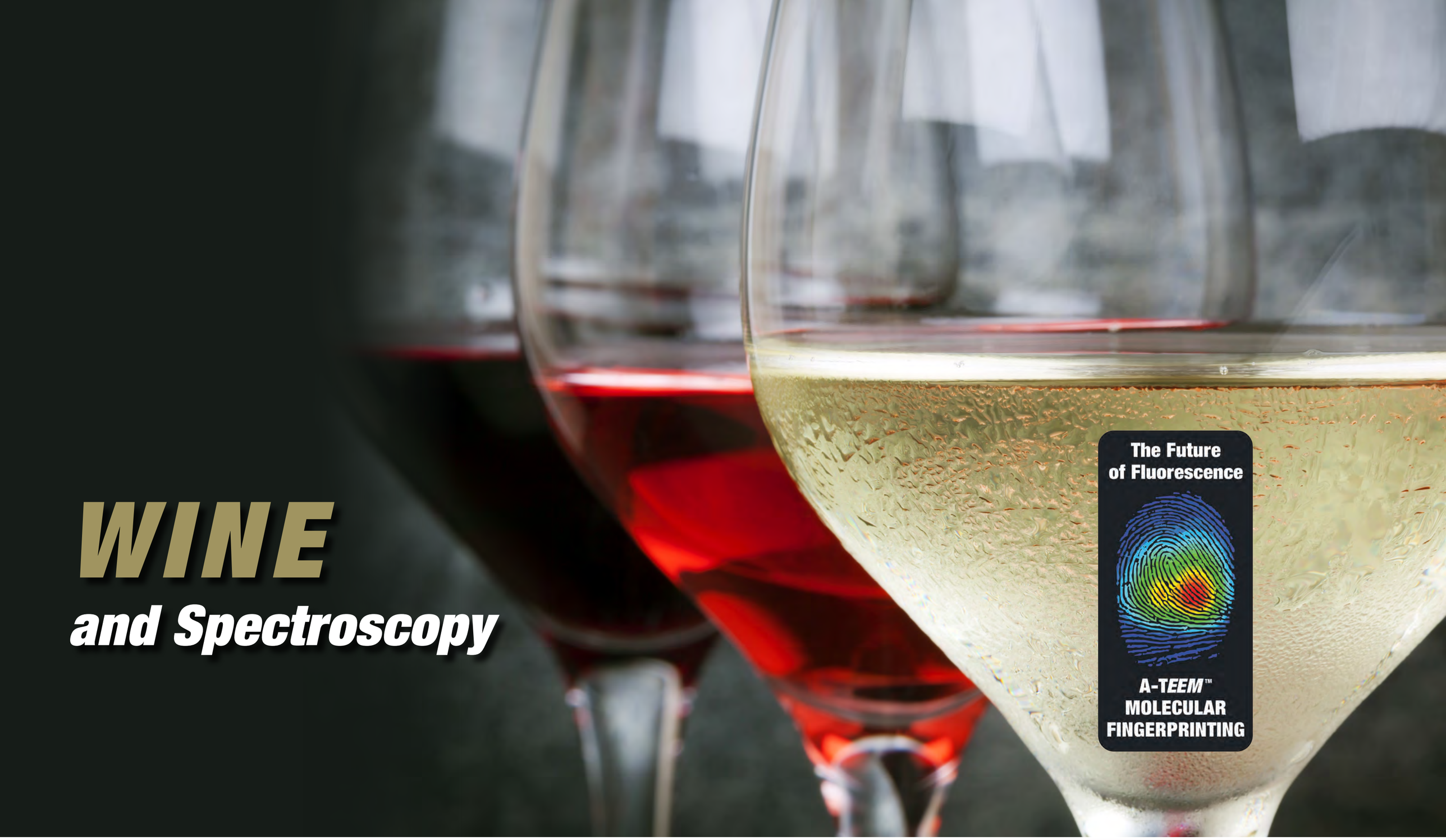
WINE
and Spectroscopy



Introduction to Wine and Spectroscopy

The wine industry has long employed analytical tools to characterize grapes and the finished product. Yet the most popular methods, including chromatography, are slow and costly.

The wine industry has long employed analytical tools to characterize grapes and the finished product. Yet the most popular methods, including chromatography, are slow and costly. Growers, producers and those in the distribution chain need inexpensive, rapid analytical methods to test for authenticity, quality assurance, and developing needs caused by the global spread of wildfires.

In this e-book, we have assembled a number of stories and research papers that delve into the challenges faced by the wine industry, with the newest techniques to overcome these issues. And, our applications scientists are always available to answer your questions and discuss these latest techniques.

Thank you for downloading this e-book. We hope you find it useful and informative.

There are a lot of quality characteristics that the winemakers are interested in... compounds that affect the wine's taste, color and mouthfeel.

Fine wine-making with the help of HORIBA tech

Take a typical Chardonnay. It's rich, full-bodied and buttery.

Those qualities don't happen by accident. It's the combination of a variety of ingredients; and controlling those ingredients could be the difference between a celebratory toast and a glass of vinegar.

It's also the reason that testing the grape harvest is so important as the grapes mature. But it's been a laborious, expensive process. Most wineries have multiple brands and growing fields. Each needs to be monitored for the ingredients in the grape that will give it the desired color, flavor and mouthfeel.

Equipment constraints

Wineries typically send out samples of the grapes to analytical labs to be tested on costly, hard to maintain equipment. The traditional devices are slow, and since so many samples must be run to monitor multiple harvests, it becomes an expensive and time-consuming process.

"Primarily, it's a matter of cost, time and flexibility in terms of overall characterization of the wine," HORIBA Scientific's Aqualog Product Manager, Adam Gilmore, Ph.D., said.

"The conventional methods that most laboratories employ are things like Gas Chromatography-Mass Spectrometry, Liquid Chromatography-Mass Spectrometry, and Fourier Transform Infrared Spectroscopy. Those types of equipment are quite expensive. There are significant difficulties in terms of calibrating the systems and maintaining calibration, and transferring calibrations to field locations and places where analysis is very important."

And there's always a significant work overload in terms of characterizing the grapes scientifically as they're being delivered or prepared for wine making. It's a critical period in the winemaking process.

There are a lot of quality characteristics that the winemakers are interested in that relate to the color of the wine and the phenolic content – compounds that affect the wine's taste, color and mouthfeel. Mouthfeel is usually referred to as silky, smooth, velvety and rough.

All of these characteristics, including the pH and sugar content, are routinely evaluated by instruments for each batch of grapes. The intention is to have a survey that's representative of the particular vineyard and different locations in the vineyard.

It takes between 35 and 90 minutes to analyze a single sample in an independent laboratory using the conventional methods to fingerprint the grapes. And it can cost hundreds of dollars per sample.

A typical small winery would like to test each wine field location many times during the veraison, the wine making season, and the large wine companies have many different vineyards from which they are collecting grapes. Thus, it's clearly expensive for most small vineyards, let alone very large vineyards and wine companies to have to manage a comprehensive characterization of all the key parameters.

The next innovation

HORIBA Scientific recently patented an instrument called the **Aqualog®**, which makes this process faster and less costly.

"With the Aqualog, you can actually collect the entire composition of all the colored and phenolic compounds," Gilmore said. "And the acquisition time is roughly around 30 seconds. Further, in less than a minute, the analysis can also be fully automated in terms of the phenolic identity and concentration."

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Fine wine-making with the help of HORIBA tech, cont.

The Aqualog is an instrument that acquires Absorbance, Transmittance and the fluorescence Excitation-Emission Matrices (A-TEEM) simultaneously. EEMs are acquired up to 100 times faster than with conventional scanning PMT-based fluorescence instruments. The Aqualog was initially developed for water treatment facilities, but its portability, speed, functionality, flexibility, and cost are now appealing to several world-renowned winemakers around the globe. These winemakers have begun to adopt the technology, especially since its portability and cost allow it to be deployed in multiple locations.

Decentralization and speed

Most big wineries are dealing with multiple vineyards, often in different regions. Each region may have a laboratory that can operate the Aqualog for quality control, as opposed to sending samples to one central internal or external contract laboratory. “It’s really about speed. Clearly we can do it much faster,” Gilmore said. “It's a solid state instrument that's fully externally validated with NIST traceability. Laboratories can thus establish multiple units that have matching calibrations, and they can have these at convenient field locations, as opposed to having to send everything to another laboratory and wait for results.”

Phenolic information highway

How is all this characterization information used?

Generally, winemakers have something akin to laboratory information management systems (LIMS). It catalogs this fingerprinting information, and statisticians and winemakers evaluate the data to predict the flavor characteristics, storage, stability and blending operations.

The information yielded by the grape analysis is often immediately relayed to the growers to advise them when to harvest, or hold off. It’s also used by the winemakers in terms of final processing, quality control and assurance, blending, bottling, and storage.

“The HPLC (high-performance liquid chromatography) and LC (liquid chromatography) are really the primary discriminatory methods for identifying the different phenolics and anthocyanins,” he said. “But day in and day out, for day-to-day operations, it's very difficult to train somebody in the field, or in a remote laboratory, how to maintain this (HPLC and LC) type of equipment.”

The Aqualog uses fluorescence, which produces orders of magnitude higher sensitivity compared to UV-Vis absorbance. And it also produces a molecular fingerprint in the form of the Excitation-Emission Matrix. Analysis of the A-TEEM data separates the spectrum in terms of the emission energy, as well as the absorption, which gives the user the ability to identify and create libraries with wide varieties of chemicals.

If a grape sample doesn’t meet the standards of its particular brand, it might influence a blending plan. The grape would go into another type of wine or another blend of something else the winery was making. Grapes are rarely thrown away.

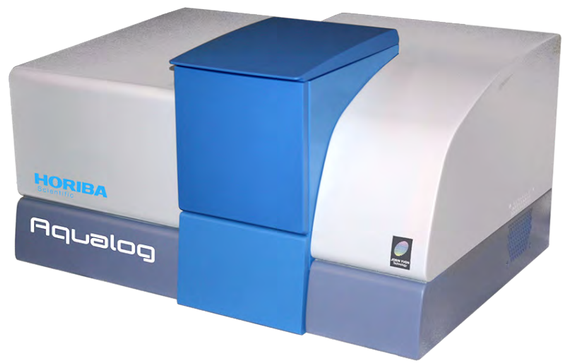
Sampling

The rate of sampling with the Aqualog is very fast.

“We can run hundreds of samples a day with the Aqualog,” Gilmore said. “They can be working in shifts around the clock.”

Wineries are finding out they can’t keep up with the samples with the costlier analytical tools, or get the discriminatory phenolic profiles they want with simpler equipment such as UV-Vis absorbance.

“With the Aqualog, it’s a very simple standard operating protocol,” he said. “It can be automated with our auto-sampling systems. We can fully automate the data collection and analysis, and we can generate complete profile reports.”



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“With the Aqualog, we've been able to classify the wine according to its geographical origin with 100 percent accuracy.”

Study validates fluorescence spectroscopy with A-TEEM for fast and precise wine authentication

Rudy Kurniawan considered himself a bon-vivant, ingratiating himself in the exclusive world of wine connoisseurs. The twenty-something collector graciously shared expensive vintages with other devotees, all the while selling millions of dollars of supposed rare wines to unsuspecting collectors.

By 2012, the bubble had burst. The FBI raided his wine cellar and discovered Kurniawan blended cheaper wines, old corks and aged labels to carry out his deception. In 2018, he achieved the dubious distinction of becoming the first person in the U.S. to be convicted of wine fraud, and received a 10-year sentence.[i]

In 2017, leading authority Maureen Downey estimated $3 billion worth of the wine market is usurped by fake wines globally.[ii] Labeling and packaging are usually the most obvious signs, but since some vintages can sell in the tens of thousands of dollars, fraudsters have become more sophisticated

Enter the world of food science, and a group of researchers working out of the University of Adelaide in Adelaide, South Australia. They set out to apply pioneering instrumentation and advanced chemometrics to identify wines by their chemical composition.

