# Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting

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The patented (US patent US8,901,513) Aqualog which facilitates simultaneous **A**bsorbance- **T**ransmission and fluorescence **E**xcitation-**E**mission **M**atrix (**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 mouth-feel, 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 analyti-

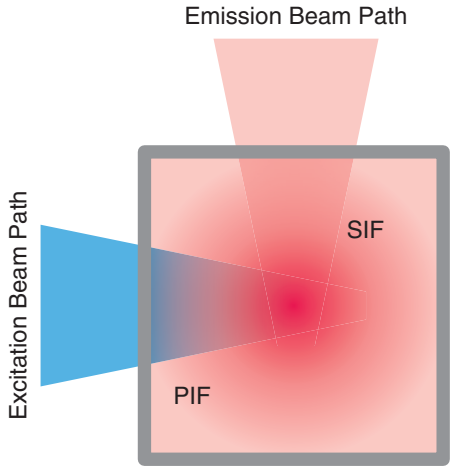


Figure 1 Inner Filter Effect (IFE)

cal services laboratories exist world-wide.

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 reabsorption 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 reabsorption of emitted fluorescent light.

Figure 2 shows spectral consequence of the inner filter effect. The shorter wavelength intensities of the fluorescence spectrum are diminished due to reabsorption 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 lot-to-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.

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, correct for color or aroma shortcomings. Both the quantity and the extractability of anthocyanins and tannins increase throughout the grape ripening. Vivid

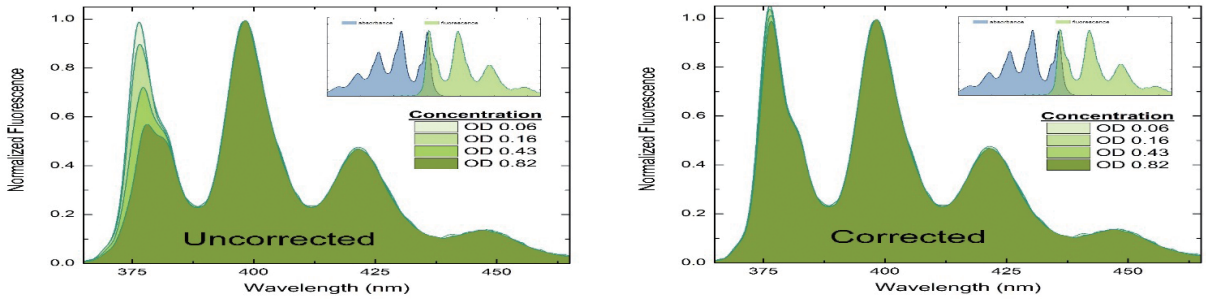


Figure 2 Spectral consequence of the inner filter effect

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 |

Table 2

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

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.

## 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.

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.

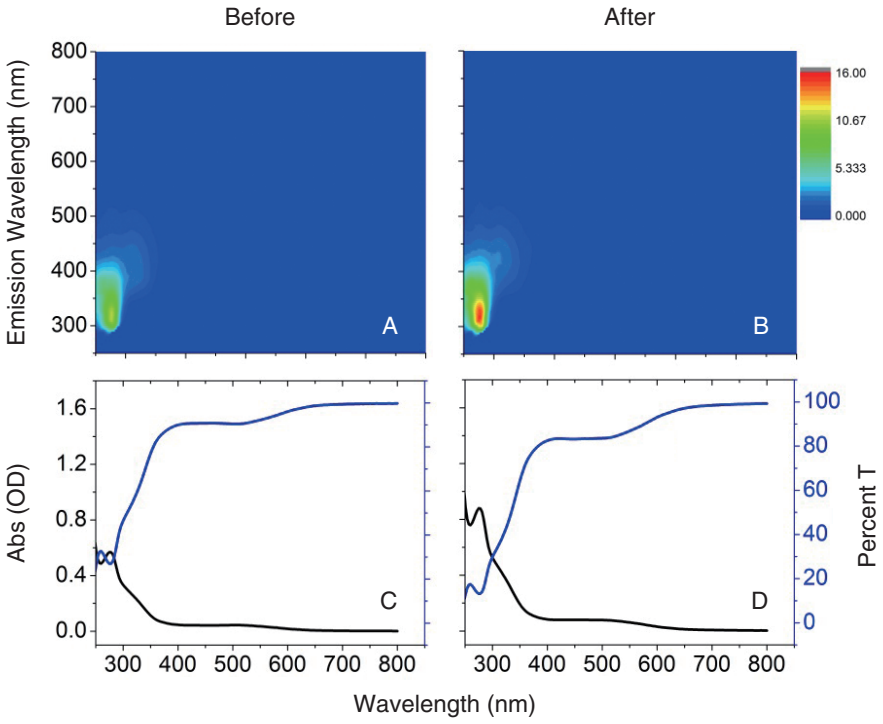


Figure 3 Simultaneously recorded EEMs (A and B) and Absorbance and percent Transmission spectra (C and D) for a typical Italian red wine from a freshly opened bottle Before (A and C) and After a one week exposure to air (B and D).

