evaluation of samples in a similar manner, thereby compromising the panelists' discrimination power. Ideally, higher F-values and low MSE values are observed.

Finally, p*MSE plot (plot of p-values obtained from the ANOVA test against the MSE) give an overall performance of a panelist. Panelists in the lower quadrants of the plot are deemed to be performing better. Other studies involving the use of one-way ANOVA for panel performance monitoring include trained panel evaluation of jelly products (Tomic *et al.*, 2013) and the use of secondary data to illustrate the use of univariate methods for evaluating panel performance (Naes, 1998; Derndorfer *et al.*, 2005). Mixed models have also been used to monitor panels and individual panelist effects in terms of variability differences, presence of disagreement, scaling differences and sensitivity differences, entire panel and individual panelist performance for discrimination, repeatability, panelist effect and the effect of panelist and

sample interaction (Brockhoff, 2003; Findlay *et al.*, 2006; Latreille *et al.*, 2006). Although univariate analyses are useful in evaluating panel performance, these analyses assess panel performance one attribute at time. Meullenet *et al.* (2007) indicated that sensory evaluation is a multivariate task and as a result, any consideration of panelist performance should use techniques that measure performance in the context which involves the use of multivariate analysis.

Multivariate analysis methods used in the evaluation of panel performance include multivariate analysis of variance hierarchical cluster analysis, consonance, RV coefficients, analysis and principal component analysis (Dijksterhuis, 1995; Cliff and King, 1999; King *et al.*, 2001; Derndorfer *et al.*, 2005; Kermit and Lengard, 2005; Findlay *et al.*, 2006; Meullenet *et al.*, 2007; Tomic *et al.*, 2013). These studies have documented how multivariate methods address panel performance. While the principal component analysis has been used to identify panelists according to how they use the attributes in the evaluation of the samples, hierarchical clustering is used to group panelists based on similarity across samples and attributes. In like manner, MANOVA tests are used for product discrimination, RV coefficients are used ascertain similarities between two datasets and consonance analysis gives an indication of the agreement in the use of attributes by panelists.

The approaches previously described only identify differences, without giving any further insight into the possible reasons for differences in panelists' ratings of the attributes of a sample. Also, since these tools are used for panelist monitoring, they are only applicable until the last day of the training before formal evaluations of the samples. The objective of this study is to estimate a panelists' bias as a matrix and then apply it to correct their evaluations to give a better approximation of the panel average intensity of an attribute of a sample. Since this bias matrix can be used to correct the panelist's evaluations, the bias (representing the psychological and

physiological differences among panelists) can be used to correct the formal evaluations so that differences seen among the samples are mostly due to the inherent chemical and physical differences associated with the product under investigation.

Theory

For a sample of any material that is to be profiled through human sensory evaluation. We suppose that the sample is characterized by some positive integer $M$ attributes. Therefore any sample is associated with some $M$ dimensional vector *z*, whose individual entries are the values of the $M$ attributes for that sample. It is assumed that there will be issues with objectivity and reproducibility of evaluations as they are evaluated by humans with differing perceptions. For example, if the sample was wine, the attributes might include fruit aroma, floral flavor, sweet taste, astringent mouthfeel, and others. In this case $z = (z_1, z_2 \dots z_M)^T$ would be made up of a "true" value $z_1$ for the fruit aroma, a "true" value $z_2$ for the sweet taste, and so on for a particular sample. Obviously $z$ for any sample is unknown; the goal is to estimate it.

One obvious choice for estimating *z* involves choosing a panel of *K* human panelists, who assign values to the sample based on their own sensory perceptions. Each evaluation is some vector we write as $X_k$ for $k = 1,2 \dots K$. The vector $X_k$ is assumed to be a random variable describing a perception of the sample by the panelist.

Unfortunately, the perceptions of the panelists are necessarily biased. The panelists might have differing sensitivities to certain flavors or aromas; they might have psychological inclinations to underrate or overrate some or all attributes; or might have some temporary impairment of their senses (Meilgaard *et al.*, 2007). As a consequence, it is likely that $E(X_k) \neq z$ for any particular $k$, and again that the variance of the random variables $X_k$ is not uniform. This introduces an inevitable bias into the evaluations of the samples. This would not matter when the

mean for very large samples is used, but for small samples, it can be significant. In this study, an attempt is made to abstract that bias as a linear operator which can be estimated, and then use this to filter the evaluations by the panelists, simultaneously obtaining a good estimate of $z$ and also characterizing the biases of the individual panelists.

The vector $z$ is considered to be a unique fixed property of any particular physical sample which can neither be known nor measured. For the purpose of this study, it is assumed that $z$ is the mean of panelists' evaluations as the number of panelists becomes very large. Thus:

$$z = \lim_{K \to \infty} \frac{1}{K} \sum_{k=1}^{K} X_k$$

This limit exists by the law of large numbers. Note that $z$ is not a random variable; only $X_k$ is a random variable, with expectation $E(X_k) \neq z$. The definition for $z$ is thus, in some sense, an expectation of expectations.

To further elaborate on this point, perhaps one way to evaluate samples would be to ask one individual to judge the sample many times. That individual would probably evaluate the sample each day for 100 days. It is assumed that this would be a biased estimate $E(X_k)$ for that sample; e.g. that individual might not be capable of sensing some bitter taste, or might have color blindness, or some other issue that would make their evaluation differ from that of another individual. Instead, the characterization we want is necessarily a mean over the evaluations of a large number of individuals -- it is not a sample mean over trials of a single random variable, but rather a mean over trials of separate, non-identically independently distributed random variables. Here, a different unbiased estimator $A_k X_k$ is derived for an individual, based on that ensemble mean.