Ruchira Ranaweera, MSc, led the project as part of her doctoral curriculum, joined by researchers Dimitra L. Capone, Ph.D.; Susan E Bastian, Ph.D.; David W. Jeffery, Ph.D. and HORIBA Applications Scientist, Adam M. Gilmore, Ph.D.

The aim of the study, titled *Authentication of the geographical origin of Australian Cabernet Sauvignon wines using spectrofluorometric and multi-element analyses with multivariate statistical modelling,* [iii], was to see if fluorescence spectroscopy, using HORIBA’s proprietary A-TEEM technology, and a novel use of a multivariate algorithm, could effectively and economically identify a number of wine samples produced from various regions in Australia.

“Wine is very susceptible to fraud because it has a very complex chemical composition,” Ranaweera said. “So it's so difficult to identify certain adulterations. If we can authenticate the geographical origin or the varietal origin of the wine, then we can give that product or the brand more value. That’s the economic side.”

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Study validates fluorescence spectroscopy with A-TEEM for fast and precise wine authentication, cont.

David Jeffery, Ph.D., Associate Professor in Wine Science at the University of Adelaide. Courtesy of David Jeffery.

"Wine is a luxury good," David Jeffery, Associate Professor in Wine Science at the University of Adelaide said. "So there's a lot of value in wine. And usually any highly valuable food product is open to fraudulent activity because there's a lot of money that can be made by taking something that's much cheaper that maybe looks like the product, and selling it for something that's much more expensive. It's a global issue."

Wine components

Elements are often studied in wine, along with isotopes, volatile compounds and phenolics.

"Those compositional aspects have an influence on the sensory properties of wines from different regions, as well," Jeffery said. "Phenolics drive color, mouthfeel, and taste and aroma compounds can originate from the berry, but yeast introduces some of its own, which relates to the amino acid profile of the grapes, as well. Together, these aspects feed into characteristics that people are looking for in terms of wine sensory profiles. So regional wines, even if they're the same variety, can have different characteristics, depending on where they come from, and we'd like to understand that. In fact, authenticity is one thing, but what we really want to get to is what's underpinning that, in terms of the chemical markers."

The researchers intended to use the underlying chemical markers, even if not identified as yet, to classify wines that are unique to different regions of Australia, in comparison to wines from Bordeaux, the birthplace of Cabernet Sauvignon. Scientists have typically used inductively coupled plasma-mass spectrometry (ICP-MS) to analyze the elements in the wine. So Jeffery and the team chose that method to compare with A-TEEM fluorescence spectroscopy molecular fingerprinting.

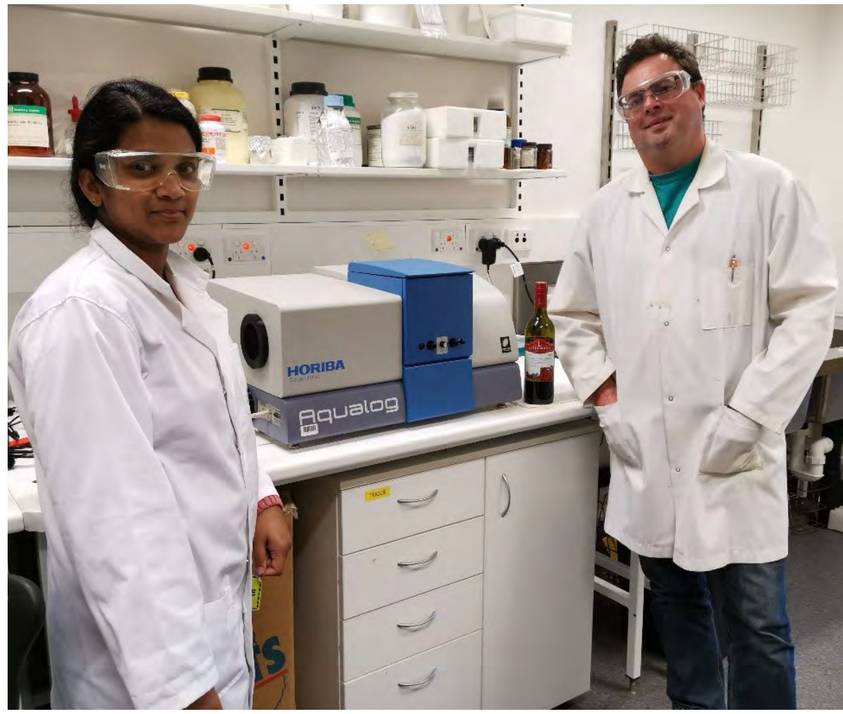
A-TEEM can quickly and simultaneously acquire Absorbance, Transmittance and a fluorescence Excitation Emission Matrix, and automatically corrects for the Inner Filter Effect. A-TEEM is exclusively featured on HORIBA's Aqualog and Duetta spectrofluorometers.

Rapid molecular fingerprinting

The aim of the project was to identify the unique characteristics that are inherent to Australian regions. The main idea was to identify a method that gave rapid results, and was relatively simple to implement. To achieve that, the researchers were looking for some chemical markers, using the two above-mentioned methods.

"I think it is really important, not only for the lab analysis, but if a method could be used in the supply chain," Ranaweera said. "So an instrument which is very user friendly and gives rapid results with high sensitivity provides an advantage."

The two analytical methods were used with commercial Cabernet Sauvignon wines from vintage 2015, originating from three wine regions of Australia, along with Bordeaux, France[iv], as a benchmark for the study. All told, the researchers sampled dozens of wines (iv) much of which was donated by Australian producers. Hundreds of EEMs were acquired with the Aqualog during the study. The A-TEEM method proved to be remarkably accurate for classification, meeting the researchers' greatest expectations.



Ruchira Ranaweera and David Jeffery in their lab with a HORIBA Aqualog. Courtesy of David Jeffery.

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Study validates fluorescence spectroscopy with A-TEEM for fast and precise wine authentication, cont.

“With the Aqualog, we've been able to classify the wine according to its geographical origin with 100 percent accuracy,” Ranaweera said.

She believes the Aqualog with A-TEEM technology has many advantages over ICP-MS.

“When we compare all the rapid methods that are available for wine analysis, the A-TEEM technique is a standout because it meshes both the absorbance and transmittance, as well as the excitation emission matrix of the sample, at the same time,” she said. “So we can obtain the molecular fingerprint of the wine using this A-TEEM technique, which is very unique for each wine.”

The Aqualog and A-TEEM provided other advantages beyond accuracy. Sample preparation and speed of acquisition were at the top of the list.

“In the analysis process, there are less preparation steps we have to use compared to, for example, HPLC or GC methods,” Ranaweera said. “This is a very quick method. We get the spectral results within minutes and don't have to spend much time on sample preparation. We just need to do dilutions with a solvent and can then use the instrument to analyze the samples. So it's very user friendly and gets us rapid results. That's what's most important.”

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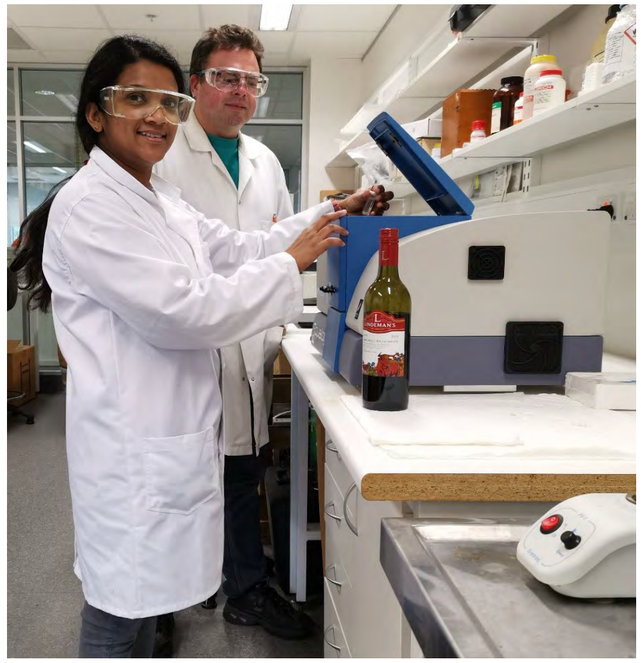

Ruchira Ranaweera loading a wine sample in the Aqualog with David Jeffery. Courtesy of David Jeffery.

Chemometrics

Ranaweera and her colleagues used the multivariate algorithm, XGBoost, or Extreme gradient boosting discriminant analysis (XGBDA), which yielded 100% correct classification. As a machine learning technique, XGBDA was an inspired choice for statistical analysis, which delivers high performance and accuracy, especially being very effective in the classification of these types of multiclass unbalanced groups of data, she said.

The researchers believe that combining A-TEEM and XGBDA has great potential for accurate authentication of wines, and could be used in the supply chain to verify the provenance of wine.

Next steps

Ranaweera continues to work on the authentication project as she pursues her Ph.D. Hesitant to give too much information away, she said she’d be going to go further into identifying the chemical drivers behind this classification. Jeffery is also encouraging his other Ph.D. students, who work on an array of different projects unrelated to authenticity, to “throw every sample they have” at the Aqualog to further learn how to exploit its unique capabilities.

Overall, wine makers and consumers should become the great beneficiaries of Ranaweera’s research.

References

[i] Bosker, Bianca, A True-Crime Documentary About the Con That Shook the World of Wine, The New Yorker, Oct. 14, 2016

[ii] Cho Lee, Jeannie, Fake Wine Is A Billion Dollar Market And Here Are The Ways To Identify Them, Forbes, Feb. 17, 2017

[iii and iv] Ranaweera, Ruchira, MSc; Capone, Dimitra L., Ph.D.; Bastian, Susan E., Ph.D.; Jeffery, David W., Ph.D.; Gilmore, Adam M., Ph.D.; Authentication of the geographical origin of Australian Cabernet Sauvignon wines using spectrofluorometric and multi-element analyses with multivariate statistical modelling, Food Chemistry, July 2020

Publications

Simultaneous measurement of Absorbance, Transmission, Fluorescence Excitation and Emission Matrix

Classification and Phenolics Analysis of Red Wines with A-TEEM Molecular Fingerprinting

Introduction

HORIBA Scientific has developed Aqualog®, an analytical instrument based on the simultaneous measurement of Absorbance, Transmission, Fluorescence Excitation and Emission Matrix (A-TEEM™).

Aqualog reports NIST-traceable A-TEEM fingerprints which can be evaluated using multivariate statistics such as PARAFAC (Parallel Factor Analysis), PCA (Principal Components Analysis), CLS (Classical Least Squares) and PLS (Partial Least Squares Analysis). Importantly A-TEEM fingerprints yield qualitative and quantitative composition of key flavor and color determinants in wine and spirits that are not discernible with simple Absorbance or Transmission data analysis.

Of the hundreds of different compounds that have been identified in grapes, it is the phenolic content of ripening grape berries that fundamentally determines the quality of a wine. The different classes of phenolics (anthocyanins, tannins, flavonols, catechins) affect the color, the mouthfeel, flavor and aroma, to various extent[1].

The individual compounds comprising these classes of phenolics contribute in concert to give the wines their unique character. The simultaneously acquired Absorbance, Transmission and IFE-corrected EEM data can be used to evaluate lot-to-lot, regional, and varietal characteristics. (See Figure 1.)

The samples were measured at room temperature and diluted 1:100 with 50% deionized water, 50% ETOH in a 1 cm path quartz fluorescence cell. Spectral A-TEEMs were automatically corrected for the influence of Inner Filter Effects (IFE), and Rayleigh masking was applied prior to PARAFAC and CLS analysis using the Eigenvector Inc. SOLO™ package.

The phenolics in Table 1 are just some of the most prevalent compounds known to affect wine characteristics like flavor, long term stability and color.

