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TITOLO DELLA TESI DI DOTTORATO DI RICERCA

Low-cost non-destructive sensors for monitoring polyphenols, volatile compounds and quality parameters in grapes, musts and wines

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Abstract

Climate change is increasingly affecting the compositional characteristics of grapes, leading to an anticipation of technological ripening in relation to phenolic ripening and making it more challenging to produce balanced, structured, and long-lived wines. In this context, the availability of rapid, non-destructive, and cost-effective tools for monitoring phenolic compounds during winemaking becomes crucial to support timely and informed oenological decisions. This research addresses this need by exploring the development and application of prototype sensor technologies for the measurement of polyphenols in modern enology, with the aim of providing effective tools to mitigate the impact of global warming on wine quality. Furthermore, the rapid monitoring of key analytical parameters in fermenting musts has long been considered a hallmark of quality in the winemaking process, allowing producers to promptly intervene in case of issues arising during vinification.

Three low-cost prototype devices were designed and tested: a spectrophotometer operating in the visible (VIS) region, one in the near-infrared (NIR) region, and a quartz crystal microbalance with dissipation monitoring (QCM-D) sensor functionalized with selective peptides. The VIS-based spectrophotometer was employed to non-destructively monitor phenolic extraction during skin maceration in red wine production. Trials were carried out on three grape varieties subjected to different fermentation protocols and validated through parallel destructive chemical analyses. Results demonstrated the potential to develop predictive models for key parameters such as anthocyanins and total polyphenols, although limitations in robustness and sensitivity were observed, likely due to the reduced spectral resolution and limited UV range coverage.

To address these limitations, a second prototype was developed based on NIR spectroscopy, capable of providing more comprehensive information on the wine matrix's chemical-physical structure. The use of the NIR region significantly improved the predictive capacity of the system, enabling more accurate estimations of polyphenol content and phenolic ripeness indices. This approach proved more suitable for operational deployment, thanks to enhanced response stability and the ability to collect real-time data without sample preparation. The implementation of

chemometric models further enhanced the device's capacity to accommodate the natural variability of grapes and fermentation conditions.

In parallel, a completely different technology was explored, based on QCM-D sensors functionalized with synthetic peptides designed to selectively interact with specific classes of phenolic compounds. This system allows for label-free analysis with high molecular specificity, while maintaining the non-destructive nature of the measurement. Laboratory tests demonstrated the sensor's ability to selectively recognize phenolic acids and flavonoids, offering a novel perspective for advanced qualitative monitoring of tannins and their evolution. However, challenges remain in terms of signal reproducibility and functionalization stability, requiring further optimization of surface chemistry and operating protocols.

1. Chapter I - Introduction

1.1 Current Context and Prospects of the Wine Sector

In recent years, the approach to production processes has increasingly shifted towards a precision-oriented vision. This approach is driven by the globalized world's desire to optimize production, adopting processes that are less impactful on the environment, minimize resource waste, and lead to higher-quality products. The parameters defining quality are now well established across various sectors, including primary, secondary, and tertiary industries. The primary sector is among the most involved in the expansion of precision technologies, partly due to political efforts aimed at modernizing and increasing the competitiveness of this segment. This is particularly relevant given its traditionally rural and archaic nature, often sustained solely by long-standing traditions passed down through generations (Koohafkan et al., 2011). The conventional narrative surrounding agri-food technology calls for a new green revolution aimed at small farmers and driven by publicly funded research. This would address the needs of emerging commercial farmers, many of whom require support in managing high information and high skill innovations (Tripp et al., 2001).

The enology sector is one of the branches of the primary agricultural industry that is benefiting the most from these policies aimed at improving equipment and infrastructure. Additionally, private companies are showing significant interest, recognizing the potential for substantial technological transfer to industries within this segment. This sector is undergoing significant market evolution, increasingly attracting the attention of private enterprises, which see great economic potential in developing new technologies for wineries.

Indeed, the growth of the wine trade during the 1990s was driven by an increase in wine consumption in Northern Europe and North America, which offset the decline in consumption in Mediterranean countries. Additionally, the expansion of exports from the so-called New World wine producers contributed to this growth (Banks et al., 2010).

According to OIV data from 2024, global wine production levels have remained relatively stable in recent years, with fluctuations depending on the harvest. As shown in Figure 1, from 2000 to 2023, global wine production decreased from 279 Mhl to 237 Mhl, marking a decline of approximately 15.05% over 23 years.

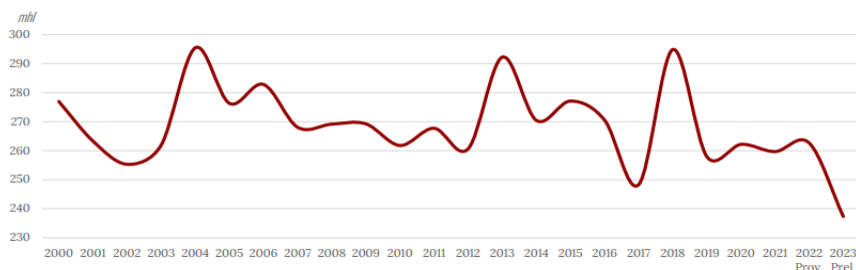


Figure 1. Global wine production volumes from 2000 to 2023 (OIV., 2024).

