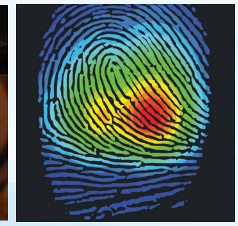
HORIBA

Fluorescence

Is My Wine Red Enough: Detecting Other Additives Through A-TEEM



Application Note
Agricultural Sciences
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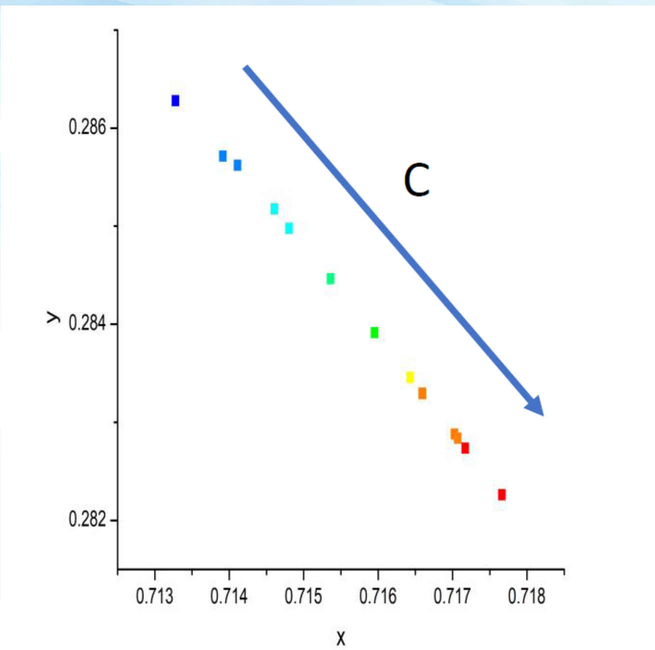
Adam Gilmore, Ph.D., Aqualog Product Manager and Senior Scientist, and Lyufei Chen, Ph.D.

In the world of winemaking, color is one of the first sensory experiences, setting the stage for the entire tasting journey. Yet, various winemaking practices and environmental factors can diminish the intensity and richness of a wine's color. This has led some winemakers to covertly enhance their wines with concentrates from intensely colored (Teinturier) grapes. Detecting these additions is challenging due to the complex pigment compositions in red wine grape varieties.

Introducing A-TEEM Spectroscopy

Absorbance–transmittance excitation-emission matrix (A-TEEM™) spectroscopy is making waves in the wine industry. This technique rapidly (<1 minute) provides coordinated measurements of UV-Vis absorbance spectra, extinctions, and fluorescence quantum efficiencies, along with excitation-emission matrix (EEM) spectral contours. This comprehensive data set enables chemically specific detection of color additives, overcoming the complexity of varietal wine matrices.



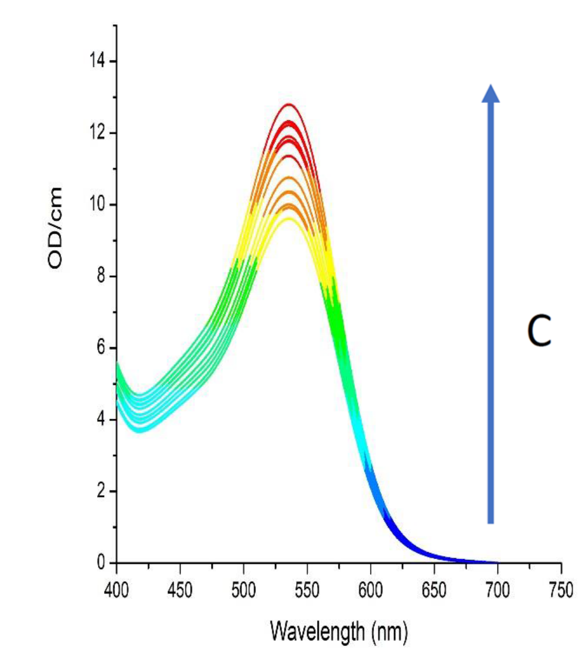


Figure 1 and 2: Commercial Additive Concentrates (CAC) of Teinturier grapes with intensely colored skins and flesh, enriched in Malvidin-3,5-diglucoside, are commonly covertly used to enhance color in finished wines from Vitis vinifera sp. grapes. The target CAC concentration is typically at least 0.2% by volume.