*The Bias Matrix:* Suppose there are $N$ separate samples to work with, leading to $N$ distinct attribute vectors $z$. It is assumed that $N \geq M$. A matrix whose columns are these attribute vectors known as $Z$ is formed. For each of these samples, some evaluation $W_{kn}$ by the individuals on the panel is made. These individual evaluations are the columns of a matrix $W_k$. Both $Z$ and $W_k$ are $MxN$. Let $B_k$ be some $MxM$ matrix (called the bias matrix) which maps $W_k$ to the actual values of the attributes for the samples; and solved for as follows:

$$minimize\left|\left|B_k W_k - Z\right|\right|_2$$

Unfortunately, both $Z$ and $B_k$ are not known

Let:

$$\bar{W} = \frac{1}{K}\sum_k W_k$$

Observe again that the law of large numbers implies that:

$$\lim_{K\to\infty} \bar{W} = Z$$

The goal is to estimate $B_k$ using the mean $\bar{W}$ of the matrices $W_k$. Physically, $B_k$ is an abstraction of the physiological and psychological biases the $k^{th}$ panelist brings to the evaluation, so that if we can estimate it, that will have value in itself.

First observe that $\left|\left|B_k W_k - Z\right|\right|_2$ is minimized when all its rows are orthogonal to all the vectors in the span of the rows of $B_k W_k$; i.e:

$$B_k W_k (B_k W_k - Z)^T = 0$$

$$\Leftrightarrow B_k W_k W_k^T B_k^T = B_k W_k Z^T$$

$$\Leftrightarrow B_k^T = (W_k W_k^T)^{-1} W_k Z^T$$

Thus, $\left|\left|B_k W_k - Z\right|\right|_2$ is minimized when $B_k = ZW_k^T(W_k W_k^T)^{-1}$ because $W_k W_k^T$ is a symmetric matrix.

However, since $Z$ is not known, the bias matrix can only be estimated using the mean: $A_k = \hat{X}W_k^T(W_kW_k^T)^{-1}$. Then $A_k$ is an estimate for $B_k$.

$$F_k = W_k^T(W_kW_k^T)^{-1}W_k.$$

The matrix $F_k$ is called the filtered matrix.

**Materials and Methods**

Materials

Tartaric acid, 6-propyl-2-thiouracil, tannic acid, alum, quinine sulfate were purchased from Sigma (St. Louis, MO, USA). Materials for standard recipe preparation for aroma and flavor evaluation included pure cane sugar (C&H sugar company, CA, USA), Kool-Aid (cherry artificial flavor, Kraft Foods Group Inc., Northfield, IL, USA), whole berries (black berries, raspberries and strawberries), cloves (McCormick & Co., Inc. Hunt Valley, MD, USA), iron tablets (65mg, Pharmavite LLC, Mission Hills, CA, USA) and olive brine (Safeway black olives, large, pitted, ripe, Pleasanton CA, USA), violet aroma (Wine Awakening Inc., Canada) and oak chips (Gusmer Enterprises, Inc., CA, USA). MilliQ water was obtained through purification (Millipore Corporation, Billerica, MA, USA).

Wine Samples

Twelve (12) wines were selected from a set of 61 wines purchased from local retail shops and analyzed for their physicochemical characteristics. The selected wines were a representative sample of the 61 wines (from Washington State and California) based on the agglomerative hierarchical clustering of the chemical characteristics. The ranges of the physicochemical parameters for these samples were: alcohol (12-16 %), pH (3.22-3.36), titratable acidity (0.47-0.753 g/100 ml), soluble solids (7.03-8.8°Brix), tannins (89-924 mg/l Catechin Equivalent [CE]), large polymeric pigments (0.312-1.448 Absorbance Units [AU]), small polymeric pigments

(0.734-1.211 AU), phenolics (93-293 mg/l CE), protein (49-106 mg/l) and mannoprotein (nd-231 mg/l Mannan Equivalence [ME](Diako et. al., in review)).

Trained Panel

Panelists (n=13) were recruited from the Washington State community through electronic advertisement. Previous training in wine or sensory evaluation was not a requirement for participation. The panel was composed of 54% males and 46% females with ages between 21 and 60. Most of the panelists (77%) were between 21-30 years while the rest were between 41 and 60 years. The wine consumption patterns of the panelists varied with most panelists consuming wine once to a few times a month. The panelists received minimum background information about the study to reduce potential bias and were simply informed they would be evaluating red wines over 12 training sessions followed by two formal evaluation sessions. The project was approved by the Washington State University Institutional Review Board for human subject participation. On the first day of training; all panelists signed an informed consent form and received nonmonetary incentive after each training and formal evaluation sessions.

Inconsistency in the evaluation of taste and mouthfeel compared to flavor evaluation of model solutions has been documented (Ott and Palmer, 1990). Because genetic and individual differences could account for this, the saliva flow rate and taster status as dictated by individual sensitivity to 6-n-propylthiouracil (PROP of each panelist were determined on the first day of training. PROP taster status was determined as previously described (Tepper *et al.*, 2001), as was saliva flow rate (Mialon and Ebeler, 1997).