When these data are considered alongside wine consumption trends over the past 23 years, where consumption decreased from 225 Mhl in 2000 to 221 Mhl in 2023 (OIV., 2024), they may suggest a downward trend in the wine market. However, what has significantly increased during this period is the added value of the product itself. The global market grew from a value of €14 billion in 2000 to €36 billion in 2023 (Figure 2), along with an increase in the average export price of wine, which reached €3.62/L in 2023, more than a 50% rise compared to €2.30/L in 2000 (OIV, 2024).

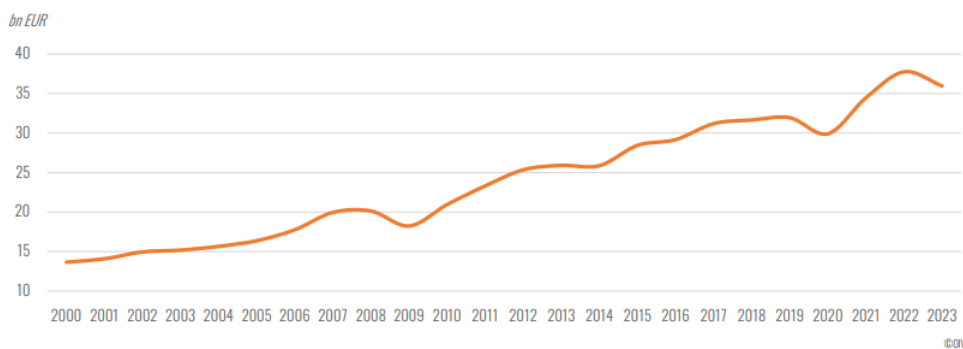


Figure 2. Increase in the global wine market value from 2000 to 2023.(OIV., 2024)

Similarly, the evolution of consumer preferences in the wine market has been profoundly changed. This change has been influenced by several factors, including increasing attention to quality, authenticity, traceability, and environmental and social sustainability. These elements are

redefining the dynamics of the wine sector, pushing producers to adopt more responsible and innovative practices.

One of the most significant aspects is the growing demand for high-quality products. Consumers are increasingly willing to pay a higher price for wines with distinctive sensory characteristics and those produced using traditional methods (Galati et al., 2019; Scozzafava et al., 2021). This pursuit of excellence is accompanied by a demand for authenticity and transparency. The ability to verify the origin of wine through traceability systems is becoming a fundamental requirement for many buyers (Pasvanka et al., 2019). At the same time, environmental sustainability is becoming an increasingly important selection criterion for consumers. Interest in wines produced using ecological methods is constantly growing, with a strong demand for organic and biodynamic wines made with minimal use of chemical products and with greater respect for the ecosystem (Scozzafava et al., 2021; Schäufele et al., 2017). Many consumers, especially younger generations, are willing to pay more for bottles that carry environmental certifications, recognizing the value of low-impact production practices (Sellers et al., 2016). A particularly significant role in this transformation is played by Millennials, who show a preference for wines with ethical and environmental attributes. This generation is more sensitive to sustainability and social responsibility issues and tends to favor products that stand out for their environmental and social commitment (Gallenti et al., 2019; Pomarici et al., 2014).

Globalization has led to increased competition and the need to reconfigure national industries to adapt to more sustainable production, supply chains, distribution, marketing, and consumption on an international scale (Overton et al., 2012; Aylward et al., 2008). This process requires strategic responses to address the evolution of consumer preferences and market conditions driven by increasingly sustainability-oriented approaches (Duarte Alonso et al., 2014; Del Rey et al., 2021).

In a market where the use of the Internet and social media has become crucial for attracting new consumers and promoting high-quality wines with good value for money (Sykalia et al., 2023; Tsiakis et al., 2022), the ability to showcase the use of modern technologies for process monitoring can be a valuable asset, both for the product itself and from the consumer's perspective.

Innovative analytical technologies are progressively transforming the enology sector, offering more efficient solutions compared to traditional methods. Tools such as chromatography and mass spectrometry, while providing high precision in analyses, present significant limitations. These approaches require long analysis times, high costs, and highly skilled personnel for their execution (Littaru et al., 2025; Buglass et al., 2013). Moreover, they often involve destructive testing, making

it impossible to reuse samples after analysis (Gayda et al., 2019). To overcome these issues, in recent years, a new generation of non-destructive analytical techniques has emerged, capable of providing reliable results without altering the sample. These include spectroscopic instruments employed in the monitoring of grapes, musts, and wines, as well as electronic noses (E-noses), which simulate the human olfactory system to evaluate the sensory characteristics of products (Gonzalez Viejo et al., 2022). Non-destructive technologies are particularly advantageous as they do not require highly specialized skills. This makes them accessible to a larger number of operators within wine companies, contributing to the broader diffusion of innovation within the sector (Sergeeva et al., 2024; Ranaweera et al., 2021). An equally relevant aspect is sustainability. The use of non-destructive analytical techniques fits perfectly into the new enological practices aimed at reducing environmental impact. By minimizing the use of chemical reagents and drastically reducing laboratory waste, these technologies represent a more ecological and responsible choice (Clodoveo et al., 2016; Comuzzo et al., 2022). Furthermore, the introduction of faster and more reliable analytical tools allows producers to make more informed and timely decisions during fermentation, ensuring better control over the final quality of the wine. The ability to intervene promptly on process variables enables the production of more consistent products, aligning better with market expectations (Lambrecht et al., 2024).

