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Innovative Optical Methods for Analytical Monitoring of Bionanoagents with Emphasis on Georgian White Wine Authentication

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Abstract: The integration of bionanoagents in the food industry has highlighted the need for accurate analytical methods to verify and authenticate products. This study introduces innovative optical methods for monitoring bionanoagents, with a focus on classifying and authenticating Georgian white wines. Through comprehensive spectral analysis, we have developed reference models for quick product verification. The application of Parallel Factor Analysis (PARAFAC) to 3D fluorescence spectra enables precise differentiation of wine components. Results confirm the effectiveness of our approach, marking significant progress in combating food and beverage fraud and indicating the potential for further application in bionanoagent research.

Keywords: Spectroscopy, Bionanoagents, PARAFAC, Wine authentication

Introduction

In today's rapidly evolving world, the quality of food and beverages significantly impacts human health and well-being. Analytical control plays a crucial role in ensuring the safety and integrity of consumables, particularly as the scale of production and the complexity of food systems increase (Smith & Johnson, 2019). The proliferation of industrial technologies and the expanding volume of production necessitate not only improvements in production efficiency but also rigorous enforcement of safety and quality standards (Wold, 1976). As production expands, so does the potential for fraud, especially in high-value commodities like wine, where counterfeiting can damage the reputation of brands and pose significant health risks to consumers (Urbano et al., 2006).

Optical analytical methods are pivotal in identifying and quantifying the chemical composition of substances, including emerging bionanoagents, which are increasingly relevant in modern technological and environmental research (Murphy et al., 2013). The challenge lies in selecting and adapting the most appropriate optical methods to the unique properties of each material, ensuring the accuracy and reliability of the analyses (Airado-Rodríguez et al., 2011).

The objective of this study is to develop a novel analytical method that utilizes optical measurements, leveraging a database created from existing laboratory equipment. This method aims not only to identify various types of fraudulent activities in the food and beverage sectors but also to establish reference models for the

classification of Georgian white wines based on their spectral characteristics (Azcarate et al., 2015). By addressing these issues, this research will enhance the level of analytical control in production processes and in the circulation of food products, while also facilitating the exploration and application of bionanoagents in various scientific and technological domains (Tauler et al., 2015).

The integration of bionanoagents into the food industry requires precise methods to verify the authenticity and safety of products. Optical analytical methods are pivotal in this context, identifying and quantifying the chemical composition of substances, including bionanoagents, which play a crucial role in technological and environmental research (Khajishvili et al., 2023a).

The challenge lies in selecting and adapting the most suitable optical methods to the unique properties of each material, ensuring the accuracy and reliability of the analyses. This study aims to develop a novel analytical method that utilizes optical measurements, leveraging a database created from existing laboratory equipment. This method is designed to identify fraudulent activities in food and beverages and establish reference models for the classification of Georgian white wines based on their spectral characteristics. Such innovations are crucial as they enhance analytical control in production processes and in the distribution of food products, facilitating the exploration and application of bionanoagents across various scientific and technological domains (Khajishvili et al., 2023b).

Methodology

Our research involves the utilization of three-dimensional fluorescence spectroscopy (3DF), a technique that has proven effective in analyzing various types of wine. This method enables us to dissect the 3D fluorescence signal into a fixed number of statistical components, which are predefined for each wine type and are conventionally referred to as standards. These benchmarks comprehensively describe the excitation/emission spectra.

The 3DF method offers advantages over other statistical methods, such as principal component analysis (PCA), due to its unique capability to unfold spectra. This feature allows further in-depth analysis of wine fluorescence spectra by PCA. Subsequently, to streamline the number of benchmarks, we employ the Tolerant Benchmark Selection Method (TES), which assesses how well a wine sample conforms to specific benchmarks.

The utility of TES in wine classification is significant, as the types and concentrations of molecules—such as polyphenols, vitamins, and amino acids—vary depending on the wine's type, maturity, and production techniques.