The panelists were instructed on the techniques to use for the evaluation of color, aroma, flavor and taste of wines. The first three training sessions were devoted to building consensus and defining appropriate standards that defined the Merlot wines. Training was conducted

through presentation of standard solutions prepared in base wine (Livingston Red Rosé, Gallo, Modesto, CA USA). The recipes for the standards are presented in **Table 13**. In subsequent sessions, panelists were presented with these standard solutions to illustrate attributes, followed by the evaluation and subsequent discussion of commercial red wine samples. Panelists were gradually introduced to the different taste and mouthfeel attributes. For both training and formal evaluation, wine samples (25 ml) were pre-poured into ISO/INAO (International Standards Organization) tasting glasses and covered with petri dishes for one hour before tasting to allow for equilibration. The samples were labeled with three-digit codes and presented to panelists one at a time in a randomized serving order. Scores for each attribute were summarized using boxplots to provide a graphical representation of outliers. Both individual and panel means and standard deviations were obtained after each training session. Panelists who scored attributes much lower or higher than the overall panel mean were provided with additional training. Data from the taster status and saliva flow rate helped to better understand the rating trends of panelist for tailored feedback on performance.

Panelists rated the perception of intensity of six aroma and flavor attributes (artificial fruit, herbaceous, earthy, fruity, floral, woody and spicy), three taste attributes (sweet, sour, bitter) and three mouthfeel attributes (astringent, burning, metallic) along a 15-cm structured line scale anchored at 1.5 (low) and 13.5 cm (high). Samples were assigned three-digit codes and presented to the panelist one after the other in individual booths. Twelve samples were evaluated in two replicates over the three formal evaluation sessions, with 8 samples evaluated per session

**Table 13.** . Recipes and standards used in panel training for the sensory evaluation of samples.

| Category | Attribute | Preparation |
|---|---|---|
| Aroma | Artificial fruit | 1 ml Kool-Aid liquid (red fruit) in 20 ml base wine[a] |
| and flavor | Fruity | 2 ml of mixed fruit juice in 20 ml base wine [Mixed fruit juice: Blackberries(~ 25g), strawberries (~ 13.5g) and raspberries (~ 18.5g) crushed and strained through a cheese cloth] |
| | Woody | Three oak chips (~2.0g) in 10 ml deionized water + 5 ml base wine. Kept overnight at room temperature. 15 ml of base wine added prior to training |
| | Spicy | 3 whole cloves (~. 0.2g) soaked in 20 ml deionized water for 30 min. Ground black pepper (~ 0.1 g) added. After 10 min, 5 ml of this solution added to 20 ml base wine and allowed to sit overnight at room temperature. |
| | Herbaceous | 3 ml of olive brine added to 15 ml base wine prior to training |
| | Floral | 2 drops of violet aroma (Wine Awakening) to 100 ml deionized water. 1 ml of this solution was added to 50 ml of base wine |
| | Earthy | Freshly uprooted roots of backyard weeds |
| Taste | Sweet | 3.3% (w/v) cane sugar in base wine |
| | Sour | 0.3% tartaric acid in base wine |
| | Bitter | 0.001% (w/v) quinine sulfate in base wine |
| Mouthfeel | Astringent | 0.78 g tannic acid + 0.35g alum in 300 ml base wine |
| | Burning | 60 ml 95% proof ethanol in 240 ml base wine |
| | Metallic | 8 iron tablets (~ 3.0 g) dissolved in 300 ml base wine and filtered |

[a]Red Rosé, Livingstone Cellars, Modesto, CA.

in a completely randomized design.

Panelists were required to rest for 1 min between samples, with a 10-min forced break after the fourth sample to refresh their palate and minimize fatigue. Panelists were provided with crackers and distilled water for palate cleansing. All instructions, scale presentations, and data collection were carried out using Compusense *five*, release 5.2 (Guelph, Canada).

**Results and Discussion**

One of the methods for tracking panelist performance is through visualization of the raw data (Meullenet *et al.*, 2007). Such graphical techniques provide a general overview of the relationship among panelists, attributes and samples. For example, the plot of the evaluation of herbaceous aroma of the wines shows some agreement among panelists and also with the panelists' average, but on the whole, some problems still exist with uniformity of evaluations among panelists (**Figure 9A**). The evaluation of wine 11 was the most consistent among panelists except for two panelists who rated it at a higher intensity. As seen from the results, panelists' evaluations still vary, with panelist and product differences observed. A similar graphical technique used in sensory profiling which allows comparison among panelists is the eggshell plot (Hirst and Næs, 1994; Lea *et al.*, 1995; Naes, 1998; Kermit and Lengard, 2005; Tomic *et al.*, 2007). Eggshell plots graphically represent and compare panelists' ranking results with the panel average. Although this approach handles ranked data, the similarity with **Figure 9A** is that both allow the evaluation of panelist performance compared to the overall panel average.