In this historical context, non-destructive technologies that do not destroy the sample (Bellincontro et al., 2015), generate no laboratory waste for disposal (Wen et al., 2024), and are particularly fast in execution have increasingly gained traction (Chapman et al., 2019). Among these technologies the following paragraphs will describe the application of spectroscopy, electronic noses, and quartz microbalances with various functionalization as innovative tools for wine analysis.

1.2 Foundations of Spectrophotometry

By definition, originating from Maxwell's early treatises, spectrophotometry refers to the study of the interaction between electromagnetic radiation and matter. It involves the absorption, emission, or scattering of radiation by the system under investigation, resulting in molecular spectra that provide detailed information about the system's structure and chemical properties (Pérez-Juste et al., 2015).

The interaction of electromagnetic radiation with matter is a quantum phenomenon that depends both on the properties of the radiation and on the structural characteristics of the sample involved. This is not surprising, as the origin of electromagnetic radiation is linked to energy changes within

the matter itself (Maxwell, 1873). The transitions occurring in matter are quantum phenomena, and the resulting spectra are mostly predictable (Hofmann et al., 2010). Electromagnetic radiation consists of an electric field and a perpendicular magnetic field (two vectors), both oscillating in planes at right angles to the direction of propagation, as shown in Figure 3

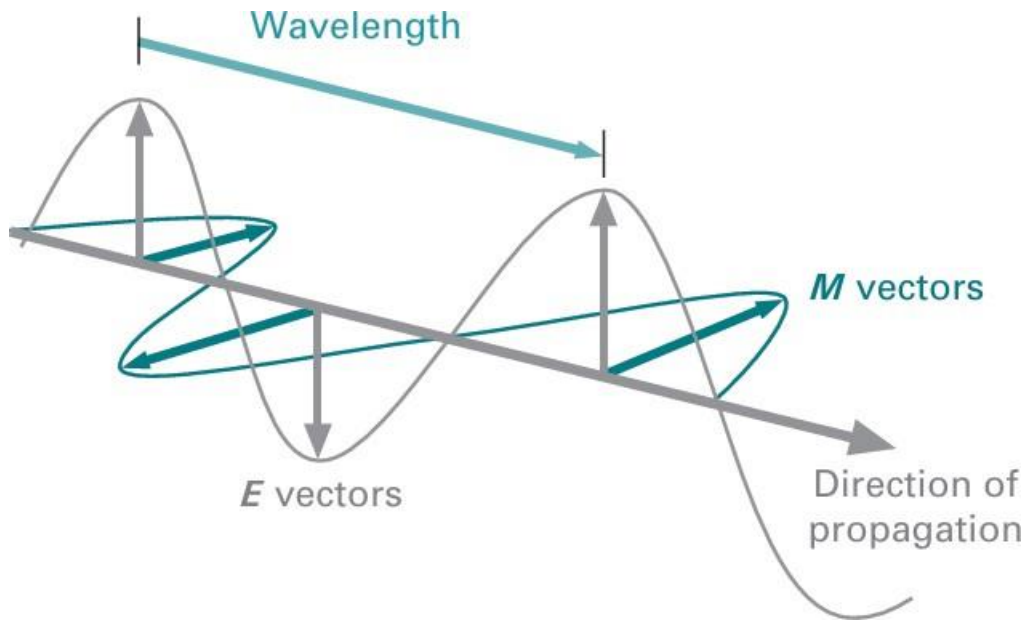


Figure 3. Light is electromagnetic radiation and can be described as a wave propagating transversally in space and time. The electric (E) and magnetic (M) field vectors are directed perpendicular to each other (Hofmann et al., 2010).

Wavelength (λ) is the spatial distance between two consecutive peaks in a sinusoidal waveform, typically measured in nanometers (nm). Amplitude refers to the maximum length of the vector, while the frequency of the radiation is the number of oscillations completed by the wave in one second, measured in Hertz (Hz). The frequency is related to the wavelength through the speed of light, c , where: $\nu = c/\lambda$.

The electromagnetic spectrum covers a range of frequencies spanning several orders of magnitude, as shown in Figure 4 .