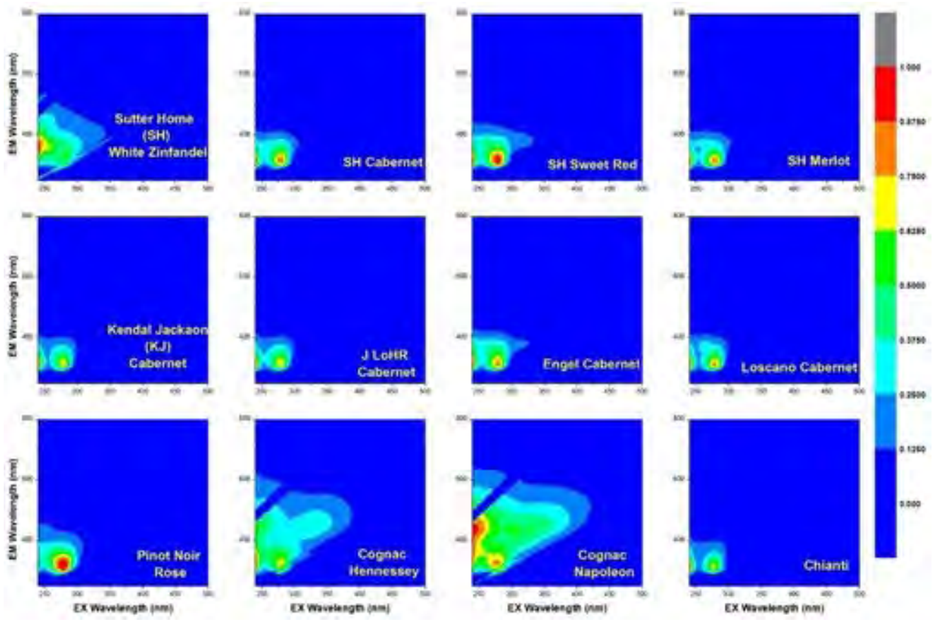

Figure 1: An array of A-TEEM molecular fingerprints of various wines and spirits displaying perceptible differences.

Molecular Groups	Main Contribution	Examples of individual molecules
Anthocyanins	Color	cyanidin-, petunidin-, delphinidin-, malvidin (-3-glucosides)
Catechins	Bitterness	monomeric flavon-3-ols catechin, epicatechin, epicatechin-gallate
Tannins	Astringency	polymers of flavon-3-ols catechin, epicatechin
Non-flavonols	Antioxidants, sun screen	coumaric,caffeic, ferrulic,gallic acids, resveratrol
Flavonols	Photoprotection	quercetin, myricetin, kaempferol, isorhamnetin, syringetin

Table 1

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Classification and Phenolics Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.

CLS analysis of the wines and spirits in Figure 2, based upon a library of 9 phenolic compounds for illustrative purposes, yields their relative contribution to the total phenolic fingerprint normalized to 100%. Significant differences between wines are reflected visibly in their phenolic compound fingerprints. Figure 2.

CLS analysis of spirits only, as seen in Figure 3 (cognacs and scotches) yields a different distribution, consistent with their being prevalently matured in oak casks that impart Quercetin and Myricetin compounds to them.

Monitoring the phenolics content in grapes, and in grape juice after harvest, allows the winemaker to determine the optimal period of fermentation and consequent extraction of these compounds from the skin and seeds of the berries. Adjustment of these values in the finished product by deciding on a course of blending, contributes to flexibility in producing wine with the desired characteristics, and also corrects for color or aroma shortcomings.

Traditional analysis relies on the use of HPLC, GC/MS techniques that require sophisticated laboratory equipment and expert preparation by trained personnel. UV/Vis optical density measurements are being used to provide bulk assessment of component molecules, but lack specificity.

In contrast, the A-TEEM technique with IFE, provided by Aqualog, requires only a single scan of a diluted sample, lasting a few seconds. Subsequent application of a predetermined multivariate model, calibrated using a library of reference compounds, is the fastest and simplest technique to classify and compare wines, detect adulteration, spoilage, quantify SO_2 treatment[2], etc.

1. (Waterhouse et al. Understanding Wine Chemistry ed. Wiley and Sons 2016)
2. Coelho, C. et al. Anal. Chem. 2015, 87, 8132–8137

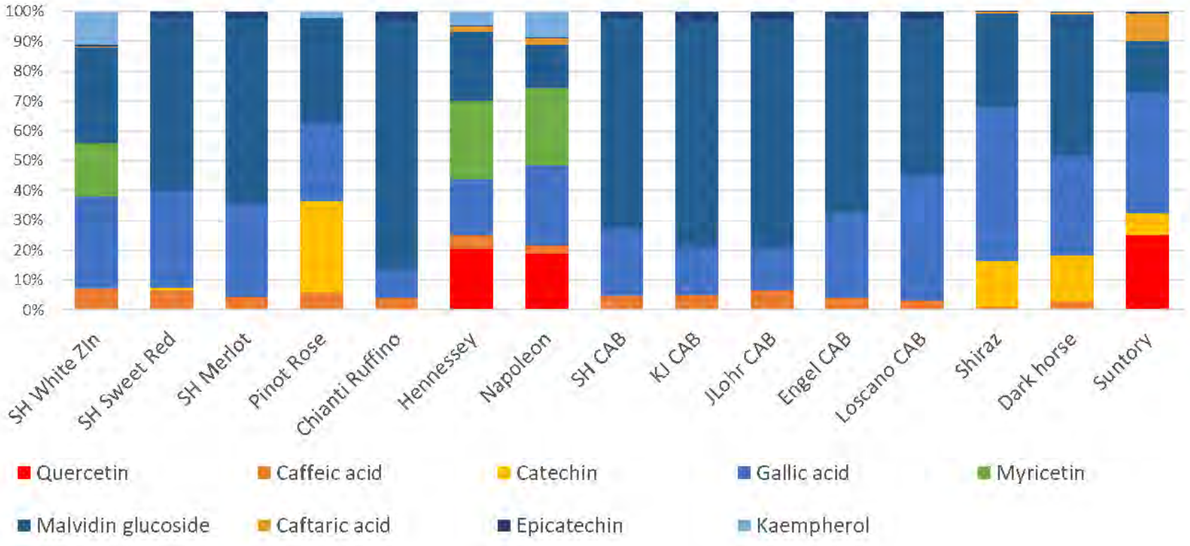

Figure 2

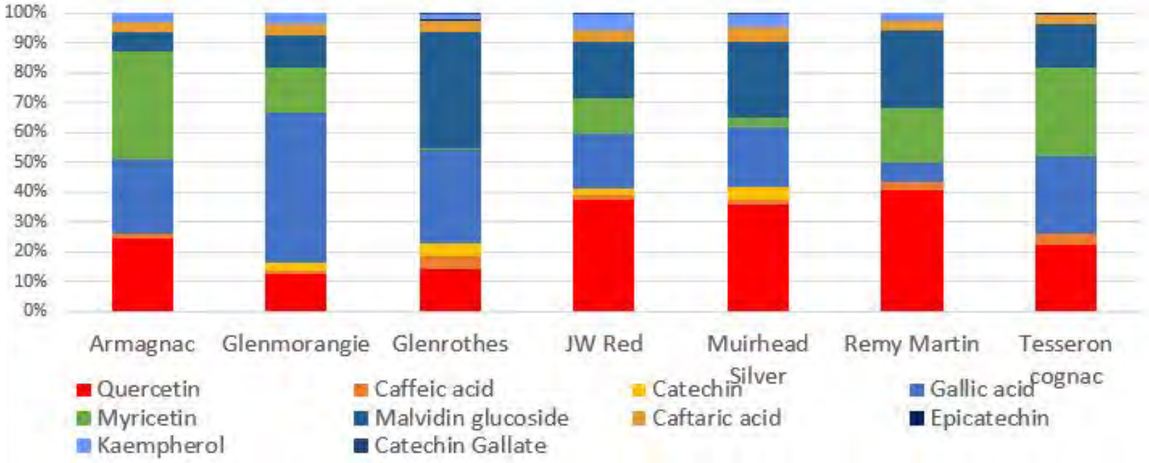

Figure 3

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Wine is perhaps the most researched and analyzed beverage after water.

Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting

The patented (US patent US8,901,513) Aqualog which facilitates simultaneous Absorbance-Transmission and fluorescence Excitation-Emission Matrix (A-TEEM) technology, provides rapid access to a wide range of parameters of significance in water treatment, drug and protein analysis, as well as the food and beverage industry. We think of Aqualog as an instrument for water analysis, but find that it is adept at wine analysis and more. Aqualog acquires a complete UV-VIS spectrum including the wine industry-standard Absorbance wavelength values at 280, 420, 520 and 620 nm, which are important to evaluate a wine's phenolic content, and derive characteristic Hue and Intensity values. Aqualog also reports the Transmission spectrum which can be used to determine the CIElab Tri-Coordinate color descriptions. Aqualog reports a NIST-traceable EEM fingerprint which can be evaluated using multivariate statistics, such as PARAFAC (Parallel Factor Analysis), and PCA (Principal Components Analysis). Most importantly, A-TEEM fingerprints yield qualitative and quantitative composition of key flavor and color determinants in grape juice and wine that are not discernible with simple Absorbance or Transmission data analysis.

Introduction

The A-TEEM technique enables Molecular Fingerprinting of complex chemical mixtures, and the identification of their components with high precision and speed. This study details the use of Aqualog in the analysis of red wines. Wine is perhaps the most researched and analyzed beverage, after water. With the advent of inexpensive transportation, global trade in wine, both in bulk and bottles, has skyrocketed. This increase in global trade has also facilitated a significant hazard of adulteration by using easily accessible cheap, lower quality wine and other adducts. Hence, the need for a relatively simple and fast, localised analysis of the provenance and composition of wine becomes paramount.

The global economic impact of the wine industry is approximately $ 300 Billion,[1] with $ 32 Billion in the US alone, and most of that $ 32 Billion is generated in California. Whereas the wine market comprises different wine types and styles, red wines dominate the concern with analysis of the constituents, both after grape fermentation, and before. Particular attention is paid to the issue of red wine color; its stability during aging seems to be the overarching determinant of value when comparable quality wines are evaluated.

Of the hundreds of different compounds that have been identified in grapes, it is the phenolic content of ripening grape berries that fundamentally determines the quality of a wine. The different classes of phenolics (anthocyanins, tannins, flavonols, catechins) affect the color, the mouthfeel, flavor and aroma, to various extents.[2] The individual compounds comprising these classes of phenolics contribute in concert to give the wines their unique character. Interventions during the onset of ripening (veraison) and the 30 to 60 days thereafter, play a significant role in determining the final quality of the wine. These interventions include leaf thinning to influence the insolation of berries and consequently phenolic composition,[3] removal of grape clusters to control yield and thereby channel fruit growth to a limited number of grape berries, and post-veraison irrigation protocols. It is therefore of great significance to be able to assess the phenolic profile of the crop during the critical veraison period to plan for the optimal harvest time. Bulk measurements of sugar content, pH and total acidity are simple measurements that can be carried out even by the lay person with little training, In contrast, more involved and detailed phenolic analyses using HPLC, GC/MS, UV/Vis, that provide more actionable information, require trained laboratory staff and expensive equipment. In fact many wine analytical services laboratories exist worldwide.

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Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.

A key feature and capability of Aqualog is automatic Inner Filter Effect (IFE) correction. The IFE correction is meant to compensate for both the Primary Filter Effect (PIF) where the excitation light intensity is gradually diminished due to absorption as a function of the optical pathlength of the liquid sample before reaching the fluorescent volume, and the Secondary Filter Effect (SIF) where the emitted fluorescence intensity is diminished due to re-absorption even by the portion of the sample that is not excited directly by the excitation beam.

As shown in Figure 1, blue excitation light intensity is gradually diminished due to sample absorption outside the measurement volume. Red emission light is diminished due to re-absorption of emitted fluorescent light.