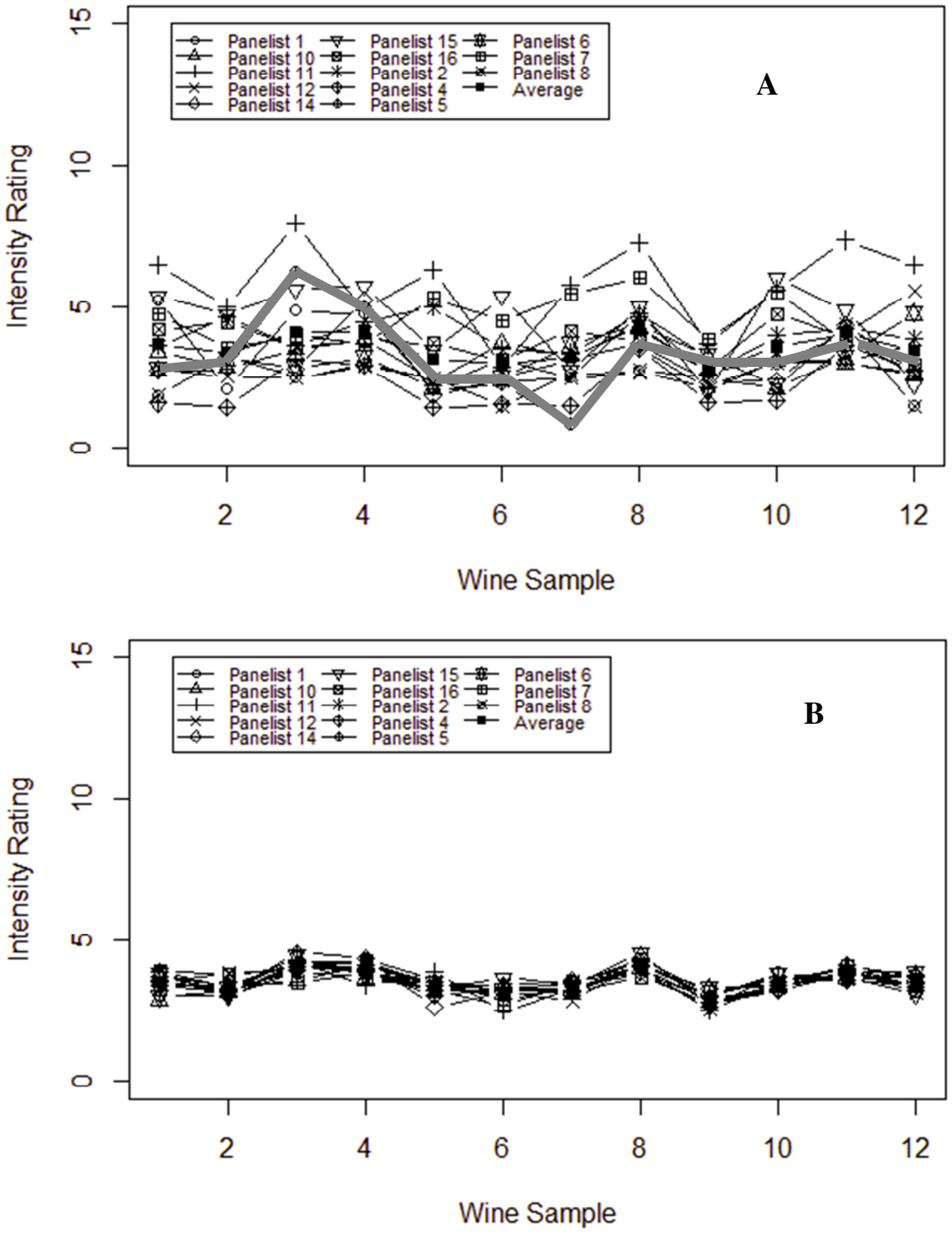


**Figure 9.** Intensity evaluation of herbaceous aroma of 12 commercial Merlot wines (n=13). (A) Panelists' unfiltered evaluations with Panelist 5 highlighted **(B)** Panelists' filtered evaluations after applying bias matrix.

Given that there are still some variations in the evaluations of the panelists, there are likely still some effects due to individual differences arising from the panelists' physiological and psychological dispositions. In descriptive analysis, panel performance is directly related to training time. Chambers *et al.* (2004) observed that training a panel for 120 hours increased the discrimination capabilities of the panelists through improved accuracy and precision of the panel leading to reduction of variability in the results. Munoz (2008) outlined time requirement for a descriptive trained panel as dependent on the type of descriptive method (free choice, QDA, flavor or texture profiling), type of panel (universal or product specific), type of category (complex or simple), number of attributes to profile and the required level of training (semi- or well-trained). Clearly, there is no simple means of improving panel performance. However, in this study, each panelist's bias (presenting individual variations which will take time to reduce) was computed and used to correct their original evaluations, thus leading to more consistent evaluations among panelists (**Figure 9B**). These evaluations were tightly clustered around the average, making the differences among the samples more related to the physico-chemical composition of the samples. Wines 4 and 8 had the highest overall average for herbaceous character while sample 9 had the least.

Using panelist 5 as a case study and taking another look at the herbaceous aroma evaluations, the ratings of panelist 5 are highlighted (**Figure 9A**). Obviously, panelist 5 had a very high rating for the perception of herbaceous aroma for samples 3 and 4 while having a low intensity rating for sample 7. The bias matrix is a $pxp$ square matrix of the attributes with diagonal entries ≤1.0 and some high off-diagonals. The bias matrix for panelist 5 is shown in **Table 14**. The columns are the evaluations for panelist 5 while the rows represent the average evaluation of the entire panel.

**Table 14.** Bias matrix for Panelist 5 for the evaluation of aroma of commercial Merlot wines (n=12).

| | | Evaluations of Panelist 5 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Attributes** | **Artificial Fruit** | **Herbaceous** | **Earthy** | **Fruity** | **Floral** | **Woody** | **Spicy** |
| **Average panel evaluations** | **Artificial Fruit** | **-0.006** | 0.117 | 0.209 | 0.254 | 0.196 | 0.166 | -0.061 |
| | **Herbaceous** | -0.484 | **0.371** | -0.239 | 0.754 | -0.115 | 0.812 | 0.058 |
| | **Earthy** | -0.410 | 0.207 | **-0.139** | 0.562 | 0.006 | 0.668 | 0.068 |
| | **Fruity** | -0.496 | 0.034 | 0.025 | **0.835** | 0.131 | 0.561 | 0.110 |
| | **Floral** | -0.304 | 0.252 | 0.034 | 0.543 | **0.272** | 0.301 | -0.211 |
| | **Woody** | -0.235 | 0.248 | -0.088 | 0.502 | 0.130 | **0.570** | -0.177 |
| | **Spicy** | -0.138 | 0.140 | 0.078 | 0.317 | 0.129 | 0.363 | **-0.149** |

A panelist with whose evaluations agree perfectly with the entire panel would have a bias matrix that would look like an identity matrix (a matrix with zeros off diagonals and ones on the diagonal). In the case of panelist 5, the diagonal entry for herbaceous was 0.371. As this was less than 1.0, some deficiency in the evaluation of herbaceous aroma as compared to the overall panel average was revealed. If the evaluation of panelist 5 for herbaceous aroma was more closely aligned to the panel average, this diagonal entry would be very close to 1.0. The off-diagonals for the herbaceous row showed high entries for fruity aroma (0.754), woody aroma (0.812) and a negative entry for artificial fruit (-0.484). These off-diagonal entries showed that panelist 5 was confusing herbaceous aroma for fruity and woody, and also evaluated herbaceous aroma as the absence of artificial fruit aroma.