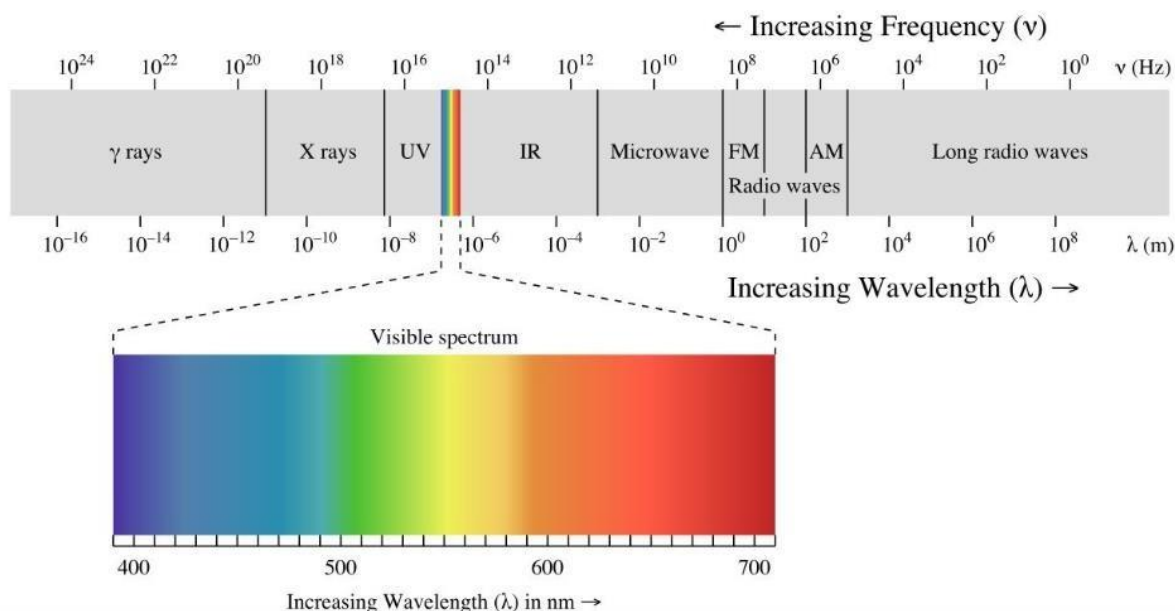


Figure 4 Graphical representation of the entire electromagnetic spectrum (www.leadertechinc.com).

This type of radiation is generally referred to as light, although only a small portion of the spectrum is visible (VIS) to the human eye. The frequency (ν) and wavelength (λ) of light are inversely proportional: $c = \lambda\nu$, where c is the speed of light in a vacuum. The spectrum ranges from the low-energy region of radio frequencies, with frequencies varying from MHz to kHz (Hz = Hertz, a unit of frequency equivalent to cycles per second or s^{-1}), to the high-frequency gamma ray region, characterized by frequencies up to 10^{22} Hz. The corresponding wavelengths range from thousands of kilometers to less than 10^{-15} m (McHale et al., 2017). The wavelengths of VIS light, which represent only a tiny fraction of an essentially limitless spectrum, vary from about 400 to 700 nm, from violet to red. These wavelengths correspond to frequencies ranging from 7.5×10^{14} Hz (violet) to 4.3×10^{14} Hz (red), or, in terms of wavenumber, from about $25,000 \text{ cm}^{-1}$ to $14,000 \text{ cm}^{-1}$. The frequencies and wavelengths of the individual spectral regions are listed in Table 1.

Table 1 Regions of the electromagnetic spectrum

Wavelength range (nm)	Frequency range (s^{-1})	Description
<0.1 nm	10^{20} – 10^{23}	Gamma rays
0.1–10 nm	10^{17} – 10^{20}	X-rays
10–400 nm	10^{15} – 10^{17}	Ultraviolet
400–700 nm	10^{14} – 10^{15}	Visible
700 nm to 1 mm	10^{11} – 10^{14}	Infrared
1 mm to 1 cm	10^{10} – 10^{11}	Microwaves

1 cm to 100 km	10^3 – 10^{10}	Radio waves
100–1,000 km	10^2 – 10^3	Audio frequency

Electromagnetic phenomena are explained in terms of quantum mechanics, and the photon is the elementary particle responsible for electromagnetic phenomena (Davydov et al., 2020). It carries electromagnetic radiation and possesses both wave-like and particle-like properties, despite having zero mass (Bialynicki-Birula et al., 2017). As a particle, the photon interacts with matter by transferring its energy E , as described by the equation:

$$E = hc/\lambda = h\nu$$

Considering a diatomic molecule, rotational and vibrational levels have discrete energies that merge into a continuous spectrum only at very high energies (Korobenko et al., 2014). Each electronic state of a molecule has its own set of rotational and vibrational levels. Since the schemes of such systems can be complex, the Jablonski diagram is used, where electronic and vibrational states are schematically represented as horizontal lines, and the possible transitions are indicated by vertical lines.

For a transition, energy must be absorbed. Electrons, both in atoms and molecules, can be distributed across different energy levels, but they primarily reside in the lower levels (ground state). To promote an electron to a higher level (excited state), energy must be provided to the system (Cohen-Tannoudji et al., 1998). If this energy $E = h\nu$ is derived from electromagnetic radiation, an absorption spectrum is generated, and the electron is transferred from the electronic ground state to the first electronic excited state. The molecule will also be in an excited vibrational and rotational state (Medvedev et al., 2017). Subsequently, the molecule relaxes to the vibrational ground state of the first excited electronic state, and finally, the electron can return to the electronic ground state (Tennyson et al., 2016).

Spectroscopic techniques represent one of the main sources for determining molecular geometries, such as bond lengths, bond angles, and torsion angles, and they can also provide, as will be evident, significant information on molecular symmetry, the distribution of energy levels, electronic densities, or electrical and magnetic properties (Svanberg et al., 2001; Pavia et al., 2015; Vitha et al., 2018).