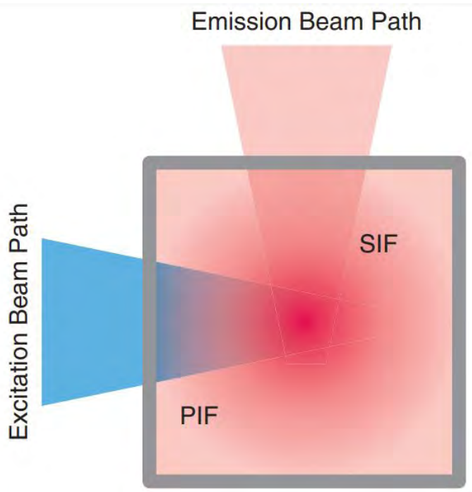

Figure 1: Inner Filter Effect (IFE)

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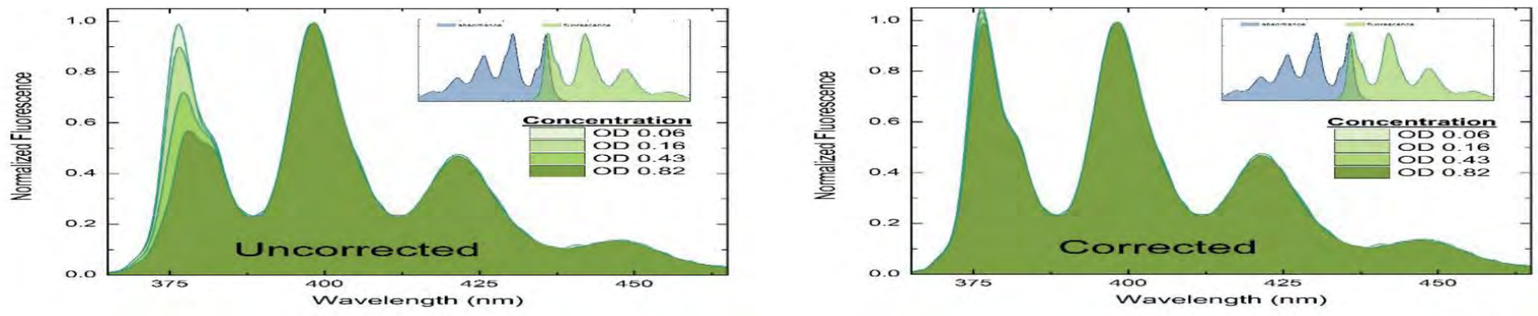

Figure 2: Spectral consequence of the inner filter effect

Figure 2 shows spectral consequence of the inner filter effect. The shorter wavelength intensities of the fluorescence spectrum are diminished due to re-absorption of fluorescence. Fluorescence spectra of increasing optical density samples are without IFE correction on the left, and with IFE correction on the right.

It is precisely because of the real-time IFE correction capability that the Aqualog has become the industry standard for quantitative water analysis.

The simultaneously acquired Absorbance-Transmission and IFE-corrected EEM data can be used to evaluate lotto-lot, regional, and varietal characteristics, as well as detect the effects of oxidation and sulfite treatment, thus making the Aqualog a valuable tool for industrial wine characterization.[4] It provides a detailed analysis of the array of phenolics present in wine, track its evolution, and use it to determine the geographical origins of wines, as well as distinguish between vintages, SO_2 treatments, and detect spoilage or forecast problems in the finished wine.

The phenolics in Table 1 are just some of the most prevalent compounds known to affect wine characteristics, like flavor, long term stability and color.

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Table 1

Molecular groups	Main contribution	Examples of individual molecules
anthocyanins	color	cyanidin-, petunidin-, delphinidin-, malvidin (-3-glucosides)
catechins	bitterness	monomeric flavon-3-ols catechin, epicatechin, epicatechin-gallate
tannins	astringency	polymers of flavon-3-ols catechin, epicatechin
non-flavonols	antioxidants, sun screen	coumaric,caffeic, ferrulic,gallic acids, resveratrol
flavonols	photoprotection	quercetin, myricetin, kaempferol, isorhamnetin, syringetin

Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.,

Monitoring the tannin and anthocyanin content in grapes and in grape juice after harvest, allows the winemaker to determine the optimal period of fermentation and consequent extraction of these compounds from the skin and seeds of the berries. Adjustment of these values in the finished product by deciding on a course of blending contributes to flexibility in producing wine with the desired characteristics, corrected for color or aroma shortcomings. Both the quantity and the extractability of anthocyanins and tannins increase throughout the grape ripening. Vivid graphs of the dramatic changes occurring over 6 weeks in the catechin to tannin ratio that affects astringency, or the 4-fold increase in polymeric anthocyanins that govern color can be viewed at reference.[5]

In the present study, the patented method of simultaneous Absorbance and fluorescence excitation-emission matrix (EEM) spectroscopy with Aqualog was employed. The main goal was to investigate and document the synergistic information gained by the A-TEEM method with respect to statistically significant resolution of wine samples, in terms of color and component composition. The Absorbance and Transmission spectra were evaluated with respect to key wavelength parameters (at 280, 420, 520 and 620 nm) and CIE color index information commonly used by the wine industry for process evaluation and tolerance settings in quality control programs. The IFE-corrected EEM data were analyzed using multivariate chemometric analyses including PARAFAC and PCA. PARAFAC and PCA were used to determine their effectiveness to resolve both spectral and concentration information of the colored wine components. The results are discussed in light of their significance for lot-to-lot, varietal, developmental and/or any other type of wine process characterization based on color composition analysis.

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Materials and Methods

Seven types of the following red wines were prepared for the analysis. Each wine production area and the grape cultivar % are described in Table 2.

Table 2

Production region	Grape cultivar
Italy (Italy)	50% Cabernet Sauvignon 50% Merlot
Italy (Italy N/A)	ND
Chile	Merlot
California (CA)	Merlot
Argentina (Arg)	Malbec
France (France)	55% Cabernet Sauvignon 40% Merlot 5% Petit Verdot
Spain (Spain)	50% Tempranillo 50% Gamacha

The samples were analyzed at room temperature and diluted with deionized water in a 1 cm path quartz fluorescence cell for an adjusted optical density (OD) of 0.6 cm^{-1} at 278 nm. Each sample's EEM and Absorbance spectrum were measured in triplicate. The samples were measured both when the bottle was freshly opened (within 1 hour), and after one week exposure to air.

All EEMs were normalized based on water Raman scattering units for the defined emission conditions. NIST-traceable spectral EEMs were corrected for the influence of Inner Filter Effects (IFE) and Rayleigh masking prior to PARAFAC and PCA analysis, using the Eigenvector Inc. SOLO package.

Results and Discussion

Typical changes in EEMs, Absorbance and Percent Transmission Curves for Red Wine after bottle opening are shown in Figure 3.

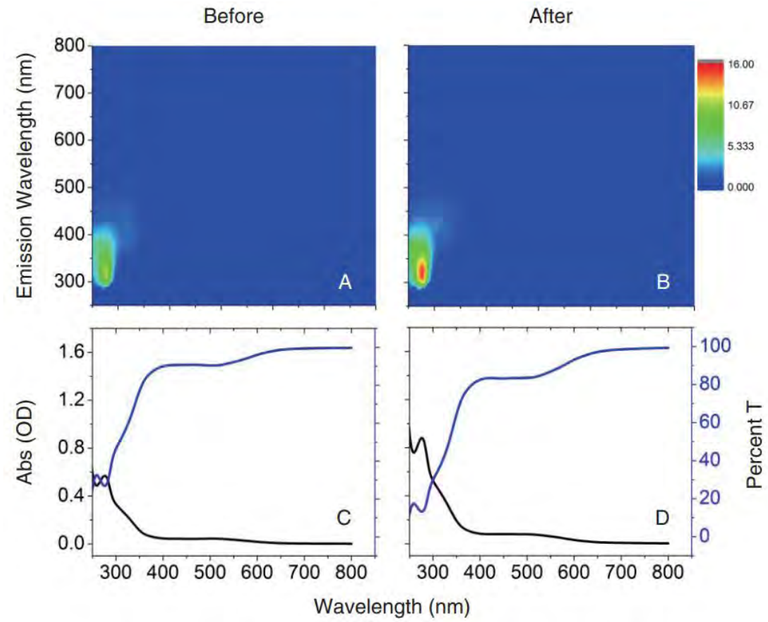

Figure 3: Simultaneously recorded EEMs (A and B) and Absorbance and percent Transmission spectra (C and D) for a typical Italian red wine from a freshly opened bottle Before (A and C), and After a one week exposure to air (B and D).

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Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.

The complexity of the EEM spectral contours, which comprise multiple overlapping excitation and emission components, limits qualitative and quantitative visual interpretation to major contour elements. The complex EEMs clearly exhibit major contours in the UV excitation emission range with the major excitation/emission (Ex/Em) peak around 275/309 nm. For both the Before and After samples, the Absorbance (and Transmission) spectra exhibited a major extinction peak around 275 nm, a smaller shoulder peak around 320 nm, and a second minor peak around 520 nm. The 275 nm peak region is commonly associated, at least in part, with phenolic compounds, and the 520 nm peak region is generally associated with anthocyanin compounds. The fluorescence from anthocyanin at 520 nm in the absorption spectra is rather weak and is not evident in the EEM in this scaling. In the component fingerprint it would show a major peak in the 500-600 nm region (cf. Figure 6).

Compared to the Before sample (Figure 3C), the After samples (Figure 3D) exhibited increased extinction across the entire Absorbance spectrum associated with the oxidation phenomenon. Likewise, the After EEM (Figure 3B) showed stronger emission Intensity than the Before EEM (Figure 3A) for all spectral contour features.

Absorbance, Transmission and CIE Lab Analysis

The wine industry has adopted a conventional analysis involving three discrete Absorbance wavelengths, namely *A420, A520* and *A620* nm, to rapidly characterize basic color characteristics of wine, including the wine's redness, brownness or yellowness, Hue and Intensity. The Aqualog's complete UV-Vis spectrum provides access to these wavelength parameters to facilitate these analyses.

The *Hue* parameter is calculated as:

Hue = A420/A520

and the *Intensity* is calculated as:

Intensity = (A420+A520+A620).

In addition, the *A280* value is commonly used as a metric for the presence of phenolics. It may, however, also include Absorbance from many other compounds in this wavelength region.

The samples in Figure 4A and 4B were arranged from left to right according to the decreasing average *Intensity* in Figure 4B. The Italy N/A sample showed the highest Intensity value and the France sample showed the lowest. The *Intensity* parameters *A420, A520* and *A620* in Figure 4A did not necessarily correlate with the *A280* values, which always exhibited the highest Absorbance peak in each wine. Likewise the Hue parameters, which ranged from around 0.82 to 1.07, more or less randomly among the samples in Figure 4B, did not correlate strongly with the Intensity parameters.

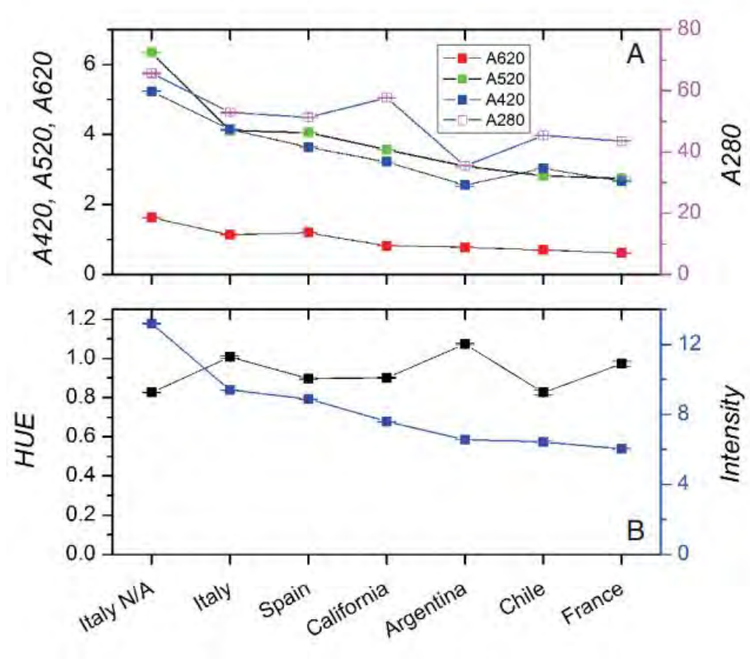

Figure 4: Comparison of the Absorbance parameters (A) and Hue and Intensity parameters (B) defined above, measured with Aqualog for a series of freshly opened red wines from various countries.

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Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.

