**Low-cost non-destructive sensors for measuring polyphenols and quality attributes in musts and wines**

Gianmarco Alfieri (gian.alfieri@unitus.it)

Dept.  Dipartimento per l’Innovazione nei sistemi Biologici, Agroali­mentari e Forestali (DIBAF),

University of Tuscia, Viterbo, Italy

Tutor: Prof. Andrea Bellincontro

For the second year of my PhD, I developed a collection of low-cost and non-destructive sensors for monitoring polyphenols fractions in musts and wines. Two prototype spectrophotometers (VIS-NIR) are used to acquire spectra of wines and musts which were then compared with total polyphenol index, anthocyanin, tannin, polymer pigment and colour intensity content, analysed on the same samples, with destructive analytical approaches. Moreover, the concentrations of the different fractions of phenolic compounds in wines have been measured with wet chemistry analysis (HPLC-DAD) and through sensor based on acoustic microwave. Specifically, new chemical functionalization strategies were developed for gold-based acoustic transducers.

**Sensori non distruttivi a basso costo per misurare polifenoli e attributi di qualità in mosti e vini.**

Nel secondo anno di dottorato ho sviluppato una serie di sensori a basso costo e non distruttivi per il monitoraggio delle frazioni polifenoliche nei mosti e nei vini. Due prototipi di spettrofotometri (VIS-NIR) sono stati utilizzati per acquisire spettri di vini e mosti che sono stati poi confrontati con l'indice di polifenoli totali, il contenuto di antociani, tannini, pigmenti polimerici e l'intensità del colore, analizzati sugli stessi campioni con approcci analitici distruttivi. Inoltre, le concentrazioni delle diverse frazioni di composti fenolici nei vini sono state misurate con analisi di chimica umida (HPLC-DAD) e attraverso sensori basati su microonde acustiche. In particolare, sono state sviluppate nuove strategie di funzionalizzazione chimica per i trasduttori acustici a base di oro.

**Key words**: Low-cost spectrophotometers, polyphenols, wine, quartz crystal microbalance, VIS-NIR, acoustic microwave.

# **Introduction**

In accordance with the PhD project previously described (Alfieri, 2022), this poster reports the main results of the first two-years activities

(A1) Original and polyphenols-enriched wine samples have be analysed through classical analytical approach (HPLC-DAD) to quantify polyphenols content. Quantification has be performed by using calibration curve built with 33 standards. The same samples have been then analysed with the Lab-on-a-Chip biosensor. At this stages, the collected data have been used to perform functionalization tests of quartz microbalances. Two funzionalizations were selected as promising tool to measure polyphenols: Gel-A and MP5 ;

(A2) Spectral acquisitions with VIS and NIR prototype spectrophotometers have been taken on fermenting musts and wines. Spectral measurement were correlated with the total polyphenol index, colour intensity, tannin content, anthocyanin content and poilimer pigment concentration.

1. **Materials and Methods**

For the first operational objective (A1), the identification, characterization and quantification of the different polyphenols of grapes and wine was carried out by high-performance liquid chromatography (HPLC). The method used for HPLC analysis is reported by Watherouse (1999). Briefly, an HPLC system (Dionex Corporation Sunnyvale, Sunnyvale, CA, USA) with four pumps (P680) of solvent and PDA 100 as detector was used. A C-18 column (Dionex Acclaim® 120 C18, 5 μm, 4.6 × 250 mm), maintained at 40 °C, with a mobile phase flow rate of 0.5 mL/min was used as the stationary phase. The HPLC measurements were then correlated with those obtained with the QMB-D instrument functionalized with i) bovine serum albumin (BSA); ​ii) type A gelatin for porcine skin (Gel-A); iii) ​synthetic low-molecular-weight peptide called istatine-5 (Ist-5) and iv) ​a peptide fragment of the murine salivary protein-5 (MP-5)) (Gagliardi, 2022).

For the operational objective A2, sixteen different micro vinification were conducted with daily sampling until the end of fermentation. On collected samples spectral acquisition were take by using NIR and VIS low-cost prototype spectrophotometers developed by Nature 4.0. On the same samples, wet chemistry analyses were performed (i.e. total polyphenol index, anthocyanin, tannin, polymer pigment and colour intensity as reported by Ribereau-Gayon, 1965; Glories, 1984; Iland, 2000; Mercurio, 2007 respectively). Analytical and nondestructive approaches were then used to built predictive models of the different quality parameters. Moreover, a study of how winemaking condition (i.e. presence of stalks and CO2) can affect spectral acquisition has also been performed.

**3. Results and Discussion**

## **3.1 Measurements with VIS-NIR prototype spectrophotometers**

The VIS-NIR spectral acquisition was taken on 160 must/wine samples from 3 different varieties at different degrees of ripeness and on wines made with two different winemaking process. Correlation data between spectra and destructive measurements of the various polyphenols are currently being processed. The figure 1 shows some results of the spectra of the VIS prototype.

***Figure 1.***  *Spectral trends of the 160 must/wine samples, the VIS prototype covers 8-point wavelengths between 410nm to 860nm.*

## **3.2** **Modelling of the QCM-D sensor**

For measurements with gravimetric sensors based on acoustic wave QCM-D, monitoring data of changes in resonance frequency, Δf, are used as units of measurement, allowing small changes in crystal thickness (mass) to be detected. The dissipation, ΔD, gives information about the energy losses in the system and are particularly useful in the study of soft layers, where this information is used for quantification of the layer properties. Via an applied voltage, the crystal can be excited to resonance, and the resonance frequency is related to the thickness (mass) of the disk. Particularly, it has been observed that microbalances functionalised with MP5 and Gel-A effectively detect polyphenols in commercial wines. In particular, MP5 can predict polyphenols content (with an R2 of 0.759 – data not shown) as well as different classes of polyphenols such as the level of hydroxybenzoic acids (figure 2).

**Figure 2.**  *Correlation model for hydroxybenzoic acids using the ΔD of QCM-D functionalised with MAP-5*

**4. References**

Alfieri G. Low-cost non-destructive sensors for monitoring polyphenols, volatile compounds and quality parameters in grapes, musts and wines. In Proc.s of the 26th Workshop on the *Developments in the Italian PhD Research on Food Science, Technology and Biotechnology Università degli Studi di Torino – Asti (Italy), 19th -21st September 2022*, pp. 23-24.

Gagliardi, M.; Tori, G.; Agostini, M.; Lunardelli, F.; Mencarelli, F.; Sanmartin, C.; Cecchini, M. Detection of Oenological Polyphenols via QCM-D Measurements. Nanomaterials 2022, 12, 166

Glories, Y. La Couleur Des Vins Rouges. 2éme Partie: Mesure, Origine Et Interpretation. Connaissance De La Vigne Et Du Vin. 1984, 18, 253–271

Iland, P. (2000). Techniques for chemical analysis and quality monitoring during winemaking. Patrick Iland Wine Promotions.

Mercurio MD, Dambergs RG, Herderich MJ, Smith PA. High throughput analysis of red wine and grape phenolics-adaptation and validation of methyl cellulose precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format. J Agric Food Chem. 2007 Jun 13;55(12):4651-7.

 Ribereau-Gayon, P.; Stonestreet, E. Le dosage des anthocyannes ́ dans Je vin rouge. Bull. Soc. Chim. Fr. 1965, 9, 2649−2652.

Waterhouse, L. A. and JRitchey, J. G. A standard red wine: monomeric phenolic analysis of commercial cabernet sauvignon wines, Am. J. Enol. Vitic., 1999, 50, 91–100.