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, 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 5A illustrates the proximity of all the red wines in

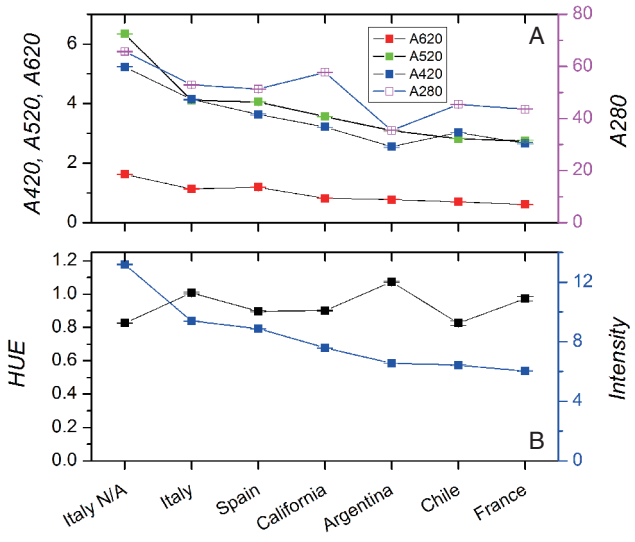


Figure 4 Comparison of the Absorbance parameters (A) and *Hue* and *Intensity* parameters (B) defined above, measured with Aqualog for a series of freshly opened red wines from various countries.

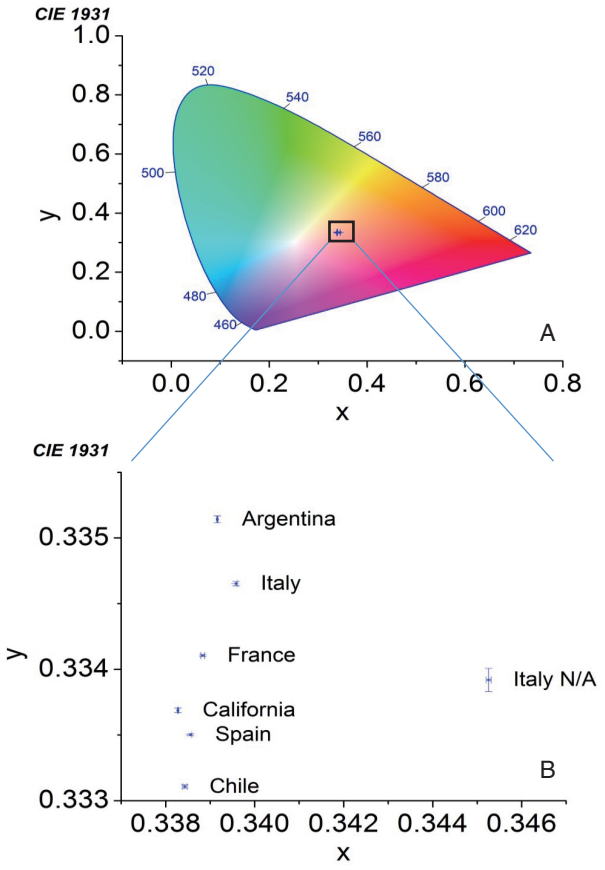


Figure 5 CIE 1931 x and y coordinates for each of the wine samples from Figure 4.

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

| 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 |

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.

## EEM PARAFAC Analysis

PARAFAC is a tri-linear matrix decomposition method. In the following it is used to yield 1) an excitation and 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 ($r^2$: % 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]

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.