The primary objective of this study is to enhance the capability of fluorescence spectroscopy by incorporating Parallel Factor Analysis (PARAFAC) for the excitation/emission matrix (AEM) analysis. We also plan to apply PCA and model TES analog classes based on the hematological and optical characteristics of erythrocytes. Our methodology is supported by a progressively modernized hardware setup and the development of new analytical approaches.

Fluorescence spectra will be captured using the Black Comet spectrometer (200-950 nm), manufactured by StellarNet. Various frequencies of LED lamps will serve as the light source. A 100 µl sample of erythrocyte substitute treated blood will be placed in a quartz cuvette for spectrum recording at room temperature. We will perform multiple scans to mitigate any drift effects, calibrating the standard at the start of each experimental day. The excitation wavelengths will range from 250-500 nm, and the emission wavelengths from 275-600 nm, with measurements taken at 5 nm increments. Each day, the wavelength system is recalibrated based on peak combinational scattering (Raman scattering) to compensate for potential instrument wavelength drift. Total scan time per sample is approximately 10 minutes, and measurements are conducted over a period of no more than two months to minimize atmospheric and instrumental fluctuations, such as lamp intensity variations.

For data classification, PCA is used for an initial descriptive analysis of spectral features, followed by TES for light-independent modeling of analog classes. Graphical visualization of spectra is facilitated using the SpectraWiz and Mathematica software packages.

To model the excitation and emission data, the wavelengths for N samples are arranged in a three-dimensional array sized $I \times J \times K$, where I is the number of samples, J is the number of emission wavelengths, and K is the

number of excitation wavelengths. In the PARAFAC model, the array is decomposed into its components such that the norm of the residuals, E, is minimized:

$$x_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk}$$

Elements a_{in} of matrix A (often termed the stroboscopic matrix) represent the concentration of fluorophores n in sample i, while elements b_{jn} and c_{kn} of matrices B (radiation) and C (excitation) respectively are scaled to estimate the emission spectrum and absorption coefficients of the fluorophores. To ensure the model's feasibility, a non-negativity constraint is applied to the concentration and excitation/emission coefficients. Finally, by analyzing the agreement percentage of the active zones relative to a diagnostic sample, we estimate the optimal number of components needed for effective analysis.

In a simple model, fluorescence data can be viewed as a set of signals obtained from independent fluorophores. PARAFAC - the analysis is practically the sum of the signals obtained from the fluorophores. The e_{ijk} elements represent the deviation from the statistical mean for each sample. The PARAFAC expansion is performed so that the norm of the E array is minimal. Within this model, the elements of the matrix a_in can be interpreted as the concentration of fluorophores n in sample i. The load matrix elements b_{jn} are the basis for the scaled spectrum estimation of the n-th fluorophore at the j-frequency, while the c_{kn} matrix element is proportional to the absorption coefficient of the fluorophore at the k-th frequency. It is necessary to introduce a negativity boundary condition because the concentration and excitation/emission array (AEM) coefficients cannot be negative. We need to calculate the percentage of agreement of the active zone in all cases according to the diagnostic sample in order to get an initial idea about the optimal amount of components

Experiment Statement

Spectrometer installation: Connect the BlackComet detector to the cuvette holder with fiber optic cables, and to the recording device with the USB port. We used StellarNet's SpectraWiz software for signal registration. Install the software and configure the BlackComet detector license code, specifying the attached parameters.

- **Sample preparation:** Place the sample in the cuvette and make sure it is properly aligned with the light beam.
- **Capture spectra:** record the spectrum of the sample using the spectrometer software.
- **Photographing reference or standard spectra:** we placed the control sample in the so-called reference in the cuvette and capture the spectra. It should be noted that the control sample contained the same solvent as the test sample to exclude the influence of solvent-induced background absorption.