Recent studies, including those by Gilmore et al., have demonstrated that A-TEEM spectroscopy, combined with chemometrics, can offer precise analysis of various colored and fluorescent compounds in grape extracts and wine. This method can accurately discriminate grapes and wines based on production region, variety, blend, and vintage, providing a molecular fingerprint for each sample.

Understanding Anthocyanins

Anthocyanins are the primary red-colored compounds in red wine. In Vitis vinifera, the predominant anthocyanin is malvidin-3-O-glucoside, whereas Teinturier grapes feature other di-O-glucosidic anthocyanins, such as malvidin-3,5-di-O-glucoside. Commercial color additive concentrates (CACs) are enriched in these di-O-glucosidic anthocyanins, making them a target for detection.,

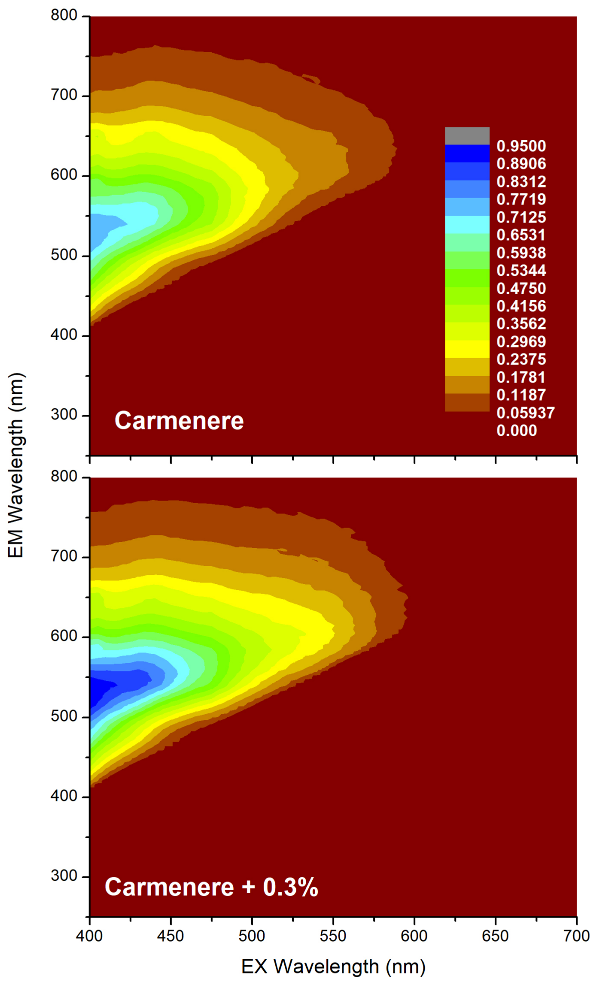
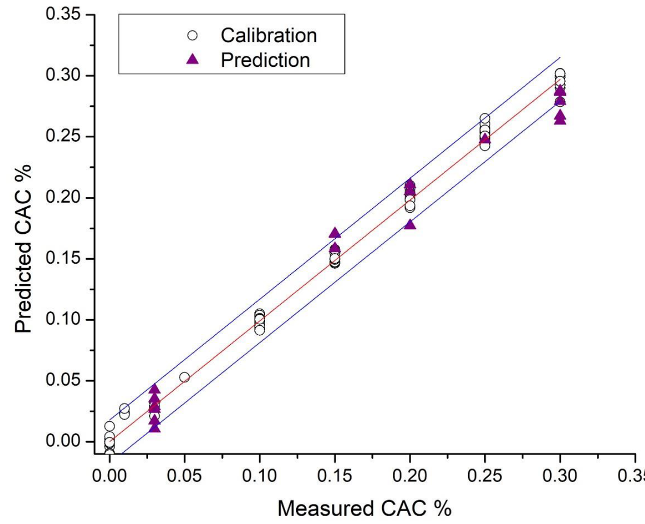