## Varietal Fingerprint-Cluster Analyses with PARAFAC and PCA

The three major fluorescence PARAFAC and two-way

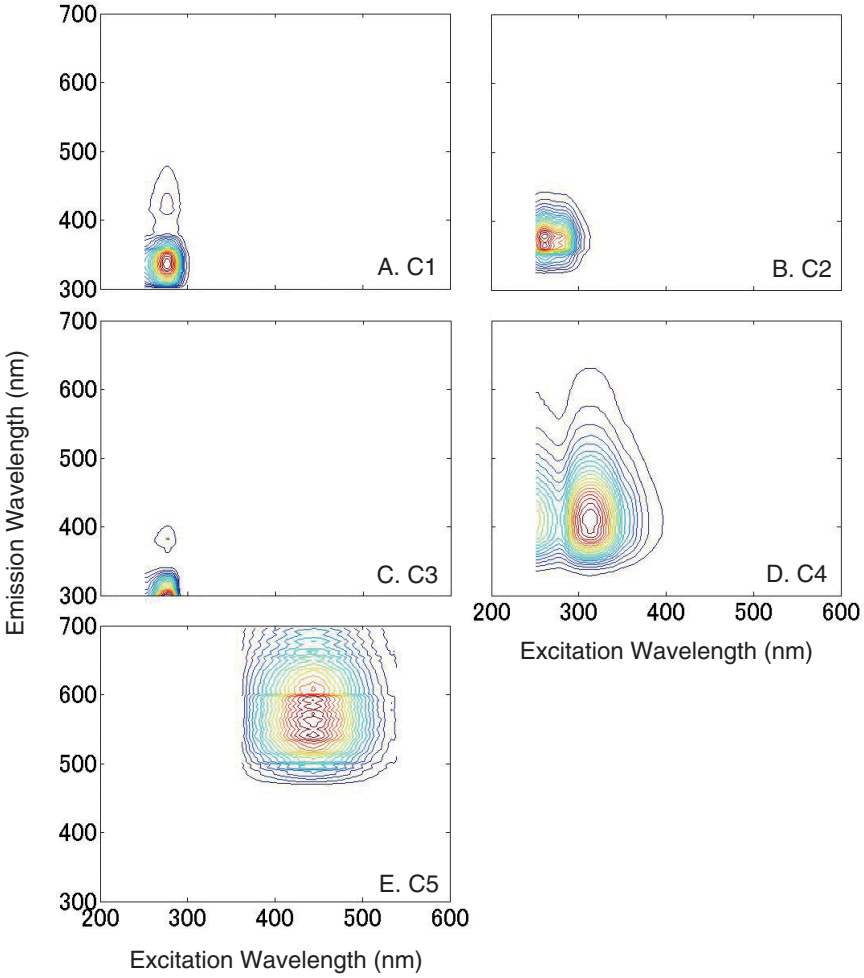


Figure 6 IFE corrected excitation and emission spectral contour loadings for the five component model evaluated for all wine sample replicates before and after oxidation. The PARAFAC model (n=84) ) was described with an $r^2$=0.997 and a split half validation score $r^2$=0.906 (where the model is tested with two halves of the dataset separately) .

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.

Table 4 Qualitative assignment of PARAFAC components

| 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 |

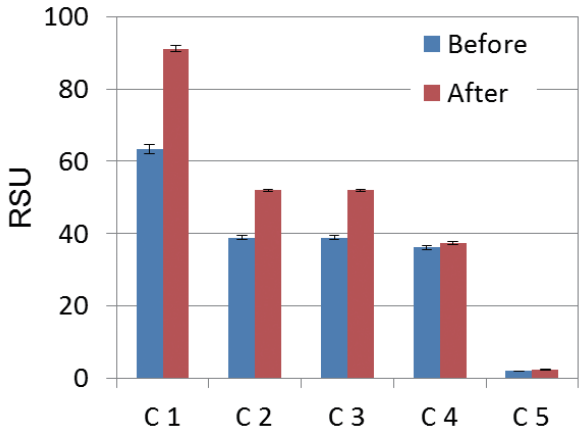


Figure 7 Comparison of the five PARAFAC component scores in the Italy wine samples ($n$= 3 replicates per sample) before and after oxidation. Component scores are reported normalized water Raman scattering units (RSU).