Our research involves the formation of three-dimensional fluorescence spectra, that is, we rely on 3D fluorescence spectroscopy (3DF), which have been successfully used in the analysis of various types of wine. In this method, the 3D fluorescence signal is divided into a fixed number of statistical components. For each type of wine, its strict definition is carried out and we conventionally call it standards. The benchmarks describe the excitation/emission spectra in detail.

Fig.1.a Illustrates the impact of cuvette path length on optical density, showing that increasing path length (0.05, 0.5 and 1 cm) reduces optical density (indicative of increased absorption) and vice versa. Fig. 1.1b depicts the linear relationship between optical density and cuvette path length at varying concentrations (0.1, 0.5, 1 mg/mL), with higher concentrations resulting in steeper increases in optical density. The concentration of the solution was chosen so that the absorbance fell within the linear range of the detector, typically 0.2 to 1.0. The concentration affects the accuracy of the measurement, because the absorption of light is affected by factors such as solvent activity, temperature, and the composition of other compounds. BlackComet spectrometers typically use a 1 cm path length cuvette. However, for samples of low concentration or high absorbance, a shorter path length cuvette is suitable to avoid saturation of the detector signal. Conversely, high concentration or low absorbance samples may require a longer path length cuvette to obtain a measurable signal.

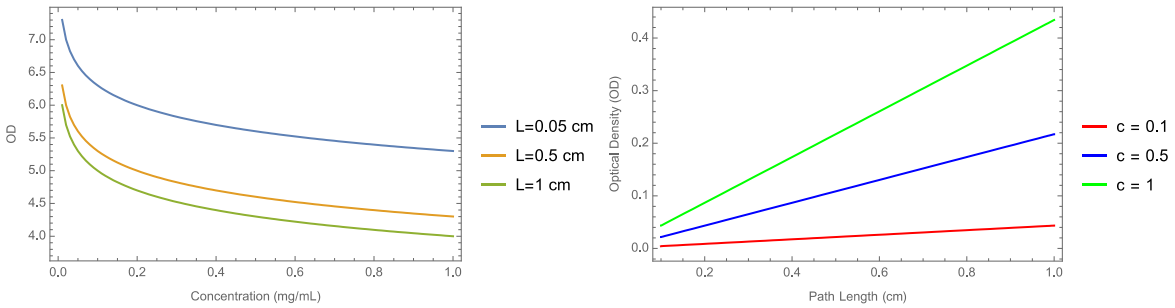


Figure 1. a) OD vs concentration, b) OD vs path length

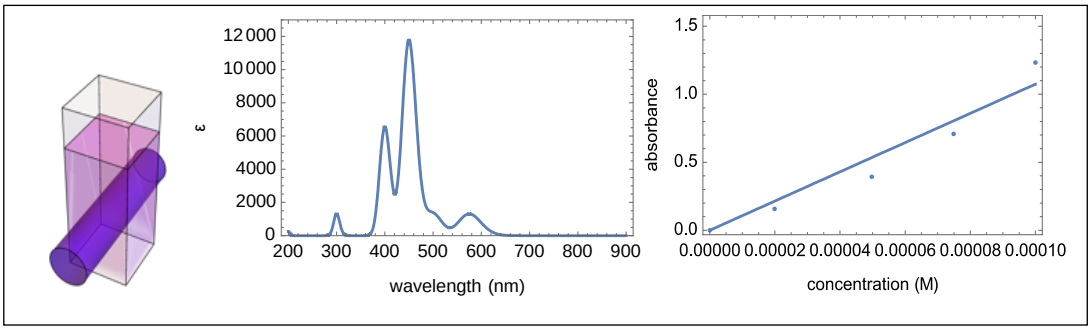


Figure 2. OD vs the molar ratio spectrum of the sample

Figure 2 shows the dependence of the optical density on the cuvette path length at different concentrations. This relationship is linear, with a steeper graph of optical density corresponding to a higher concentration. The absorption spectra of ultraviolet and visible light, when the visible region of the spectrum includes photon energies from 36 to 72 kcal/mol, and in the near-ultraviolet region (up to 200 nm), this energy range increases to 143 kcal/mol. Ultraviolet spectra with wavelengths shorter than 200 nm are difficult to process, so they are rarely used for structural analysis of substances.