1.2.1 Spectroscopy UV-VIS-NIR

Ultraviolet-visible (UV-VIS) spectroscopy deals with the interactions between electromagnetic radiation in the UV-VIS region and matter. The ultraviolet (UV) region approximately covers the wavelength range between 10 and 380 nm. It is commonly divided into three main sub-regions: UVA (320-380 nm), UVB (280-320 nm), and UVC (100-280 nm). Additionally, the range from 10 to 200 nm is defined as vacuum ultraviolet (VUV), although it is only explored under vacuum conditions (Piccolo et al., 2019). The VIS region includes the spectrum from 380 to 750 nm, while the near-infrared (NIR) region extends from 780 to 2500 nm.

UV-VIS spectroscopy is related to the excitation of the outermost electrons of atoms involved in molecular bonding, which is why it is often referred to as "electronic spectroscopy" (Gauglitz et al., 2001). Measurements in the UV-Vis region are typically performed in transmittance, reflectance, and photoluminescence (fluorescence and phosphorescence) modes. Transmittance and reflectance measurements require a reference material serving as a blank, whereas photoluminescence acquisitions can be considered absolute measurements. Electromagnetic radiation in the UV-Vis region has sufficient energy to induce electronic transitions. Electrons in conjugated double bonds or chromophoric groups absorb energy and transition to excited electronic states (Nassauk et al., 1983). This phenomenon is typical of compounds such as polyphenols, anthocyanins, and carotenoids, which exhibit strong absorption in the UV-Vis region (Castañeda-Ovando et al., 2009).

UV-Vis spectrophotometry exploits the electronic properties of molecules. When a photon in the UV or VIS region interacts with a molecule containing a chromophore, it can provide the necessary energy to promote an electron from a bonding orbital (π or n , non-bonding) to an anti-bonding orbital (π^*) (Schmid et al., 2001; Esfandiary et al., 2012). The most common transitions are $\pi \rightarrow \pi^*$ (typical of conjugated double bonds) and $n \rightarrow \pi^*$ (observed in compounds containing heteroatoms with lone electron pairs, such as carbonyls) (Piccoli et al., 2009). This energy absorption results in a peak in the UV-Vis spectrum, which can be used to identify and quantify specific compounds.

The probability that a photon is absorbed by matter is given by an extinction coefficient, which depends on the wavelength (λ) of the photon. If a beam of light with intensity I_0 passes through a sample with appropriate transparency and a path length (d), the intensity (I) decreases exponentially along the path. The ratio of the transmitted light to the incident light ($T = I/I_0$) is called transmittance. An essential aspect is the distinction between transmittance and absorbance,

two complementary concepts used in different contexts. Transmittance represents the fraction of light that is not absorbed by the sample but instead passes through it. Since transmittance always ranges between 0 and 1, $T = 1$ corresponds to no absorption (all light passes through the sample), whereas $T = 0$ means that all light has been absorbed. Absorbance, on the other hand, is a direct measure of how much of the incident light is absorbed by the sample. It is related to transmittance through a simple logarithmic relationship:

$$A = -\log T = -\log (I/I^0)$$

From this expression, it is evident that absorbance increases as transmittance decreases, and vice versa. When the sample fully absorbs the light (zero transmission), absorbance is very high, whereas if the sample does not absorb at all (complete light transmission), absorbance is zero. Biochemical samples usually consist of aqueous solutions where the target substance is present at a molar concentration (c). The algebraic transformation of the exponential correlation between absorbance and concentration into a logarithmic expression leads to the Beer-Lambert law:

$$-\log(I/I^0) = \log(T/1) = \epsilon cd = A$$

where:

$d = 1$ cm,

$c = 1$ mol/dm³,

$\epsilon = 1$ dm³/mol·cm,

ϵ is the molar absorption coefficient (also known as the molar extinction coefficient),

A is the sample absorbance, as displayed by the spectrophotometer.

The Beer-Lambert law is valid only at low concentrations. At higher concentrations, molecular association may occur, causing deviations from linear behavior. Absorbance and extinction coefficients are additive parameters, complicating the determination of concentrations in samples containing multiple absorbing species at the same wavelength. It is important to note that in suspensions, scattering effects increase absorbance, as scattered light does not reach the detector (Hofmann et al., 2010). Consequently, the absorbance recorded by the spectrophotometer is overestimated and requires appropriate corrections.

NIR spectroscopy provides information on combination bands and harmonic overtones of highly anharmonic vibrations, particularly those involving X–H bonds (C–H, N–H, and O–H) (Zapata et al., 2018). Combination bands involving C-H or O-H stretching vibrations appear in the NIR

region (4000-10,000 cm^{-1}). A detailed analysis of the frequency and intensity of NIR combination bands enables the elucidation of vibrational coupling mechanisms in the condensed phase in terms of mechanical and electrical anharmonicity (Nishida et al., 2012). Stretching vibrations occur when the distance between two atoms in a molecule changes along the bond axis. These vibrations can be further divided into symmetric and asymmetric stretching. For instance, the O-H stretching vibration in water is a prominent feature in NIR spectra (Han et al., 2023; Futami et al., 2016). The first overtone of the O-H stretching vibration appears around 6900 cm^{-1} , while the combination of bending and stretching vibrations occurs around 8600 cm^{-1} (Han et al., 2023).