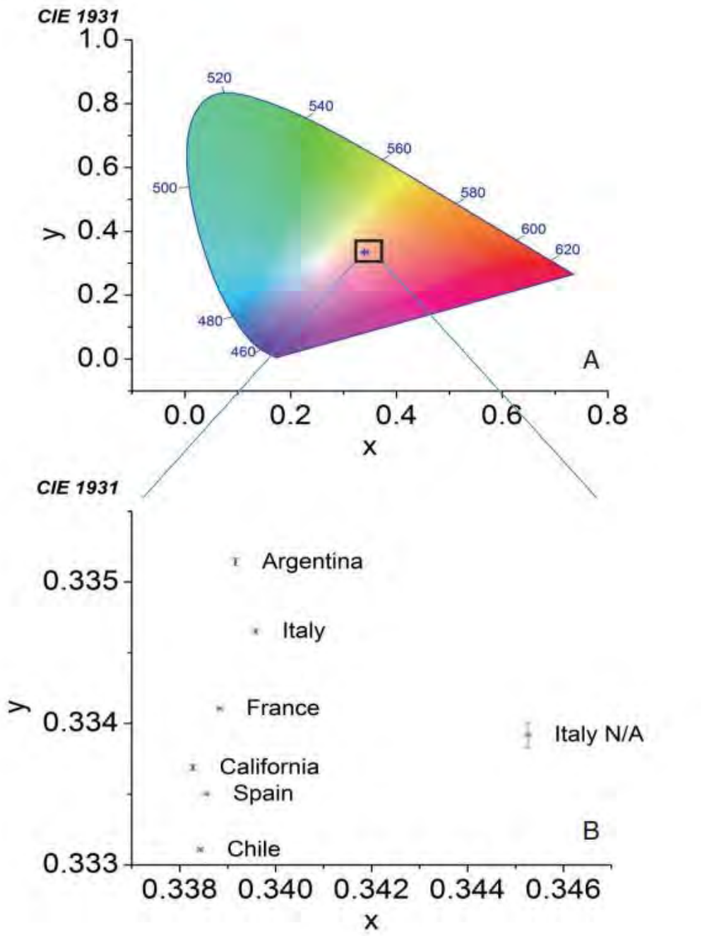

Figure 5: CIE 1931 x and y coordinates for each of the wine samples from Figure 4.

Figure 5A illustrates the proximity of all the red wines in the full scale of the CIE 1931 index, and the approximate region (indicated by the square frame) expanded in Figure 5B. All the samples fell within a narrow range on the CIE 1931 scale shown ranging from (x=0.337 to 0.347) to (y=0.333 to 0.3355). The data points are shown with corresponding x and y standard deviations in Figure 5B Table 3 is Absorbance, Hue, Intensity and CIE Lab color index parameters for the Italy wine sample shown in Figure 3 Before and After oxidation.

Consistent with the EEM, Absorbance and Transmission data in Figure 3, the Before – After=Δ samples were negative indicating a significant ($p<0.05$) increase in all Absorbance parameters and hence the Intensity parameter. The Hue parameter also increased significantly. It follows that all of the CIE Lab color indices also registered significant changes ($p<0.05$) associated with the oxidation treatment.

Absorbance	Before	σ	After	σ	Δ	σ
A620	1.14E+00	1.09E-02	2.11E+00	1.17E-02	-9.78E-01	1.60E-02
A520	4.12E+00	1.50E-02	7.15E+00	1.70E-02	-3.03E+00	2.27E-02
A420	4.14E+00	1.86E-02	7.60E+00	1.14E-02	-3.46E+00	2.18E-02
A280	5.30E+01	1.38E-01	8.21E+01	1.36E-01	-2.92E+01	1.93E-01
HUE	1.01E+00	5.82E-03	1.06E+00	2.99E-03	-5.69E-02	6.54E-03
Intensity	9.39E+00	2.63E-02	1.69E+01	2.36E-02	-7.47E+00	3.53E-02
CIE Lab						
X	1.10E+02	3.00E-02	1.06E+02	3.39E-02	4.79E+00	4.53E-02
Y	1.09E+02	3.29E-02	1.03E+02	3.85E-02	5.69E+00	5.07E-02
Z	1.06E+02	5.09E-02	9.72E+01	2.14E-02	8.57E+00	5.52E-02
x	3.40E-01	2.71E-05	3.45E-01	2.25E-05	-5.50E-03	3.52E-05
y	3.35E-01	1.59E-05	3.37E-01	3.30E-05	-2.25E-03	3.66E-05
X+Y+Z	3.25E+02	1.14E-01	3.06E+02	8.89E-02	1.91E+01	1.44E-01
L*	1.03E+02	1.21E-02	1.01E+02	1.46E-02	2.12E+00	1.89E-02
a*	1.18E+01	4.74E-03	1.32E+01	7.58E-03	-1.40E+00	8.94E-03
b*	6.57E+00	1.12E-02	8.45E+00	1.80E-02	-1.88E+00	2.12E-02
C*ab	1.35E+01	9.48E-03	1.57E+01	7.02E-03	-2.17E+00	1.18E-02
h*ab	5.09E-01	5.68E-04	5.70E-01	1.16E-03	-6.11E-02	1.29E-03
S*	1.31E-01	1.07E-04	1.55E-01	6.70E-05	-2.42E-02	1.26E-04
Q*	1.33E+02	1.08E-02	1.31E+02	1.31E-02	1.91E+00	1.70E-02

Table 3 Absorbance, Hue, Intensity and CIE Lab color index parameters for the Italy wine sample shown in Figure 3 Before and After oxidation.

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Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.

EEM PARAFAC Analysis

PARAFAC is a tri-linear matrix decomposition method. In the following it is used to yield 1) an excitation; 2) emission spectral shape loading; and 3) concentration loading score for each assumed model component. PARAFAC results are evaluated by a least-squares fitting figure of merit (r2: % variance captured), in addition to residuals analysis, core-consistency and split-half validation tests to evaluate the model fit and parameter redundancy. The PARAFAC model evaluated in this study was constrained to yield non-negative values for all loading and score parameters. Figure 6 shows the excitation-emission contours for the five spectral loading components resolved in the PARAFAC model developed using all the fresh and oxidized wine sample replicates.

Component 1 exhibited one major Ex/Em contour (275/330 nm) and a minor contour (275/425 nm). Component 2 exhibited one contour (260/370 nm). Component 3 showed the deepest UV emission with a major contour (275/300 nm) and a very minor contour (275/370 nm). Component 4 was broad in emission and bimodal in excitation with the major contour (325/410 nm); the deeper UV excitation band may not have been fully resolved above the 250 nm scale used in the analysis. Component 5 was present at low levels in all samples with a broad contour (430/560 nm). Based on the resolved Ex/Em coordinates, the components were compared to literature values for tentative identification, as shown in Table 4.[6]

Table 4

PARAFAC Component	Excitation Max, nm	Emission Max, nm	Name
C1	278	340	Caffeic Acid
C2	263	380	Flavonol Like
C3	280	300	Epicatechin
C4	315	405	Gentisic Acid
C5	445	568	Anthocyanin

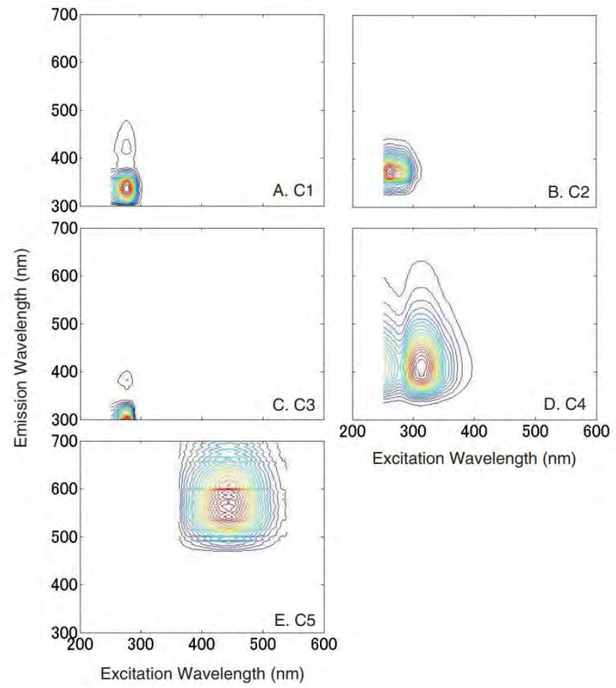

Figure 6: IFE-corrected excitation and emission spectral contour loadings for the five component model evaluated for all wine sample replicates, before and after oxidation. The PARAFAC model (n=84)) was described with an r2 = 0.997 and a split half validation score r2 = 0.906 (where the model is tested with two halves of the dataset separately).

Significant changes occur during oxidation in an opened bottle of the Italy wine sample, as shown in Figure 7. Component 1 was the dominant component, before and after oxidation. While all five components increased significantly ($p<0.05$) in Intensity loading after oxidation, the deeper UV emitting components 1-3 increased relatively more than the longer emission wavelength components, 4 and 5.

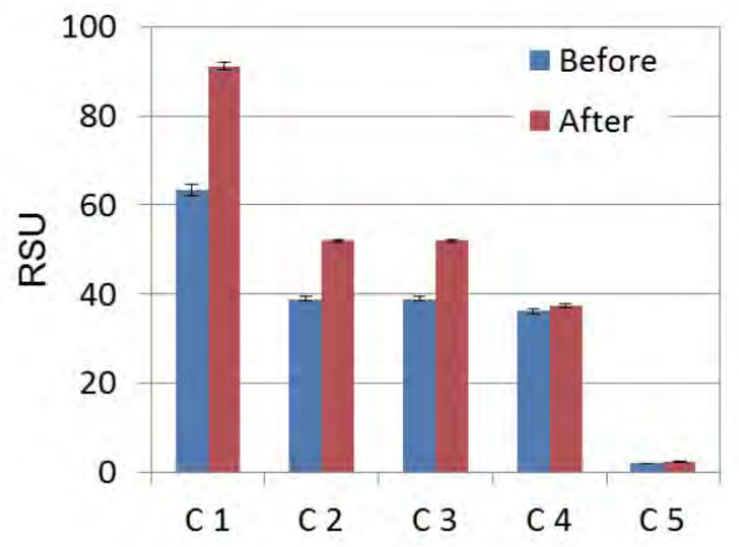

Figure 7: Comparison of the five PARAFAC component scores in the Italy wine samples (n=3 replicates per sample) before and after oxidation. Component scores are reported normalized water Raman scattering units (RSU).

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Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.

Varietal Fingerprint-Cluster Analyses with PARAFAC and PCA

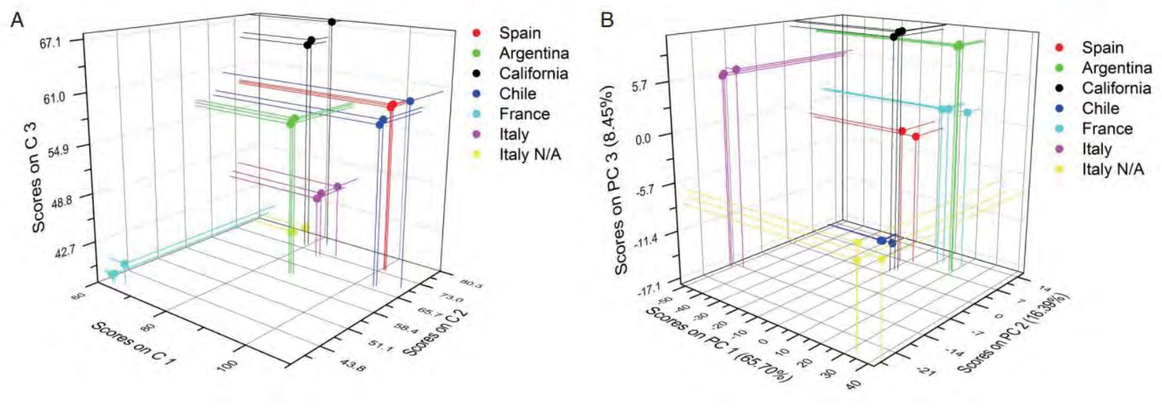

Figure 8: Comparison of the three major component cluster plots for PARAFAC (A) and PCA (B) analyses of the freshly opened wine samples (n=3 replicates per sample).