The presence of light-absorbing molecular groups - chromophores - in a molecule is best confirmed by UV-visible spectroscopy, but most spectroscopic instruments for wavelengths below 200 nm are practically problematic in terms of detecting isolated chromophores. Fortunately, electron coupling generally causes the absorption maxima to shift to longer wavelengths (for example, in the case of isoprene).

It is shown that the molar absorptivity (ε) can be very large for strongly absorbing chromophores (>10000) and very small for weakly absorbing chromophores (from 10 to 100). The magnitude of ε reflects both the size of the chromophore and the probability that light of a given wavelength will be absorbed by the chromophore when light falls on it::

$$\varepsilon = 0.87 \cdot 10^{16}\, PS$$

where P – is the transition probability, it is placed between 0 and 1, S- is the area of the chromophore (in m^2).

3D Analysis of the Detected Signal

The quantum efficiency or sensitivity function for a particular CCD (Charge Couple Device), such as StellarNet's CCD detector in the BlackComet spectrometer, will usually be provided by the manufacturer. It usually varies with wavelength and can be quite complex, often requiring calibration against a light source with a known spectrum. The quantum efficiency curves of the CCD of the BlackComet spectrometer are roughly Gaussian in shape, peaking where the CCD is most sensitive and tapering off towards the edges of its range.

Let's say the CCD sensitivity function in the Black-Comet spectrometer is given by Gaussian form:

$$g(\lambda) = exp\left[\frac{(\lambda - \lambda_0)^2}{2\sigma_\lambda^2}\right]$$