C-H and N-H stretching vibrations also contribute to NIR spectra, with second and third overtones observable in the mid-infrared region (Shenk et al., 1994). Bending vibrations, on the other hand, involve changes in the angle between two bonds. These can be classified as in-plane or out-of-plane bending vibrations. The combination of stretching and bending vibrations provides detailed information about molecular structure (Hosseini et al., 2020; Han et al., 2023). Fundamental frequencies observed are characteristic of the involved functional groups, leading to the concept of a "fingerprint" region. As the number of functional groups increases in more complex molecules, absorption bands become harder to assign. However, groups of specific bands consistently appear near the same wavelengths and can be attributed to specific functional groups (Hofmann et al., 2010). These group frequencies are therefore highly useful for molecular structural diagnostics.

The instruments used for this type of analysis are spectrophotometers, composed of different integrated parts.

The first is the light source, which provides the electromagnetic radiation necessary for the analysis. The choice of the source depends on the spectral region of interest:

- Deuterium (Xue et al., 2008) or xenon lamps (UV) (Monarumit et al., 2023);
- Tungsten, halogen, or xenon lamps (VIS-NIR) (Zhuang et al., 2011);
- Specific lasers or LEDs (NIR).

The second component is the monochromator or dispersion system, which selects a specific wavelength of radiation.

It can consist of:

- Diffraction gratings, such as concave holographic gratings like the Seya-Namioka type, which are used to disperse light into its spectral components. These gratings are essential for achieving high spectral resolution and accuracy (Wan et al., 2006).
- Prisms, less common in modern spectrophotometers but still used in some devices due to their

ability to disperse light based on wavelength through refraction (Andrews et al., 2009; Zhao et al., 2010).

The third component is the optical system (mirrors and lenses). Mirrors and lenses direct and focus the light toward the sample and the detectors, optimizing the efficiency of light transmission (Sablowski et al., 2018).

The fourth segment is the cuvette or sample holder (Genovesi et al., 2015). The sample to be analyzed is contained in a cuvette, which must not interact with the wavelength used. It can be made of:

- Plastic for VIS analysis, generally limited to the VIS range due to its higher absorbance in the UV region (Parimi et al., 2021).
- Glass, mainly used in the VIS and NIR regions.
- Quartz for UV, VIS, and NIR analyses (May et al., 2016). These cuvettes are used in high-precision measurements, such as nucleic acid and protein quantification (Lang et al., 2017; Cole et al., 2018).

The fifth component is the detector, which converts the transmitted, reflected, or emitted light from the sample into an electrical signal. The main types of detectors are:

- Semiconductor Detectors for the UV Region.

Semiconductor detectors represent a category of devices sensitive to UV radiation, used in various scientific and industrial applications. Among these, AlGaN-based detectors (Aluminum Gallium Nitride) stand out for their insensitivity to VIS and infrared radiation, making them particularly useful in contexts where the isolation of UV signal without interference from other wavelengths is necessary (Malinowski et al., 2011; Barkusky et al., 2009; Malinowski et al., 2010).

- Diode-Array Detectors.

Diode-array detectors (DAD) are widely used in spectrophotometry over a broad wavelength range, generally from 300 to 1710 nm, covering both the VIS-NIR regions (Mouazen et al., 2009; Es'haghi et al., 2011). Due to their ability to simultaneously acquire data at multiple wavelengths, these detectors are particularly valued for their speed and practicality, making them highly suitable for portable spectrophotometers (Huck et al., 2021; Cozzolino et al., 2009).

• Photomultiplier Tubes (PMT).

Photomultiplier tubes (PMTs) are among the most sensitive detectors available for detecting UV and VIS radiation. Their operating principle is based on the multiplication of the electronic signal generated by the impact of photons on the photosensitive surface of the tube (Faham et al., 2015). This amplification process makes them particularly suitable for applications where the light signal is extremely weak, such as high-precision measurements in the UV region (Wang et al., 2021). For this reason, PMTs are widely used in advanced spectroscopic instruments, ensuring high sensitivity and low noise levels.

• HgCdTe (Mercury-Cadmium-Telluride) Detectors.

For the NIR region, one of the most efficient detectors is HgCdTe (Mercury-Cadmium-Telluride). This semiconductor material offers high quantum efficiency and low electronic noise, essential characteristics for measuring weak signals in the NIR (Bechter et al., 2019).

• Interferometric Detectors.

Another class of detectors used in spectroscopy is interferometric detectors, which operate based on the principle of light interference. These devices are widely employed in mid-infrared (MIR) spectroscopy, where light sources can be weak, and absorbance is highly pronounced. Conversely, in the NIR region, interferometric detectors benefit from more intense sources and lower noise levels, making them particularly suitable for high-precision instruments (Kholiqov et al., 2017).

1.2.2 Applications of UV-VIS-NIR Spectrophotometry in Enology

The enology industry requires precise and rapid analytical methods to enable timely decision-making in the production process, preventing issues that could compromise the quality of grapes, musts, and wines. In this context, the application of UV-VIS-NIR spectrophotometry has become increasingly relevant over time, particularly due to the growing interest in non-destructive techniques that align with the current emphasis on environmentally sustainable production.