Another piece of information the bias matrix showed was the performance of the panelist with respect to each of the attributes being rated. From the Table 4, panelist 5 was observed to be very good at perceiving the fruity aroma of the wines as seen from the diagonal entry of fruity aroma (0.835). The woody evaluation for panelist 5 was fairly good (0.570). This bias matrix is represented graphically as a figure with no prominent diagonal (**Figure 10A**). However, when the bias matrix was used to filter the panelist evaluation, there was a strong agreement between panelist 5's evaluations and the mean panel evaluations, revealed in a distinct diagonal for herbaceous aroma for the samples (**Figure 10B**).

The higher agreement between the individual panelists' evaluation and the entire panel average as a result of the application of the bias matrix to filter the evaluations led to an increased likelihood of observing differences among the products, which were due to the physico-chemical characteristics of the samples, while reducing the effect of assessor variations.

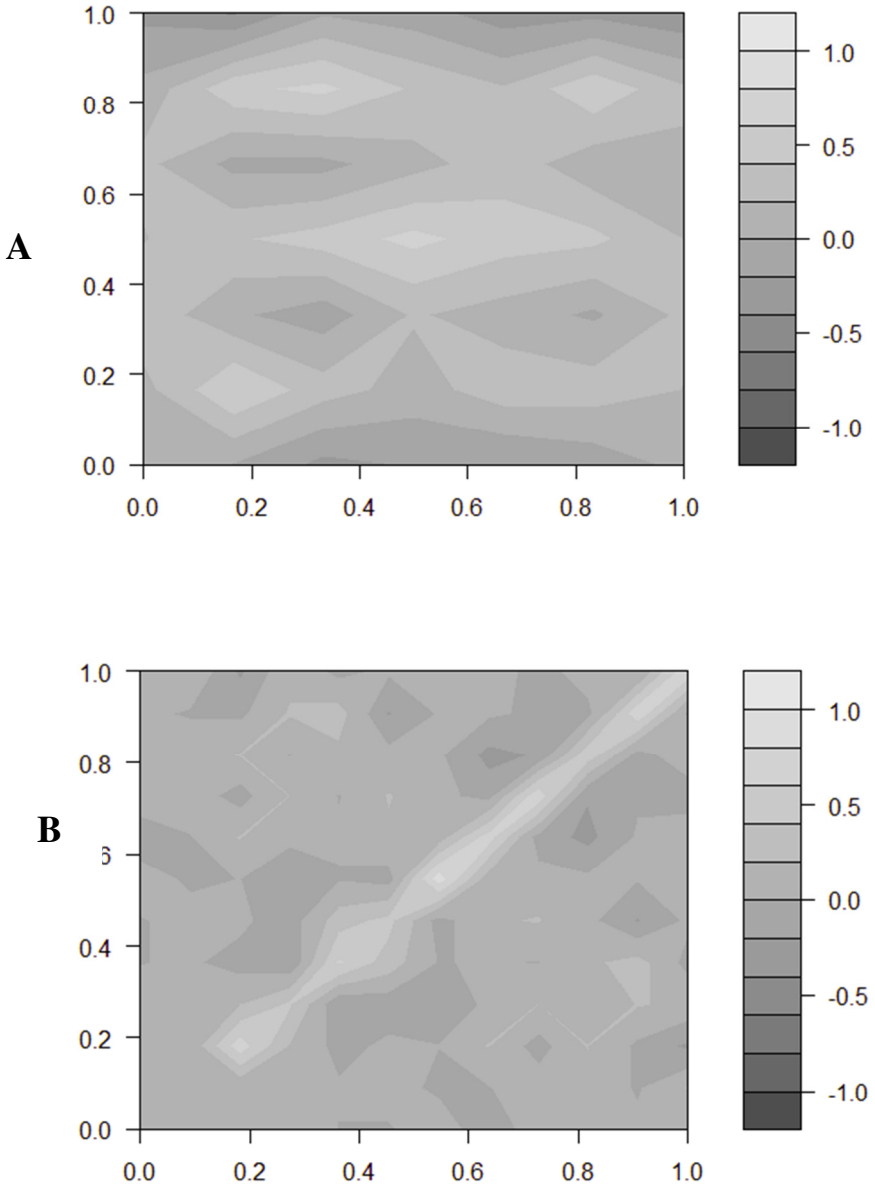


**Figure 10.** Graphical representation of the **herbaceous aroma** matrices of Panelist 5. (**A**) Bias matrix with no prominent diagonal. (**B**) Filtered matrix showing a prominent white diagonal indicating agreement with overall panel means for each panelist.

These graphs and matrices can be generated for each panelist after each training session to provide verbal, written, individual and group feedback to enhance panel training.