The three major fluorescence PARAFAC and two-way Principal Components Analysis (PCA) model component loadings were respectively evaluated in Figure 8A, 8B for all the fresh wine samples by cluster analysis. PCA is a two way analysis technique that yields component scores that can show negative amplitudes and thus may be considered physically unrealistic in terms of chemical component spectra. PCA components, however, may still be indicative of qualitative and quantitative changes in the sample's spectral composition. Visual interpretation indicates each wine exhibited a unique set of coordinate clusters for both the PARAFAC (A) and PCA data (B) and thus a unique color composition which is consistent with the CIE 1931 data shown in Figure 5. The statistical significance of the resolution for each pair of varieties was evaluated in terms of each of the three score parameters compared. The PARAFAC model exhibited significant resolution of all varietal pairs with $p<0.05$, and the PCA analysis exhibited resolution at the $p<0.1$ level.

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Discussion and Conclusion

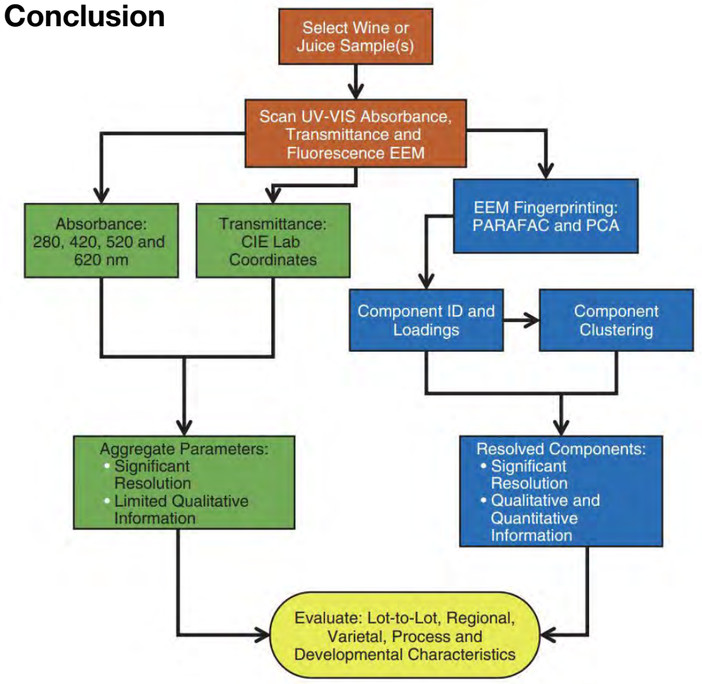

Figure 9: Schematic of the relationships among the simultaneous Absorbance and EEM data acquisition and analysis with respect to the significance of wine sample and component resolution pertaining to this study.

wine sample and component resolution pertaining to this study. The data clearly indicate that Aqualog's simultaneous Absorbance and EEM acquisition and analysis,
as described in Figure 9, can uniquely provide significant resolution of wine varieties and treatments based on the basic Absorbance parameters such as Hue and Intensity and the CIE Lab parameters derived from the Transmission data. Notably the information provided by the Absorbance and Transmission analyses represents the aggregate effects of all overlapping spectral components contributing to the processed signals. Therefore it is important to note the EEM analyses provide both valuable qualitative and quantitative information on individually resolved color components. Clearly all the analyses are of potential value to industrial wine evaluation and can be extended to a wide variety of applications beyond those described in this study.

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Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting, cont.

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Economic and cultural importance of wine is chemically supported mostly by its phenolics content...

Multivariate spectroscopy for targeting phenolic choreography in wine with A-TEEM™ and NMR crosscheck non-targeted metabolomics

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Abstract

Present work comprises the use of different multivariate spectroscopic methods for tracking novel metabolomics' signatures related to red wine chemistry. It is presented for the first time, the proton nuclear magnetic resonance metabolomics fingerprint of a monovarietal Mexican Merlot, obtained with acquisition improvements recently proposed to the OIV Methods of Analysis sub-commission. Effective multi-presaturation solvent schemes have revealed a rich (poly)-phenolics aromatic region, so far not exploited for wine-fingerprinting or – targeted profiling routines. It is presented, as well, for the first time, the use of simultaneous absorbance-transmission and fluorescence excitation-emission matrix "push-one-bottom" method (A-TEEM™) at specific chemical conditions for a rapid, effective and high-sensitivity characterization of phenolic choreography in wines, as novel observables to quantify oenological practices and aging.

Introduction

Economic and cultural importance of wine is chemically supported mostly by its phenolics content, as said, primary and special metabolites' family contribute to wines' organoleptic properties such as colour, taint, mouthfeel and aromas [1]. Standard quantification of phenolics comprises colorimetric and/or chromatographic approaches, whereas despite their robustness, they present certain complexity in terms of sample preparation, chemical manipulations, being in turn time consuming, laborious, costly and require some level of analytical expertise. OIV cross-commissions shall promptly evaluate the ratio between wineries and oenological research institutes *in all member states* that could provide the manoeuvre of analytical experts certified to carry out said standard methods.

This work presents a "push-one-bottom", rapid, user-friendly and non-invasive spectroscopic solution to track a robust phenolic profile in wines, with simultaneous absorbance-transmittance (A-T) and fluorescence excitation – emission matrix spectroscopy (EEM), branded as A-TEEM™ [2], wherein the simultaneous AT & EEM acquisition is carried out at each excitation increment. Immediate applications of the A-TEEM™ device comprise the characterization of human consumption water quality, in terms of quantification of Total dissolved Organic Carbon (TOC) metabolites like low-and highmolecular aromatics, as well as protein compounds from humic/fulvic sources [3]. More recently, A-TEEM™ technology has been used to determine phenolic and anthocyanin profiles in fresh and oxidized Italian red wines, in the excitation-emission range between 250–800 nm [3, 4].

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Multivariate spectroscopy for targeting phenolic choreography in wine with A-TEEM™ and NMR crosscheck non-targeted metabolomics, cont.

Construction of reliable meta-databases in terms of reproducible A-TEEM™ phenolic and anthocyanin libraries for quantitative analysis of mostly special metabolites in wine with a fast/high sensitivity/pushone-bottom solution needs an orthogonal crosscheck with robust OIV methods. For instance, high-resolution proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) [5–7] is used to obtain the first reported metabolomics fingerprint and profiling of a monovarietal Mexican Merlot (2018, Sala Vivé, Freixenet, Quer´etaro MX), targeting the most abundant primary metabolites, in agreement to an OIV resolution project under course [7]. ^{1}H-NMR metadata analysis is presented for fingerprinting some spectral regions associated to phenolics that orthogonally correlates with A-TEEM™ observables.

Materials and Methods

Wine samples

A set of eleven Mexican mono-varietal Merlot wines from Queretaro, México (Finca Sala Vivé, Freixenet Mexico), from two different years of vintage (2017 and 2018), aged at different conditions were analysed, hereafter numbered as follows: (1): Merlot 2017 aged within a 2017-Tonnellerie d'Aquitaine French barrel, (2): Merlot 2017 Gran Reserva taken from a 24-months bottled aging, (3–4): Merlot 2018 aged within a 2018-Tonnellerie d'Aquitaine French barrel with a duplicate sampling, (5): Merlot 2018 aged within a 2016-Tonnellerie d'Aquitaine French barrel, (6): Merlot 2018 directly taken from the fermentation tank, (7–9): Merlot 2018 aged within a 2016-Boutes French barrel with a triplicate sampling, (10–11): Merlot 2018 aged within a 2018-Demptos American barrel, with a duplicate sampling.

Sample preparation for A-TEEM™ spectroscopy was carried out by dissolving 30 µL of wine samples (3), (6) and (11) with a 200× dilution factor, using a 12% ethanol v/v solution as solvent at three different pH levels: 1, 3, 7. A final volume of 3 mL per sampling was versed in each case within a (1 × 1) cm path length quartz cuvette. A-TEEM™ lectures were done with a temperature of 25 °C.

Sample preparation for NMR studies comprised the addition of 100 µL of a mixture of D_2O and chemical-shift reference sodium 3-(trimethylsilyl)-propionate-2, 2, 3, 3-d_4 (TSP), phosphonate buffer KH_2PO_4 0.1% and 2% NaN_3 to 900 µL of wine sample, whereas pH was finally adjusted to a value of 3.9 for all samples. Samples were finally versed in standard 5 mm NMR tubes.

UV-Vis absorbance-transmittance coupled with excitation emission matrix fluorescence

Simultaneous UV-Vis absorbance-transmission and fluorescence excitation-emission matrix spectra were carried out on an A-TEEM™ Aqualog system (Horiba Jobin Yvon, Inc.) with simultaneous absorption – excitation wavelength spans from 240–800 nm (5 nm interval) and emission wavelengths spanning from 248–826 nm with an average increment of 4.66 nm. Analysis of the fully corrected A-TEEM™ data was carried out with the Aqualog DataStream package based on the multivariate routine known as parallel factor analysis (PARAFAC, Solo + MIA package from Eigenvector Research Inc.) [10]. Best fit of the data was achieved with a five component model.

Nuclear Magnetic Resonance (NMR) spectroscopy

All spectra were recorded on a Bruker 600 AVANCE III HD equipped with a 5 mm ^{1}H/ D TXI probehead with z-gradient.
1 D-^{1}H experiments with water-to-ethanol solvent presaturation were carried out as elsewhere reported [5].

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Multivariate spectroscopy for targeting phenolic choreography in wine with A-TEEM™ and NMR crosscheck non-targeted metabolomics, cont.

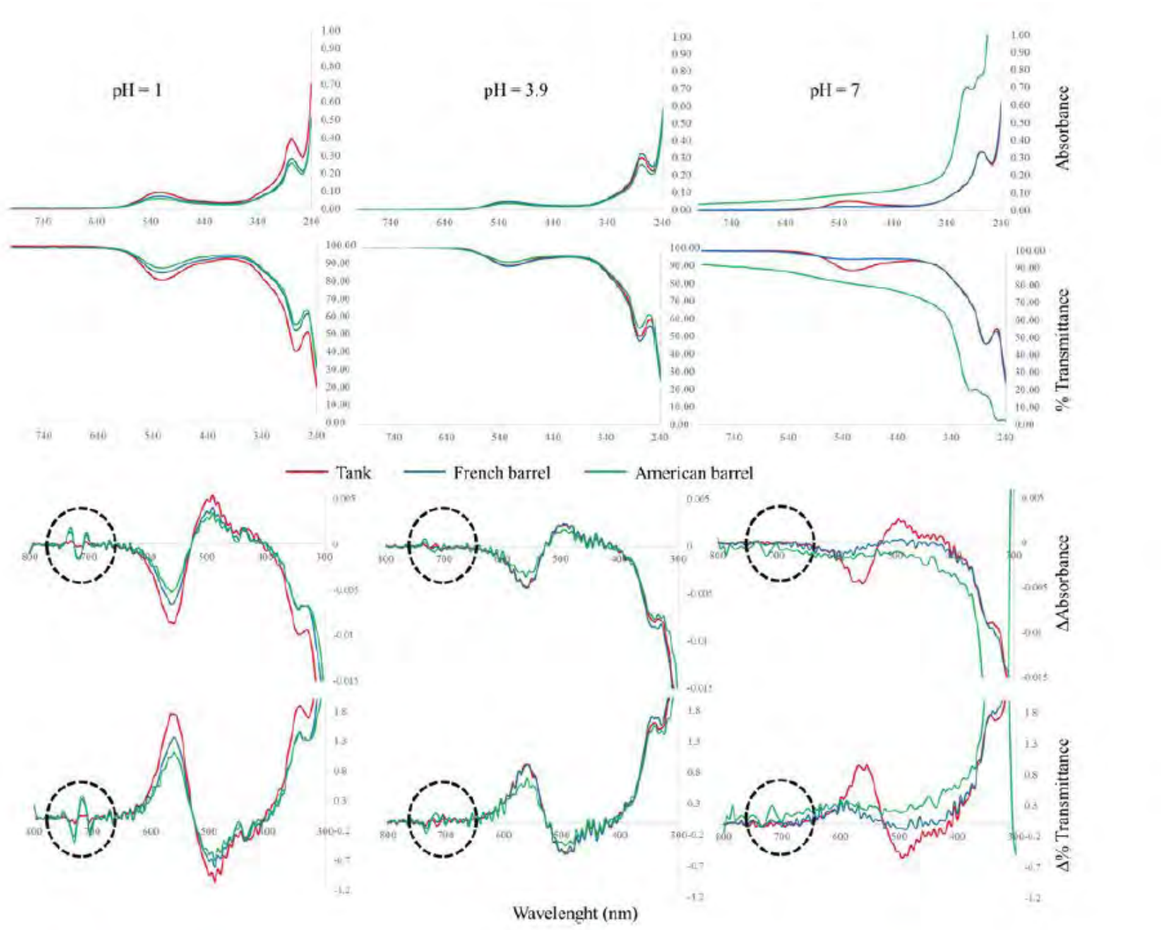

Figure 1: Raw and first derivative (Δ) UV-VIS absorbance – % transmittance spectra of Mexican monovarietal Merlot wines (samples 3 (red spectra), 6 (blue spectra) and 10 green spectra); see Materials and Methods) at three different pH values (pH = 1, extreme Left; pH = 3.9, Middle; pH = 7, extreme Right). Relevant absorption - % Transmittance peaks at 715 nm is highlighted with a dotted-line circle.