One of the main applications of these technologies is the monitoring of the phenolic fraction. Hence, phenolic compounds play a key role in the quality of grapes and wines, influencing their color, taste, structure, and antioxidant properties (Rivera-Dominguez et al., 2009; Garcia-Hernandez et al., 2025; Basha et al., 2004; Jakobović et al., 2015). Some of the most important phenols for wine quality are anthocyanins, responsible for the color of red wines, tannins, which

affect structure and mouthfeel, and other flavonoids and phenolic acids such as gallic and caffeic acids, which also contribute to the sensory characteristics of wine (Jakobović et al., 2015).

In recent years, climate change has begun to profoundly affect the phenolic composition of grapes. For instance, high temperatures can inhibit anthocyanin synthesis and alter their composition, with repercussions on wine quality (La Iacona et al., 2010; Rouxinol et al., 2023; Rouxinol et al., 2023). Moreover, there is a risk of excessive imbalances if technological ripening (based on sugar and acidity) and phenolic ripening do not progress in parallel (Chidi et al., 2018; Villangó et al., 2015). For this reason, stringent and rapid monitoring of phenolic compounds during grape ripening and winemaking has become crucial in the sector, driving researchers to apply spectrophotometric technologies for their measurement. Phenolic compounds exhibit specific absorption depending on their chemical characteristics, ranging from 280 nm for phenolic acids and tannins, to 365 nm for flavonols, and up to 540 nm for certain anthocyanins (Raja et al., 2014). For instance, wine color intensity is determined by summing the absorbance values at 420 nm, 520 nm, and 620 nm (Saerneckis et al., 2006), while the total polyphenol index (TPI) is measured by diluting the wine with water and reading the absorbance at 280 nm (González-Rodríguez et al., 2002). However, conventional methods for determining total polyphenols can take up to two hours (Singleton et al., 1965), while anthocyanin quantification via HPLC requires approximately one hour and twenty minutes (Ritchey et al., 1999). Furthermore, the high cost of these instruments often makes them unaffordable for small-scale wineries. To overcome these limitations, several researchers have developed predictive models, often with low cost prototypes, for monitoring total polyphenols during vinification using the entire VIS and NIR spectra (Aleixandre-Tudo et al., 2018; Sanna et al., 2014; Miramont et al., 2020; Liu et al., 2024; Alfieri et al., 2025).

Moreover, relevant application also regards the use of UV-VIS-NIR spectrophotometry for the rapid and non-destructive monitoring of phenolic ripeness in grapes (Tzanova et al., 2020; Xiao et al., 2018; Agati et al., 2013; Baca-Bocanegra et al., 2019; Ferrer-Gallego et al., 2011). These approaches eliminate the need for destructive sampling, allowing for in-field measurements without compromising the sample.

In addition to phenolic composition, spectroscopy can be used to assess other important parameters related to wine quality such as sulfur dioxide (SO₂) content. This parameter often needs to be measured on large quantities of samples; for instance, analyses must be carried out in a barrel cellar on every single barrel to ensure its integrity. In such cases, the possibility of using a faster

instrument for measurements could make winery operations much more efficient. For this reason, some authors have attempted to predict both free and bound sulfur dioxide content using NIR spectrophotometers (Cozzolino et al., 2007; Urbano-Cuadrado et al., 2004).

Similarly, the rapid monitoring of nutrients available for yeasts within musts is gaining increasing interest, allowing timely intervention in case of deficiencies, which are becoming more common due to climate change (De Resseguier et al., 2018; Rienth et al., 2021). In this scenario, where classical methods for monitoring yeast assimilable nitrogen (YAN) are expensive, slow to perform, and complex to apply (Baron et al., 2011; Casalta et al., 2013), the versatility of spectroscopic techniques has been evaluated. Several authors have employed NIR instruments directly to predict YAN in grapes (Walker et al., 2021), in musts (Cavaglia et al., 2019; Barnaba et al., 2014) and specifically for the determination of free ammonium nitrogen (FAN) (Manley et al., 2001).

Other important enological parameters have been monitored using UV-VIS-NIR spectrophotometry, often in combination with predictive modeling. Some studies have developed predictive models for the indirect measurement of wine alcohol content using NIR spectrophotometry (Nordon et al., 2005; Bao et al., 2012; Di Egidio et al., 2010), tartaric acid (Regmi et al., 2012), malic acid (Coernehl et al., 2023), and lactic acid (Manley et al., 2001) in wines and musts, thereby saving analysis time and generating much lower amounts of waste reagents.

Additionally, the integration of this technology into the production process can be applied to detect off flavours in wines. For instance, NIR technology has been used to monitor compounds such as acetic acid (Martelo-Vidal et al., 2015; Urbano-Cuadrado et al., 2004), ethyl acetate, and volatile phenols. UV-VIS-NIR spectrophotometry has also been utilized to ensure traceability and authentication of wine geographical origin. Some researchers have successfully distinguished wines from different geographical regions based on their VIS-NIR spectra (Liu et al., 2006; Dos Santos et al., 2017; Hu et al., 2019). Additionally, these techniques have been employed to classify wines based on aging status, distinguishing aged wines from non-aged ones (Fernández-Novales et al., 2009; Ferreiro-González et al., 2019). Another aspect of great interest in the field of rapid monitoring is related to the assessment of potential infections in grapes and musts caused by fungal pathogens. In some cases, NIR spectroscopy has been employed to monitor the presence of *Botrytis cinerea* rot in white grapes (Hill et al., 2013). Other authors have utilized NIR technology to assess infection directly on grape clusters (Berghi et al., 2017), while in other studies, infection markers such as gluconic acid, glycerol, and acetic acid have been evaluated through VIS-NIR spectroscopy (Porep et al., 2014). Moreover, NIR spectrophotometry has also been employed to

track the dehydration process in grapes during drying, providing indirect measurements of water loss in the berries and assessing the development of noble rot *Botrytis cinerea* (Modesti et al., 2024).