No significant differences were noted in the filtered and unfiltered averages for the aroma attributes of sample 1 as perceived by the 13 assessors constituting the panel ($p>0.05$) (**Table 15**). The expectations of the unfiltered means and those of the filtered were therefore not statistically significantly different. This meant that filtering the evaluations with the bias matrix did not significantly affect the original averages of the evaluations from the trained panel and hence there was no loss of information by filtering the original evaluations of the panelists with the bias matrix. However, since the noise in the data is being reduced through this process, it is expected that the differences observed among samples will be more related to the sample differences.

The spectral radius of the bias matrix is the direction in which a panelist's bias is being corrected for the overall panel evaluation. A spectral radius of 1 meant the panelist's evaluation was on target for the overall panel evaluation. A radius higher than 1 meant that the panelist was below the panel average and hence there was an upward adjustment to correct for that deficiency and vice-versa. **Table 16** shows the inverses of the spectra radii of the panelist for wine attribute categories and the penchant of panelists to underrate or overrate attributes. The ratings of panelist 7 were always on the high end for all the attribute categories. Generally, more panelists rated taste and mouthfeel attributes higher than the aroma and flavor attributes. Panelist 15 had challenges distinguishing among the aroma attributes leading to dependence on some of the aroma attributes. This panelist indicated the intensity of one or more aroma attribute as some multiple of others leading to rank deficiency. This panelist needs some more feedback to help with subsequent sample evaluation.

**Table 15.** Two sample T-test for bias corrected (filtered) averages and averages from raw (unfiltered) for the aroma attributes of Sample 1 for all panelist (n=13).

| **Attribute** | **P-value** |
|---|---|
| Artificial fruit | 0.929 |
| Herbaceous | 0.632 |
| Earthy | 0.967 |
| Fruity | 0.651 |
| Floral | 0.899 |
| Woody | 0.890 |
| Spicy | 0.993 |

**Table 16.** Inverse of spectral radii for panelists' bias matrices indicating their tendency to over-estimate or under-estimate wine attributes compared to overall panel (n=13).

| Panelist No. | Attribute Category [a] | | |
|---|---|---|---|
| | **Aroma** | **Flavor** | **Taste and Mouthfeel** |
| 1 | 0.92 | 0.62 | 0.57 |
| 2 | 0.77 | 0.45 | 0.95 |
| 4 | 0.87 | 0.47 | 1.26 |
| 5 | 0.86 | 0.83 | 0.68 |
| 6 | 0.95 | 0.91 | 0.97 |
| 7 | 1.46 | 1.22 | 1.48 |
| 8 | 0.10 | 0.55 | 0.65 |
| 10 | 0.81 | 0.69 | 0.29 |
| 11 | 1.38 | 2.22 | 0.93 |
| 12 | 1.11 | 0.84 | 1.02 |
| 14 | 0.92 | 0.93 | 1.10 |
| 15 | 0.95 | Rank deficient bias matrix[b] | 0.99 |
| 16 | 1.44 | 0.84 | 1.11 |

[a]Each category is made up of a number of attributes. Aroma and flavor have 7 attributes each while taste and mouthfeel has 6 attributes.

[b] The intensity ratings of panelist 15 are interdependent (*i.e.* the bias matrix is not full rank). The panelist has difficulty distinguishing among the flavor attributes.

The utility of this approach to analyze trained panel data was extended to predictive filtering. In this process, a given number of samples was evaluated and the bias matrices of the panelists were computed as before. These biases were then used to predict the attributes of a new sample for each panelist. For this study, one replicate of evaluations was used to compute the bias matrices for the panelists and for 11 samples. The bias matrix was then used to filter the second replicate of the $12^{th}$ sample which was being treated as a future sample. The application of this approach in sensory studies is that panelists' bias matrices can be computed after a few training sessions. This is then followed by formal evaluations. The bias matrix could then be used to filter the formal evaluations to give final results. This analysis would be as good as training the panel over an extended period based on the assumption that the bias-corrected evaluations are the best approximation of the expected attribute intensity ratings for the samples. **Figures 11A** and **11B** illustrate this concept. Samples 1 and 5 were used to show the possibility of predictive filtering for panelist 5. In both of these examples, the predictively filtered values were closer to the average of the panelist than what were the individual predicted unfiltered values.

Depending on the objective of the panel training program, one needs a few hours to 120 hours (Wolters and Allchurch, 1994; Chambers *et al.*, 2004), or up to six months (as cited by (Munoz, 2008)) to increase the number of attributes that panelists can discriminate among, reduce replicate effect and hence increase the precision and accuracy of the panel. Despite all the effort put into panels to reduce panelists effect, the hope to eliminate the ever-present panelist effect is debatable (Moscowitz, 2008). The fact that predictive filtering gives individual ratings closer to the panel average shows the possibility of having shorter training times since the