Figure 3: This study investigates the detection of CAC with A-TEEM spectroscopy using a recently introduced, single variable adjustment Classical Least Squares (CLS) method, known as Gray-CLS (Eigenvector Inc., Solo v9.3.1). The first-principles based Gray-CLS results are compared to other multivariate regression methods, including Partial Least Squares (PLSR) and Locally Weighted Regression (LWR). The experiment included several wines from 6 different varieties spiked at varying concentrations with a commercially available CAC. A-TEEM measurements were collected under Beer-Lambert linear absorbance conditions at a constant temperature (20° C) using a standard solvent (50% EtOH pH2) and filtration (0.45 M pore size). The model data included both a calibration (cross validation set) of around 80 % of the samples and an independent validation set comprising around 20%. The Gray-CLS model yielded a standard deviation (SD) of around 10% of the target CAC (0.2%) for the validation set by optimizing only the General Least Squares Weighting variable of the CLS residuals. PLSR and LWR achieved, respectively, similar to or slightly lower SD values. However, these methods all required complex, and potentially ambiguous, optimization of multiple preprocessing variables of the spectral and concentration data blocks; other algorithm tuning parameters, including, the number of latent variables and or principal components, among others, were also adjusted. These methods, being prone to under- or over-fitting, are thus potentially unreliable. We conclude the A-TEEM method can be an effective tool to quantify CAC using Gray-CLS to avoid issues with under- and over-fitting multivariate regression models, yielding results relevant to commercial wine quality evaluation



Assessing Detection

In a recent study, six wine varieties, each with unique phenolic and anthocyanin compositions, were spiked with a CAC range of 0–0.35%. A typical minimal target CAC is about 0.2%. For example, in Carmenere wine, a 0.3% CAC addition noticeably increased absorbance intensity around 520 nm, the wavelength where the red color in wine is assessed. Additionally, EEMs revealed significant increases in the intensity of complex fluorescence contours in the red emission region.

Enhancing Analytical Leverage

The study utilized multi-block (data-fusion) arrays of absorbance spectra and unfolded fluorescence EEMs for regression and discrimination analyses. This approach enhances the molecular specificity of A-TEEM by coordinating absorbance extinction and fluorescence properties. A linear regression analysis calibration with 90 measurements and a validation set of 18 samples yielded an $R^2P = 0.9931$, with a root mean square error of prediction (RMSEP) of 0.0158% CAC—less than 10% of the minimal target value of 0.2%.

The calibration and validation sets were also evaluated using discrimination models with a threshold CAC set at 0.03%, confirming a 100% detection accuracy for samples above this value.

Practical Applications

These findings are consistent with literature reports on A-TEEM's ability to accurately quantify anthocyanin and phenolic compounds across diverse grape varietals. This technique offers a valuable tool for wine quality evaluation and competitor product assessment. Moreover, these analyses can be automated for routine lab operations, supporting the wine industry's need for quick, reliable quality control measures.

info.sci@horiba.com **horiba.com/fluorescence**

USA: +1 732 494 8660 **France:** +33 (0)1 69 74 72 00 **Germany:** +49 (0) 6251 8475 20
UK: +44 (0)1604 542 500 **Italy:** +39 06 51 59 22 1 **Japan:** +81 (0)3 6206 4721
China: +86 (0)21 6289 6060 **Brazil:** + 55 (0)11 2923 5400 **Other:** +1 732 494 8660