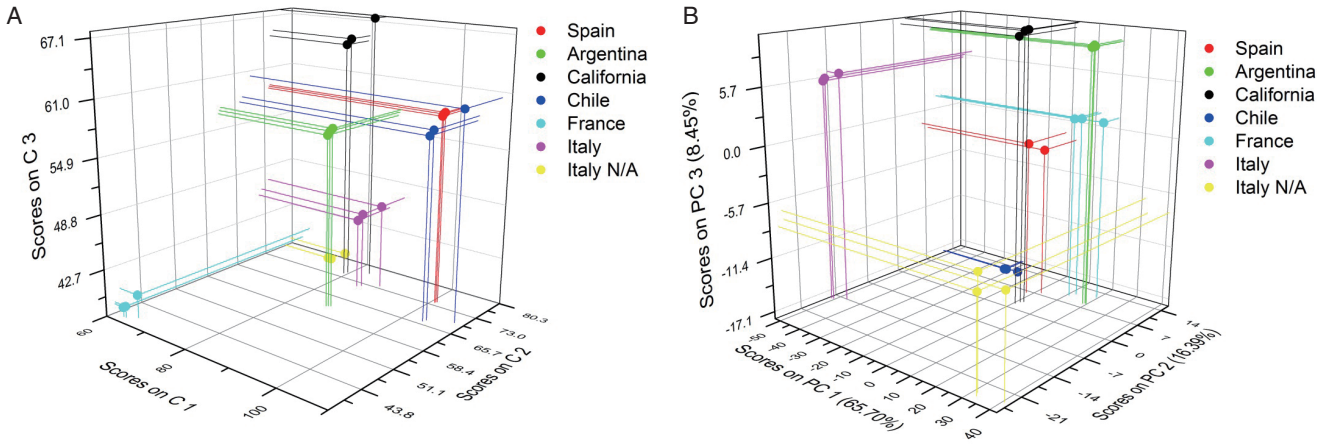


Figure 8 Comparison of the three major component cluster plots for PARAFAC (A) and PCA (B) analyses of the freshly opened wine samples (*n*=3 replicates per sample).

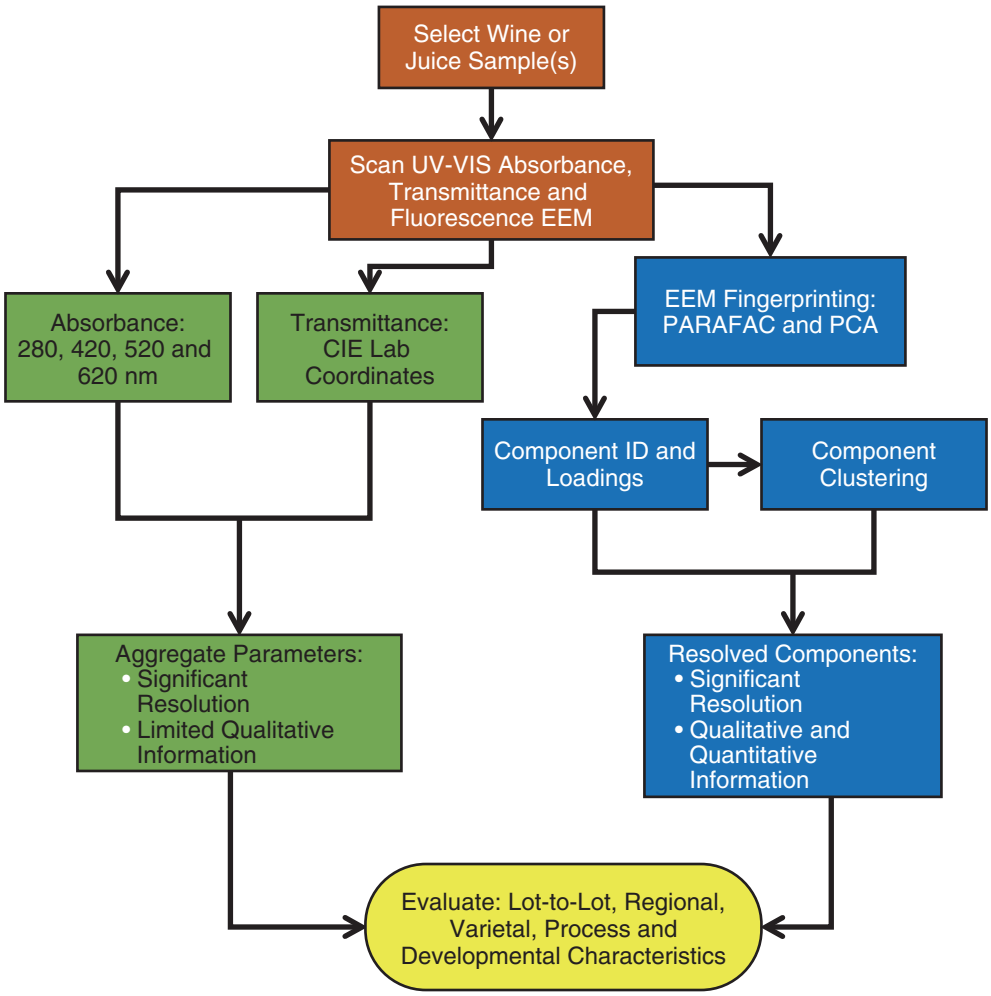


Figure 9 Schematic of the relationships among the simultaneous Absorbance and EEM data acquisition and analysis with respect to the significance of wine sample and component resolution pertaining to this study.

## Discussion and Conclusion

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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