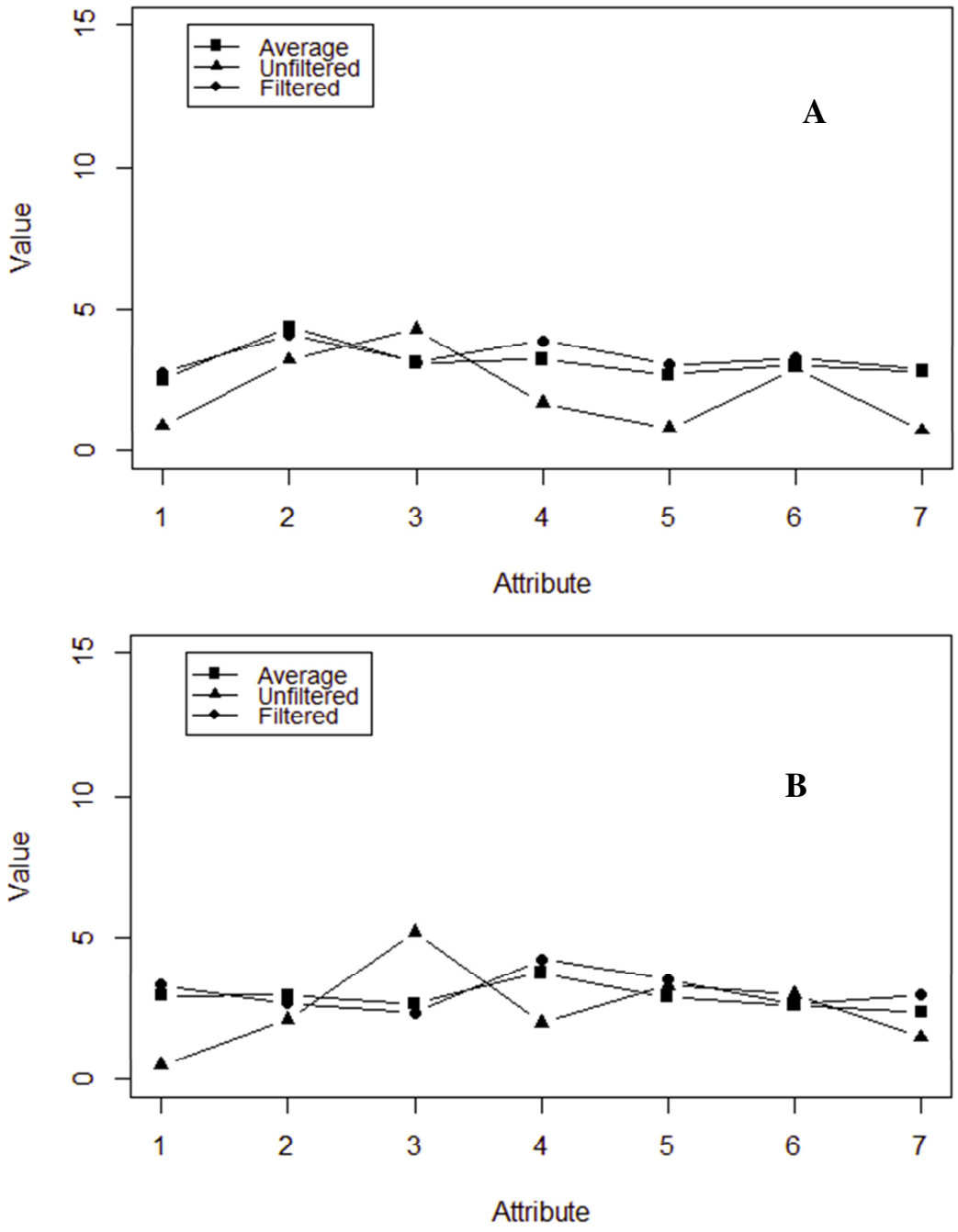


**Figure 11.** Predictive filtering of samples showing strong agreement between filtered evaluations and panel mean evaluation for seven attributes (artificial fruit, herbaceous, earthy, fruity, floral, woody and spicy respectively) of commercial Merlot wines. (**A**) Sample 1. (**B**) Sample 5

.

panelists' bias matrices can be used to correct for future evaluations within that panel and for the products being evaluated.

**Conclusions**

The results from the present study shows the possibility of abstracting a panelist's bias as a linear operator in a wine trained panel and using the estimated bias to correct their evaluations for a better approximation of mean ratings. A well-trained panel is still required as this produce a better mean $\overline{W}$, and should improve the condition of $W_k W_k^{\mathrm{T}}$. It is important that the standards used to illustrate the attributes during training are well selected to reflect sample attributes as this will improve the individual ratings of samples and will more likely produce well-conditioned $W_k W_k^{\mathrm{T}}$. In addition, the larger the panel, the better. While filtering gives us a means of "correcting" for biases of individuals, this correction is completely dependent on $\overline{W}$ being a good estimate of *Z,* which requires a good panel size. Analysis of $A_k$ for each panel member might assist in the training process. $A_k$ can indicate confusion among attributes, negative correspondences (e.g. herbaceous = absence of artificial fruit) and other effects that would otherwise be more difficult to identify and coach out of the panel. A possible advantage of this approach to panel monitoring is the possibility of spending less time on training. With two or three well-coached training sessions using the $A_k$ matrix, followed by one evaluation session to determine $W_k$, one could correct for whatever biases exist.

**Literature Cited**

Brockhoff, P. B. (2003). Statistical testing of individual differences in sensory profiling. *Food Quality and Preference, 14*, 425–434.

Chambers, D. H., Allison, A. A., & Chambers, E. I. V. (2004). Training effects on performance of descriptive panelists. *Journal of Sensory Studies, 19*, 486-499.

Cliff, M. A., & King, M. (1999). Use of principal component analysis for the evaluation of judge performance at wine competitions. *Journal of Wine Research, 10*(1), 25-32.

Derndorfer, E., Bairel, A., Nimmervoll, E., & Sinkovits, E. (2005). A panel performance procedure implemented in R. *Journal of Sensory Studies, 20*, 217-227.

Dijksterhuis, G. (1995). Assessing panel consonance. *Food Quality and Preference, 6*, 7-14.

Findlay, C. J., Castura, J. C., Schlich, P., & Lesschaeve, I. (2006). Use of feedback calibration to reduce the training time for wine panels. *Food Quality and Preference, 17*, 266-276.

Hirst, D., & Næs, T. (1994). A graphical technique for assessing differences among a set of rankings. *Journal of Chemometrics, 8*, 81-93.