Results and discussion

Figure 1 presents the raw and 1st derivative absorbance and % transmittance spectra of a set of Mexican Merlot 2018 red wines with no barrel aging (Tank, red) and three months aged, even within a 2018-Tonnellerie d'Aquitaine French barrel (blue) and with a 2018 Demptos American barrel (green), at three different acidic conditions (pH = 1, 3, 7). Peaks on raw spectra, attenuated in the derivative graphs, accentuate the following bands: A major extinction peak at 275 nm, a second minor peak at 520 nm and a third residual peak at around 715 nm, well observed at pH=1, in a lesser extent at pH=3, but only for aged samples in barrels. No residual 715 nm peak is observed, neither for Tank samples at any pH value and at a pH = 7 for the rest of the aged Merlot wines. Whereas the 275 nm absorbance – transmittance lines are commonly associated with simple phenolic compounds and the 520 nm peak region has been associated to stable anthocyanin compounds [12], the 715 nm absorbance peak could strongly be associated to flavylium cations of most common anthocyanidines in red wines that serve as dyes [13]. Deep inspection of first derivative absorbance and % transmittance spectra of Figures 1 and 2, reveals a noticeable increase of the 520 nm band for not aged, or poorly aged, species, whilst an absorption band at 715 nm is present in samples with presumably better aging processes, an effect that is better appreciated at acidic conditions. The last correlates with previous EEM studies [12] that claim the possibility to distinguish monomeric and polymeric anthocyanin species. Present results opens the venue to distinguish monomeric (at 520 nm) and polymeric (715 nm) anthocyanin species with first derivative absorption spectra, whereas at acidic conditions, said spectroscopic signature is more pronounced.

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Multivariate spectroscopy for targeting phenolic choreography in wine with A-TEEM™ and NMR crosscheck non-targeted metabolomics, cont.

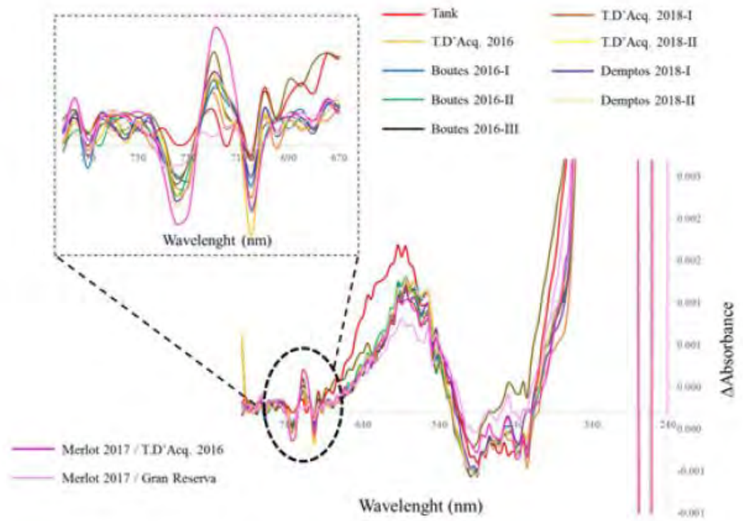

Figure 2: First derivative (Δ) UV-VIS absorbance spectra of Mexican monovarietal Merlot wines with different aging schemes (samples 1 to 11, refer colour code to figure legends and Materials and Methods) acquired at pH = 3.9. As in Figure 1, absorbance region at 715 nm is expanded.

Proton nuclear magnetic resonance (^{1}H-NMR) metabolomics fingerprint of Mexican monovarietal Merlot red wines, with the use of key methodological improvements [5,7] that noticeably increases spectral signal-to-noise ratio, is presented in Figure 3.

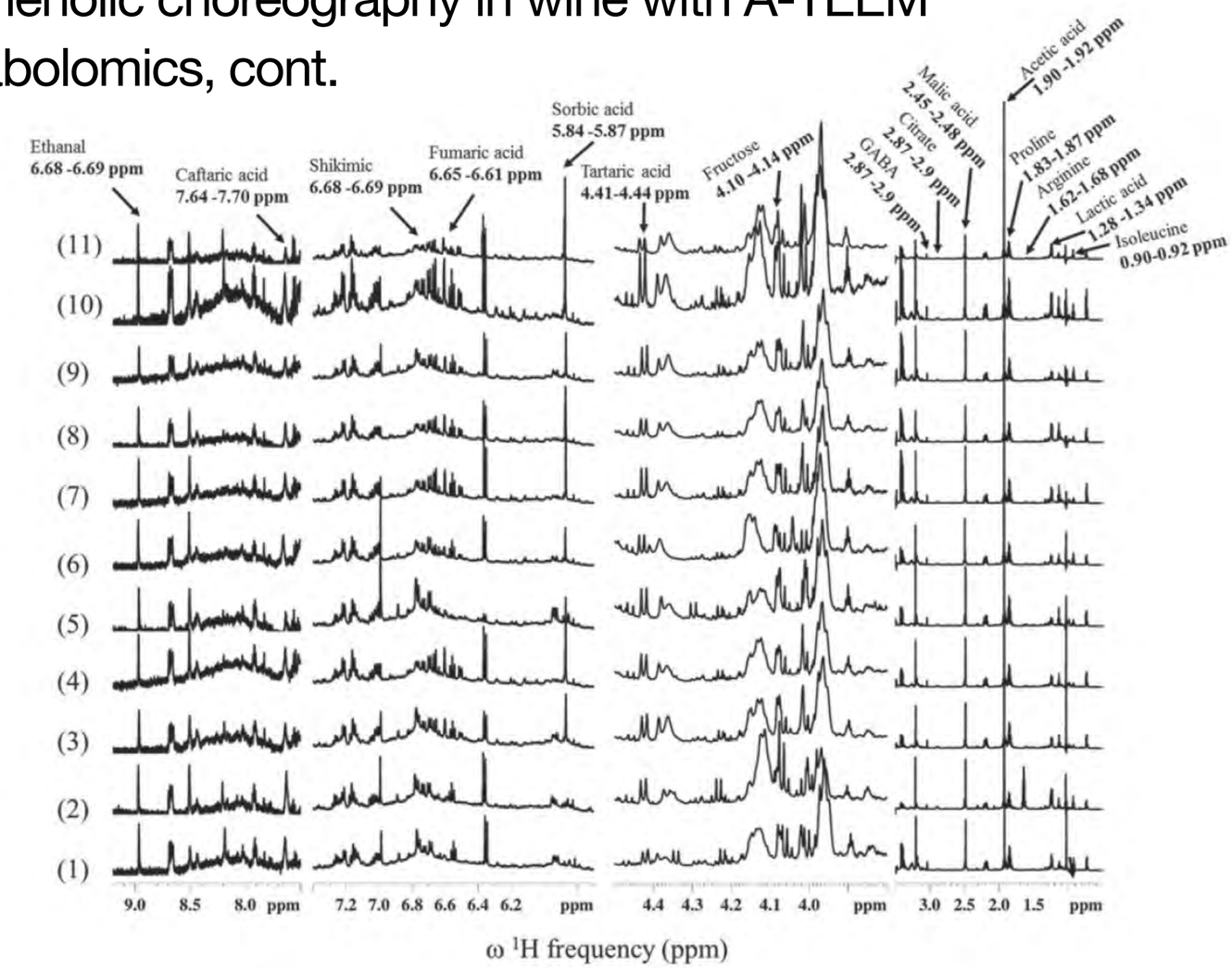

Figure 3: Stacked proton one-dimensional Nuclear Magnetic Resonance spectra (^{1}H-NMR) with improved water-to-ethanol multipresat. Scheme [5] of Mexican monovarietal Merlot wines, with different aging schemes (samples 1 to 11, refer to Materials and Methods). NMR signature of Mexican Merlot from Queretaro Region (Finca Sala Vivé / Freixenet) present signal assignment of relevant metabolites, with excellent agreements with respect foreign Merlot noir genotypes' NMR fingerprints in plant metabolomics databases [15].

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Multivariate spectroscopy for targeting phenolic choreography in wine with A-TEEM™ and NMR crosscheck non-targeted metabolomics, cont.

Improvements of water-to-ethanol multi-presaturation schemes for having a full set of proton resonances of both primary and specialized wine metabolites towards fast acquisition NMR fingerprinting & targeting, has been recently presented at OIV SCMA experts' group [7]. Advantages of the use of said methodological ^{1}H-NMR aspects, is exemplified in Figure 3 and should be read as follows: ^{1}H-NMR-OIV resolution project comprises the quantitation of solely six primary metabolites for wine analysis [7], most probably due to poor signal-to-noise ratio with the use of standard ^{1}H-NMR solventsupression schemes, that severely penalizes the limits of detection – quantification of lower concentration metabolites. Accurate water-to-ethanol multipresaturation schemes allowed the fingerprinting and profiling of at least 15 novel metabolites, having excellent agreements with respect prestigious plant metabolomics meta-data Repositories [14,15]. It is worth noting to highlight that with selected acquisition conditions [5], an important number of non-assigned resonances at the phenolics region (5.58–8.0 ppm) present an accurate signal-to-noise ratio for increasing known NMR fingerprinting & targeting of wines.

With the use of accurate multipresatuation schemes for water and ethanol intense signals, a rich (poly)-phenolics aromatic region is exposed within the full NMR-fingerprint of studied Queretaro Merlot wines, with reasonable acquisition times per sample (i.e. 4 minutes per experiment, with 64 transients). Despite a full NMR pre-processing treatment (signal bucketing,integration and quantification) of present data will be elsewhere discussed in detail, mostly from rich (poly)-phenolics aromatic region, some remarks can be done. Figure 4 present expansions of Figure 3 per data set (see Materials and Methods), whereas Querétaro, Merlot's NMR-signatures are stacked in terms of aging reservoirs and year of vintages: (1–2) Merlot 2017; (3–4): Merlot 2018/2018-Tonnellerie d'Aquitaine; (10–11): Merlot 2018/2018-Demptos; (7–8): Merlot 2018/2016-Boutes; (5) Merlot 2018/2016-Tonnellerie d'Aquitaine; (6): Merlot 2018/tank. This specific classification in Figure 4, was done by means of the UV-Vis ΔAbsorbance 715 nm peak intensity that each set of samples present (full UV-Vis ΔAbsorbance depicted in Figure 2).

