A-TEEM (Absorbance-Transmission Excitation and Emission Matrix) for quality control and fraud identification of food and beverages.

www.a-teem.com

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INTRODUCTION

All food and beverages are nothing but a complex mixture of molecular compounds. The identification and quantification of these components are the basis of the quality control of commercial products, authentication, and discovery of food fraud.

Traditionally, laboratories employ chromatographic **separation techniques**, such as GC-MS and LC-MS, to characterize samples. However, these techniques

- Require laborious calibration
- Need complex sample preparation
- Are expensive
- Are time-consuming
- Are environmentally unsustainable (consumables and waste disposal).

Especially in a manufacturing environment, where quick real-time decisions are crucial to assure quality, chromatography becomes unsuitable.

A-TEEM (Absorbance-Transmission Excitation and Emission Matrix) is an effective alternative to separation techniques in QC/QA.

1. Lakowicz J.,"Fluorescence sensing." *Principles of fluorescence spectroscopy*. Springer, Boston, MA, 1999. 55-56.
2. A Gilmore et al., "Spectroscopic Analysis of Red Wines with A-TEEM Molecular Fingerprinting" HORIBA Readout, No.49 December 2017
3. https://www.horiba.com/int/products/scientific/molecular-and-microanalysis/fluorescencespectrometers/a-teem/
4. "Rapid Extra Virgin Olive Oil Classification and Blend Quantification", HORIBA Scientific 10/2019

FLUORESCENCE AND INNER FILTER EFFECT

Fluorescence spectroscopy relies on the intrinsic ability of the sample to emit a characteristic signal when excited with the appropriate wavelength and, therefore, requires very little and easy sample preparation. Fluorescence EEM (excitation-emission matrix) is already a well-established technique, but its ability to provide a molecular fingerprint of the mixtures is dependent on the concentration of the sample. In fact, **the inner filter effect (IFE),** the absorption of the excitation photons and the reabsorption of the emitted photons by the sample itself, will change the shape and intensity of the emission spectrum[1].

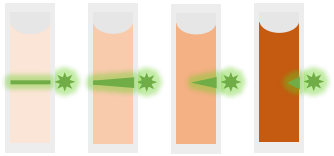

Fig. 1 **Primary inner filter effect**, the attenuation of the incident light due to the absorption by samples with increasing concentration (from left to right).

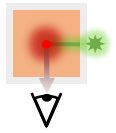

Fig. 2 Schematics of a top-down view of a cuvette, showing the **secondary inner filter effect,** the attenuation of the fluorescence signal detected due to the absorption of the emitted light by the sample itself.

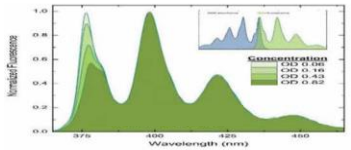

Fig. 3 Emission spectra of samples at different concentration. The signal at shorter wavelengths decreases as the sample concentration increases due to reabsorption of fluorescence[2].

A-TEEM

A-TEEM, a **simultaneous** Absorbance-Transmission Excitation Emission matrix acquisition returns a molecular fingerprint of the sample, independent of the concentration, thanks to data corrected for the IFE (Fig.4). The acquisition of fluorescence and absorbance data with the same optical configuration is essential to have a meaningful correction. Aqualog allows simultaneous acquisitions. The employment of a **CCD detector** makes the acquisition of the fluorescence data extraordinarily fast (Fig.5).

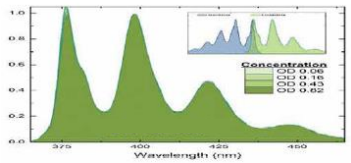

Fig. 4 Spectra of the same samples shown in Fig. 3 corrected by the IFE. The signal intensity is no longer dependent on the sample concentration[2].