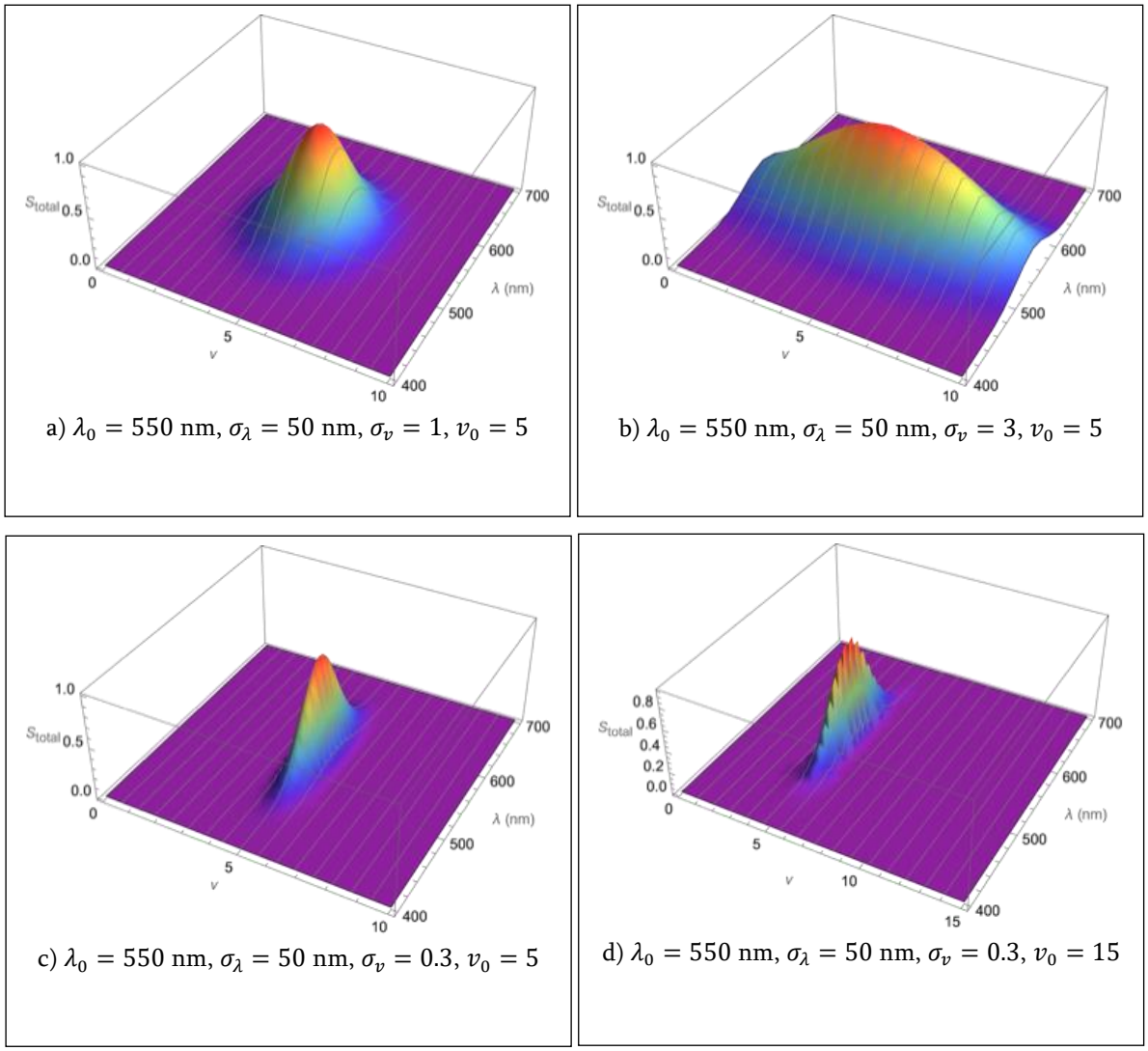
where, λ_0 – is the wavelength (in nm), where CCD sensitivity peaks, λ is the wavelength (nm), σ_λ - standard deviation of the CCD sensitivity curve.

For the simplicity of calculations, let's use the simplified representation of the Franck-Condon factor:

$$FCF(v, v_0) = exp\left[\frac{(v - v_0)^2}{2\sigma_v^2}\right]$$

v_0 – corresponds to the starting point of the vibration propagation of the CCD detector, it actually corresponds to the point of incidence of the light photon, σ_v – reflects the width of the vibrational wave. The signal detection sensitivity of the CCD detector can be calculated by a simplified formula:

$$S_{total}(v, \lambda) = FCF(v, v_0) \cdot g(\lambda).$$



a) $\lambda_0 = 550$ nm, $\sigma_\lambda = 50$ nm, $\sigma_v = 1$, $v_0 = 5$

b) $\lambda_0 = 550$ nm, $\sigma_\lambda = 50$ nm, $\sigma_v = 3$, $v_0 = 5$

c) $\lambda_0 = 550$ nm, $\sigma_\lambda = 50$ nm, $\sigma_v = 0.3$, $v_0 = 5$

d) $\lambda_0 = 550$ nm, $\sigma_\lambda = 50$ nm, $\sigma_v = 0.3$, $v_0 = 15$

Figure 3. 3D distribution of the signal detected by the CCD detector

In summary, graphical analysis indicates that both molecular characteristics (represented by Franck-Condon factors) and the wavelength sensitivity profile of the CCD play a critical role in determining the detected signal intensity. The graphs presented in Figure 3 are unique and can be used to understand the correlation between molecular spectroscopy and CCD detection capabilities, which will ultimately help to optimize spectroscopic measurements and properly plan experiments.

Graphical relationships reveal that the peak value of the absorption spectrum is directly proportional to the absorption levels. The graphs are taken at a fixed concentration of the substance to be investigated in the sample, in this case it is moles for the sample placed in the cuvette. The effectiveness of using the TES

analytical method in wine classification lies in the fact that the types of molecules (such as polyphenols, vitamins, amino acids) and their quantity depend on the specific type and maturity of the wine, as well as on the wine technology.