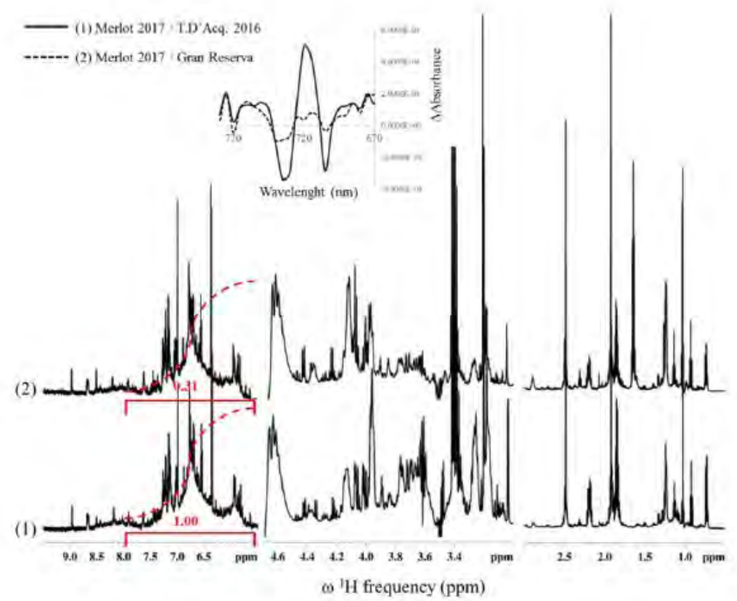

Figure 4. Proton one-dimensional Nuclear Magnetic Resonance spectra (^{1}H-NMR) with improved water-to-ethanol multipresat. Scheme [5] of Mexican monovarietal Merlot wines, with different aging schemes (samples 1 to 11, refer to Materials and Methods). Novel rich polyphenolics exposed region (5.58–8.0 ppm) with multi-preset scheme has been integrated in all cases, referenced with respect sample 1 (I = 1.0). First derivative (Δ) UV-VIS absorbance spectra of each sample, at the 715 nm region is as well exposed, per case.

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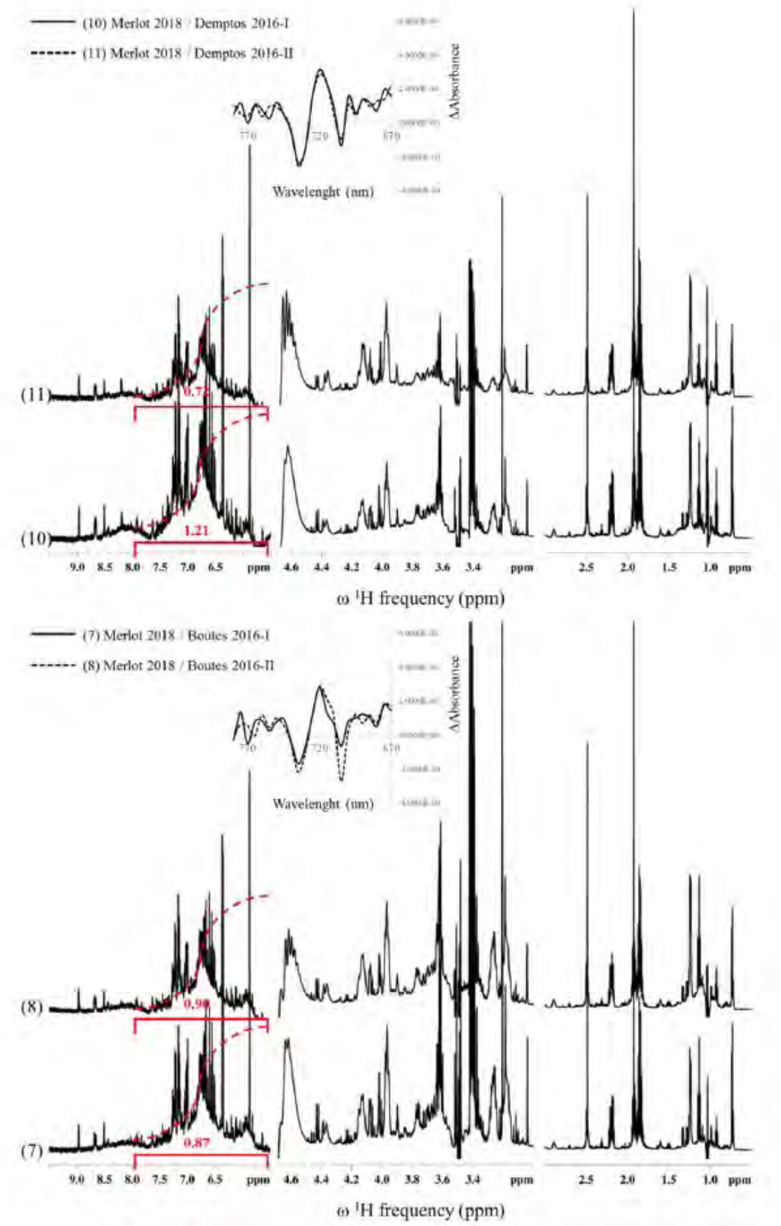
As observed and expected, aged 2017-Merlot in a 2017-Tonnellerie d'Aquitaine barrel, presents a maximum 715 nm ΔAbsorbance peak intensity. In counterpart, said sample presents a minimum signal intensity of ΔAbsorbance at 520 nm wavelength (Figure 2), strongly suggesting the presence of a major amount of poly-anthocyanin with minor amounts of mono-anthocyanin moieties. In extreme contrast, Merlot 2018/tank samples present no ΔAbsorbance signal at 715 nm and a maximum at 520 nm, confirming the UV-Vis ΔAbsorbance antagonist mono/poly-anthocyanin profile. Last observations could be cross-checked with the use of the NMR-Merlot profiling. Despite the lack of spectral resolution at the novel revealed (poly)-phenolics region around 5.58–8.0 ppm, signal integration could shed light on phenolics content in agreement with UV-Vis observations.

NMR signal integration of rich (poly)-phenolics region around 5.58–8.0 ppm within the ^{1}H-NMR spectra of wine samples exposed in Figure 4, present a coherent agreement with respect the UV-Vis ΔAbsorbance signature at 715 nm. Aged wines, such as the Merlot 2017/2017-Tonnellerie d'Aquitaine (sample 1), or Merlot 2018/2018-Tonnellerie d'Aquitaine (samples 3–4) present a normalized signal integration of the rich (poly)-phenolics region of around the unity and, in turn, a 715 nm UV-Vis ΔAbsorbance clear fingerprint. For the antagonist Merlot 2018 / Tank (sample 6), it is observed a relative signal integration of the 5.58–8.0 ppm region of 8% (with respect the reference (sample 1)) and no 715 nm UV-Vis ΔAbsorbance fingerprint, in agreement with the expected lack of complex polyphenolics. Ensemble of preliminary results suggest an aging efficiency trend corroborated by both multivariate methods: Tonnellerie d'Aquitaine (2017, 2018) French barrels promote wine aging with slightly better results of polyphenolics content. American Demptos and French Boutes barrels promote wine aging with equivalent spectroscopic results. Tested 2016-Tonnellerie d'Aquitaine produced wine samples with poor signal integration (I = 0.038) and weak 715 nm UV-Vis ΔAbsorbance signal.



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Finally, coupled A-T & EEMs could be regarded as genuine fingerprints of optical active (macro)-molecular composition of wines. As above mentioned, Absorbance – % Transmission profiles present maximum extinction peaks around 275, 520 and 715 nm (Figures 1 and 2). In turn, EEMs produce broad intense florescence emission spectra spanning from 250 to 320 nm for a 240–325 nm excitation wavelength (Figure 5, Top).

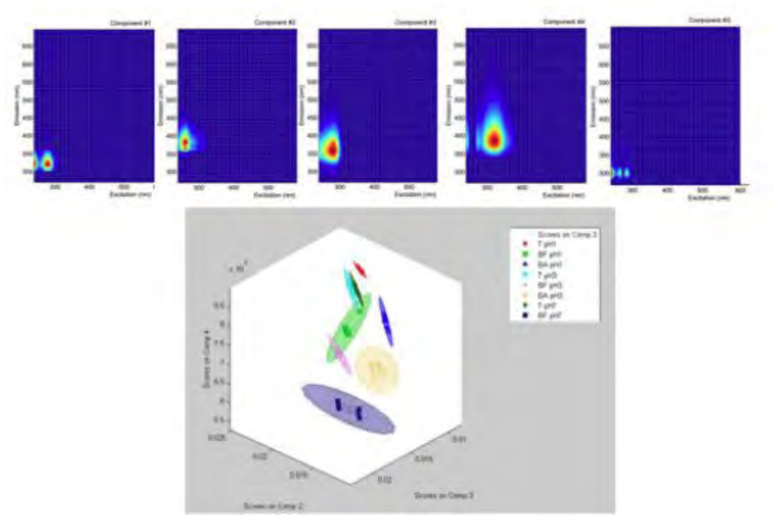

Figure 5: Five component (C5) PARAFAC model constructed from the fluorescence EEMs comprising the evaluation of 3 × 3 class groups as a function of storage vessel (tank, French barrel and American barrel) and solution pH (1, 3.9, 7) and 6 repetitions per class group (total of 54 files).

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PARAFAC model decomposes 3D-signals into a fixed number of statistical components (scores) that in turn describe the variability of acquired AT-EEM. In our study, a five component PARAFAC model (C1-C5) best explained the variability of fluorescence signatures of red wines as a function of pH. The dominant component C4 presents a maximum emission signal of 375 nm (emission) and 320 nm (excitation), whereas said emission/excitation profiles present accurate agreements with fluorescence of flavonoid like moieties [11]. C2 and C3 scores present emission/excitation peaks at respectively (375/260), (355/270) nm that most likely are due to specific polyphenol content, whereas, as appreciated by the absorbance – transmittance profile, it possess a pH dependency that can be traced within the PARAFAC cluster C4-C2-C3, depicted in Figure 5.

Conclusions

This work presents for the first time NMR/A-TEEM traceable molecular (polyphenolics) fingerprints, linked to the chemistry involved in aging processes, using a set of mono-varietal Querétaro Merlot samples as a model system. First, the use of raw and first derivative-pH dependent – UV-Vis (Δ)Absorbance spectroscopy is proposed to elucidate a simple-to-complex phenolics profile within wine samples, in terms of (Δ) Absorbance lines at 275, 520 and 715 nm. In parallel, methodological improvements allowed to obtain a proton NMR fingerprint of studied samples that, in turn, revealed a novel exploitable aromatics region, whereas signal integration of key regions, present excellent correlation with UV-Vis data, for cross-checking the novel method of analysis. Two dimensional Absorbance (Transmittance) – Excitation Emission Fluorescence Matrix and processing with a five component PARAFAC cluster accurately describe the variability of polyphenols in wines as a function of pH and different aging processes.

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In memoriam of Ernesto Ladron-de-Guevara.

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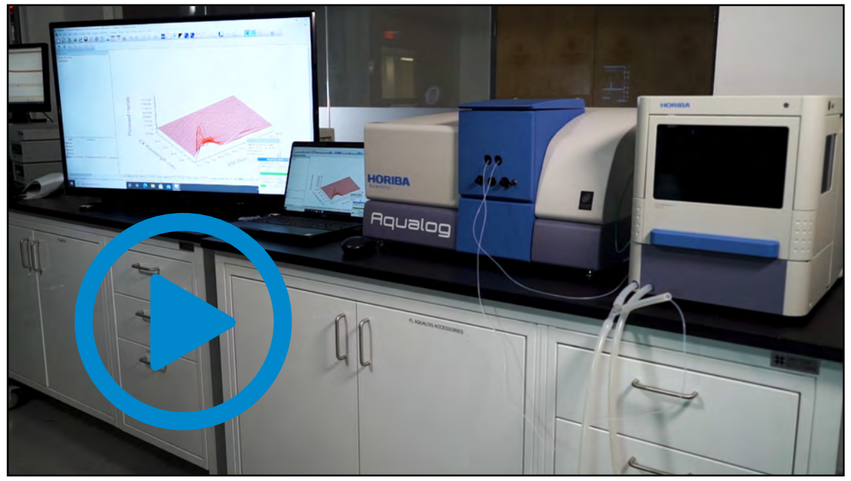

A-TEEM for Wine Compound Analysis

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