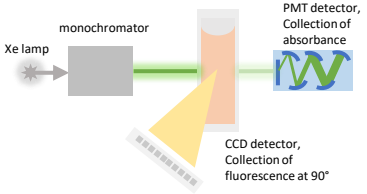

Fig. 5 A continuous light source shines onto an excitation monochromator, which selects a single wavelength. The monochromatic excitation light is directed into the sample. The fluorescence signal is recorded in a single shot by a multichannel CCD detector, at 90° to the excitation beam. Colinear with the beam, the sample's transmittance/absorbance is detected by a single-channel intensity detector.

APPLICATIONS

WINE - The phenolic content of grapes determines the quality of a wine. The simultaneously acquired Absorbance, Transmission and IFE-corrected EEM data can be used to evaluate lot-to-lot, regional, and varietal characteristics. A-TEEM data, in combination with CLS (Classical Least Squares), reveals significant differences amongst wines, reflected visibly in their phenolic compound fingerprints (Fig. 6) [2].

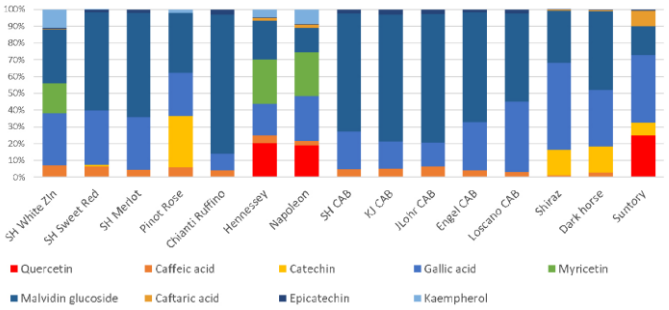

Fig. 6 CLS analysis of the wines, based upon a library of 9 phenolic compounds, provides their relative contribution to the total phenolic fingerprint normalized to 100%. .

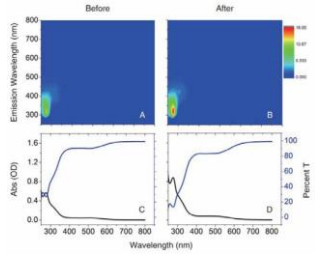

Fig. 7 Simultaneously recorded EEMs (A and B) and Absorbance and percent Transmission spectra (C and D) for a typical Italian red wine from freshly opened bottle before (A and C), and after a one week exposure to air (B and D).

OIL - In fresh, Extra-Virgin Olive Oil, emissions originate from phenols, tocopherols and chlorophylls. During oil deterioration, new fluorescence appears from oxidation products. A-TEEM can be used to screen fluorescent components during storage, to monitor extra-virgin olive oil deterioration (Fig. 8)[3]. PARAFAC (PARallel FACtor) analysis of A-TEEM spectroscopy data is also successful at distinguishing between individual varietals of Extra-Virgin Olive Oil (Fig. 9) [4].

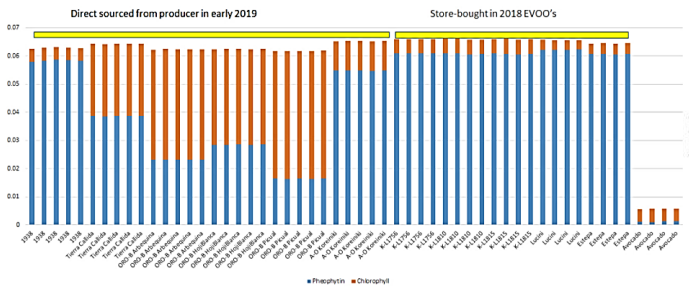

Fig. 8 Relative content of chlorophyll and pheophytin in direct sourced (2019 harvest) and store-bought EVOOs (2018 and 2017 harvest). Avocado oil results are provided for comparison.

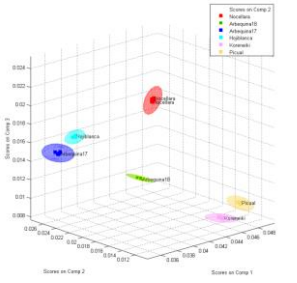

Figure 9: 3-D scoreplot of PARAFAC analysis of Mediterranean varietals.