As mentioned, the main objective was to explore the potential of fluorescence spectroscopy considering excitation/emission matrix (AEM) analysis with parallel factors (PARAFAC). In particular, we performed PCA analysis and light independent modeling of TES analogue classes according to wine product variety and origin. For this, we studied about 100 samples of four-five types of white Georgian wine. The methodology chosen by us was based on the one hand on the hardware complex, which was gradually modernized by our group, on the other hand on the development of new analytical approaches, which are quite acceptable to be used in typical laboratory control of food products and beverages. Using PCA analysis, we built tables and graphs for a specific group of samples.

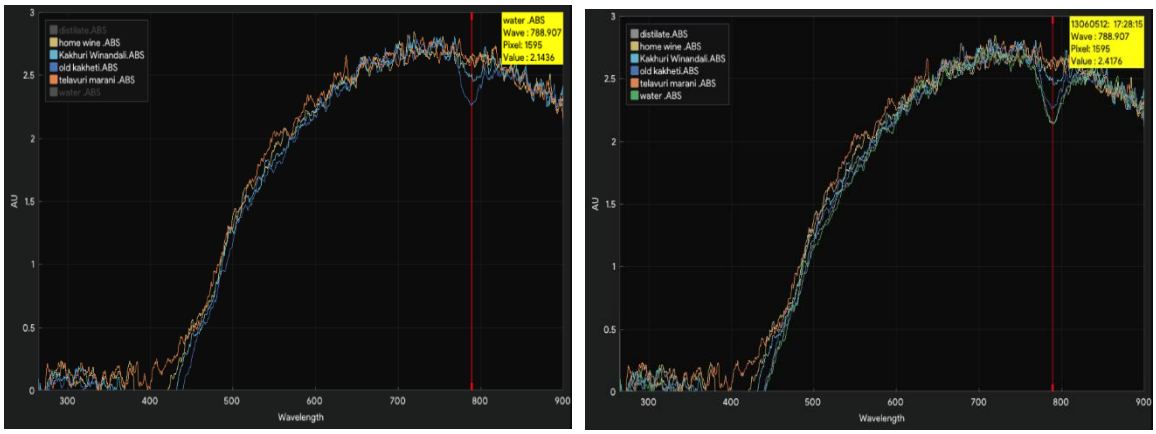


Figure 4. Absorption spectra of Georgian white wine (excitation wavelength 396 nm LED lamp)

In processing the wine spectrum, we used multivariate analysis techniques (PARAFAC) such as PCA (Principal Component Analysis) to highlight the main features of the data. We performed statistical analysis of the data to identify patterns or differences between different wine samples.

Our conclusions mainly concern the measuring device. These results refer to the CCD-detectors of the BlackCommet spectrometers manufactured by StellarNet, namely:

1. The spectra show a larger change in the average signal level at a given integration time, possibly due to thermal fluctuations between measurements. Therefore, the optimal level of thermal stabilization was selected for the spectrometer. As the measurements showed, to achieve thermal stabilization, the average signal level should be calculated with about 100 integrations in 180 milliseconds.
2. One of the main features of the spectrometer is the so-called "dark current" which averages about 0.25% of the peak value (~40,000 photons, with 100 integrations). These data were calculated in the combined spectrum of the deuterium and halogen light sources.
3. Data collected during various integrations show that until the integration time is increased to ~2000 msec, digital data acquisition of the signal continues. Further increasing the integration time leads to an approximately linear increase in the dark current.

Conclusion

The application of advanced optical spectroscopic techniques in the analytical control of bionanoagents has been comprehensively explored, with a specific focus on the identification and classification of Georgian white wines. The research has employed a multifaceted spectral analysis approach, culminating in the development of reference models for rapid identification of oenological products.