Kermit, M., & Lengard, V. (2005). Assessing the performance of a sensory panel – panelist monitoring and tracking. *Journal of Chemometrics, 19*, 154-161.

King, M. C., Hall, J., & Cliff, M. A. (2001). A comparison of methods for evaluating the performance of a trained sensory panel. *Journal of Sensory Studies, 16*, 567-581.

Latreille, J., Mauger, E., Ambroisine, L., Tenenhaus, M., Vincent, M., Navarro, S., & Guinot, C. (2006). Measurement of the reliability of sensory panel performances. *Food Quality and Preference, 17*, 369-375.

Lea, P., Rodbotten, M., & Naes, T. (1995). Mesuring validity in sensory analysis. *Food Quality and Preference, 6*, 321-326.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory Evaluation Techniques* (4th ed.). Boca Raton: CRC Press, Taylor and Francis Group.

Meullenet, J.-F., Xiong, R., & Findlay, C. J. (2007). *Multivariate and Probabilistic Analyses of Sensory Science Problems*. Iowa, USA: Blackwell Publishing.

Mialon, V. S., & Ebeler, S. E. (1997). Time-intensity measurement of matrix effects on retronasal aroma perception. *Journal of Sensory Studies, 12*(4), 303–316.

Moscowitz, H. R. (2008). Product and panelist variabilty in sensory testing. In H. R. Moscowitz, A. M. Munoz & M. C. Gaculas (Eds.), *Viewpoints and Controversies in Sensory Science and Consumer Product Testing* (pp. 375-378). Hoboken, NJ, USA Wiley-Blackwell.

Munoz, A. M. (2008). Training time in descriptive analysis. In H. R. Moscowitz, A. M. Munoz & M. C. Gaculas (Eds.), *Viewpoints and Controversies in Sensory Science and Consumer Product Testing* (pp. 351-356). Hoboken, NJ, USA: Wiley-Blackwell.

Naes, T. (1998). Detecting individual differences among assessors and differences among replicates in sensory profiling. *Food Quality and Preference, 9*(3), 107-110.

Ott, D. B., & Palmer, U. J. (1990). Ingestion and expectoration sampling methods of four tastes in a model system using time-intensity evaluations. *Journal of Sensory Studies, 5*(1), 53–70.

Rossi, F. (2001). Assessing sensory panelist performance using repeatability and reproducibility measures. *Food Quality and Preference, 12*, 467–479.

Stone, H., Bleibaum, R. N., & Thomas, H. A. (2012). *Sensory Evaluation Practices* (4th ed.). USA: Academic Press.

Tepper, B. J., Christensen, C. M., & Cao, J. (2001). Development of brief methods to classify individuals by PROP taster status. *Physiology & Behavior, 73*(4), 571–577.

Tomic, O., Forde, C., Delahunty, C., & Næs, T. (2013). Performance indices in descriptive sensory analysis – A complimentary screening tool for assessor and panel performance. *Food Quality and Preference, 28*, 122–133.

Tomic, O., Nilsen, A., Martens, M., & Næs, T. (2007). Visualization of sensory profiling data for performance monitoring. *LWT, 40*, 262–269.

Wolters, C. J., & Allchurch, E. M. (1994). Effect of training procedures on the performance of descriptive panels. *Food Quality and Preference, 5*, 203-214.

# CHAPTER VI

## CONCLUSIONS AND RECOMMENDATIONS

The influence of the interactions of key wine matrix components on the sensory and chemical quality of wines was studied. In the first study, ethanol and tannin profiles were identified as the major physicochemical parameters responsible for the main differences among the commercial Merlot wines surveyed. Here, the interactions among these chemical parameters and mannoproteins significantly affected the perception of aroma, flavor, tastes and mouthfeel attributes of the wines. Strong correlations were observed between the electronic tongue response and human taste response, indicating a high potential of the electronic tongue for being used to complement sensory evaluation in the assessment of wine quality. It is recommended that interactions be determined in different varietals to provide information about the nature of these interactions in other wines. This study has shown enhancement or suppression of aroma notes in wines as a consequence of matrix interactions. Further studies should be conducted to provide insight into the reasons for these enhancement or suppression of the perception of aromas and flavors as dictated by molecular level association between odorants and wine matrix components.

Beyond its use as a complementary method in sensory evaluation of wines, the use of the electronic tongue as an independent or stand-alone rapid method in wine quality evaluation was explored in the second study. Its discriminatory ability and the non-linear dependence of its response on the matrix components were elucidated in this study. The e-tongue was able to discriminate individually among wines from different wineries. This discrimination was accomplished based only on their characteristic response from the e-tongue using support vector machines, without the need to separate them into broad groups. Furthermore, the relationship

between the chemical composition and the output of the electronic tongue is complex and while multiple regression could predict the relationship, the relationship was more successfully predicted using artificial neural networks. Given the ease of sample preparation and high throughput of the electronic tongue, future studies should investigate quantification of wine chemical parameters to develop rapid methods for wine quality evaluation and optimization.

Finally, the third study looked at how to improve panelists' intensity ratings of wine attributes during a trained panel. The objective of training is to reduce panelists' variability so that the sensory verdict will, as much as possible, be a reflection of the profiles of the samples under investigation. This study showed that panelists' biases can be estimated and used as a monitoring tool or used to adjust the ratings of the sensory panel. Bias matrix estimation can be useful in shortening training time of sensory evaluation panels and allowing for the evaluation of more samples. With a few well executed training sessions, the panelists' biases can be estimated and used to correct for any variations in their evaluations. Future studies should explore using the same panel for a short training session using the bias matrix correction and a long training session for comparison.