Key findings from the study reveal the effective detection of critical wine constituents such as flavonoids, tannins, alcohol, sugars, and acids, utilizing the ultraviolet and visible spectroscopy facilitated by deuterium lamps. The spectral data obtained indicates that flavonoids exhibit characteristic absorption in both UV (below 400 nm) and visible (400-700 nm) regions, while tannins, being polyphenolic in nature, absorb in the

wavenumber range of 1600-1800 cm^{-1}, as identified by infrared spectroscopy. The alcohol content, pivotal to wine quality, is distinctly marked at wavelengths 210 nm and 275 nm.

The utilization of deuterium lamps for UV spectroscopy has notably enhanced the accuracy of the analysis by minimizing water interference, a vital consideration when analyzing aqueous solutions such as wine. This strategic decision has underscored the necessity of tailoring spectroscopic methodologies to the unique characteristics of the sample in question.

The research reinforces the potential of fluorescence spectroscopy, coupled with parallel factor analysis (PARAFAC) and principal component analysis (PCA), to not only distinguish between different types of wine but also to identify fraudulent activities within the food industry. The methodologies and approaches developed in this study are poised to make a significant impact on ensuring the authenticity and quality of wine, thus contributing to the integrity of the food industry and consumer safety.

In the broader context, the findings underscore the need for ongoing development of analytical methods to meet the demands of contemporary technological and environmental research. The study lays a foundation for extending the application of these methods to the investigation of bionanoagents, potentially influencing various scientific and technical domains.

The spectroscopic analysis of wine samples has not only demonstrated the capability to detect and quantify specific molecular components that determine the organoleptic qualities of wine but also showcased the robustness of spectroscopic techniques as essential tools in the arsenal against food and beverage fraud. The study's methodologies pave the way for further research and the refinement of analytical techniques that can be used in quality control and product authentication, reinforcing the indispensable role of spectroscopy in the modern scientific landscape.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

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References

Airado-Rodríguez, D., Durán-Merás, I., Galeano-Díaz, T., & Wold, J. P. (2011). Front-face fluorescence spectroscopy: A new tool for control in the wine industry. *Journal of Food Composition and Analysis, 24*(2), 257-264.

Azcarate, S. M., & others. (2015). Modeling excitation–emission fluorescence matrices with pattern recognition algorithms for classification of Argentine white wines. *Food Chemistry, 184*, 214-219.

Murphy, K. R., Stedmon, C. A., Graeber, D., & Bro, R. (2013). Fluorescence spectroscopy and multi-way techniques. Parafac. *Analytical Methods, 5*, 6557-6566.

Smith, J., & Johnson, A. (2019). Fluorescence spectroscopy in wine quality control. *Journal of Wine Research, 30*(2), 123-135.

Tauler, R., Bejarano, A., & Walczak, B. (2015). Recent advances in multivariate curve resolution-alternating least squares (MCR-ALS): Methodology and applications. *Analytica Chimica Acta, 893*, 14-33.

Urbano, M., Luque de Castro, M. D., Pérez, P. M., García – Olmo, J., & Gómez – Nieva, M. A. (2006). Ultraviolet – visible spectroscopy and pattern recognition methods for differentiation and classification of wines. *Food Chemistry, 97*(1), 166-175.

Wold, S. (1976). Pattern recognition by means of disjoint principal components models. *Pattern Recognition, 8,* 127-139.

Khajishvili, M., Shainidze, J., Makharadze, K., & Gomidze, N. (2023a). On the development of the fluorescence excitation-emission etalon matrix algorithm of wine. *The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)*, *23*, 93-99.

Khajishvili, M., Gomidze, N., Jabnidze, I., Makharadze, K., Kalandadze, L., & Nakashidze, O. (2023b). Creation 3D fluorescence spectra of wine. *Book of Abstracts JAPMED*, *12*, 100-102.

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