1.3 E-nose

The importance of human smell is often underestimated, but recent research highlights its significance in various aspects of human life. Contrary to the belief that humans have a poor sense of smell, studies show that humans have excellent olfactory abilities, being capable of detecting and discriminating an extraordinary range of odors. The human olfactory bulb is quite large and contains a similar number of neurons to that of other mammals, indicating the significance of human olfaction (McGann et al., 2017). The first process of human olfactory system is to breathe or to sniff a smell into a nose. The difference between normal breath and sniffing depends on quantity of odorant molecules that flow into the upper part of the nose (Sigeru et al., 2015). When olfactory receptors capture an odorant, the chemical reactions between them trigger electrical signals as an output. The signals are then transmitted through the glomeruli to the olfactory bulb, where mitral cells and interneurons are located (Han et al., 2022). From these electrical outputs, the brain is able to extract different types of information, including identity of objects and food, environmental hazards, and social and emotional information. These informations are probably given by distinct cortical networks within the olfactory system however, the exact arrangement of these functional networks is not fully understood (Zhou et al., 2019). As clear, the human sense of smell serves a variety of important functions in everyday life (McGann et al., 2017; Zhou et al., 2019; Devanand et al., 2015), this is the reason behind the growing interest in the development and application of instruments that mimic our noses (Borowik et al., 2020). Indeed, the application of these type of sensors is now widespread, spanning various fields from biomedicine (Zhang et al., 2017; Dragonieri et al., 2017; Wilson et al., 2018) and environmental science (Zarra et al., 2018; Prasad et al., 2022; Sayago et al., 2019) to industrial materials production (Oleneva et al., 2020; Zaytsev et al., 2023) and the food industry (Voss et al., 2020; Yavuzer et al., 2021; Rafaela et al., 2022; Cascos et al., 2023; Sanchez et al., 2021; Fujioka et al., 2021). Within the food industry, the wine industry is certainly one of the sectors most interested in developing human sense-like sensor (Gamboa et al., 2019). Hence, wine evaluation with human sense is an old story. For instance, the figure of sommeliers recovers a significant role in the world of oenology, and since time immemorial, one of the most valuable tools at their disposal is to train their sense of smell. Moreover, the wine sensory analysis includes techniques which goes far beyond simple tasting; it is a science that involves the ability to discern the most subtle bouquets and aromas that

a wine has to offer (Balda et al., 2017; Naranjo et al., 2021). A trained nose thus becomes a delicate tool for perceiving complex aromas and translates it objective evaluations (Ribereau-Gayon et al., 2021). However, sensory analysis can be imprecise and unreproducible. Furthermore, it requires time, trained panels, and certified laboratory, and it is therefore not applicable in all the contexts. On the other hand, devices such as E-nose, being fast and easy to use, can supplant possible human errors and be applied at every different stage of production, not only at the final tasting one. Numerous classes of VOCs have been identified in grapes. Among these, the principal varietal compounds encompass terpenes (predominantly found in grape skin) (Swain et al., 2023), methoxypyrazines (Xin et al., 2023), carotenoid-derived compounds also recognized as nor-isoprenoids (Boomashri et al., 2021), thiols (Di rosa et al., 2017), benzene derivatives (Robbiani et al., 2023), and compounds arising from lipid oxidation (Dey et al., 2018). The synthesis of secondary metabolites in grapes—and thus their influence on the chemical composition of the wine aroma exhibits significant variability, markedly influenced by the concept of "terroir" (Miramirkhani et al., 2017) and agricultural practices (Sorvin et al., 2018). In recent years, numerous researchers have delved into studying the interplay between the vine, soil, and climate on VOCs in grapes and wine (Sorvin et al., 2018; Graboski et al., 2018). Despite these efforts, attempts over the past decade to correlate VOCs composition with the wine aroma, as perceived, have often yielded results that are either weak or challenging to interpret (Henrique et al., 2018; Wei et al., 2019). As such, the sensory analysis of wine plays a crucial role in the wine industry, enabling the determination of perceptible differences among wines, their characterization, and relevance to consumers. The key sensory attributes employed in wine analysis include physiological, psychological, and physicochemical aspects, which are essential for evaluating wine quality and consumer preferences. The aromatic analysis of wine through panel tests involves subjective sensory evaluations by a group of trained individuals (Di Rosa et al., 2020). The evaluation by a trained panel and standardized methods ensures the objectivity of professional wine tasting by making it free of personal bias and are essential for correctly characterizing wine sensory attributes. As a result, the majority part of certifications that instruct on wine description within their tasting protocols employ standardized descriptors for various sensory parameters, including mouthfeel, color, and aroma. This practice is prevalent across well-established institutions, such as the Master of Wine Institute (MW), Wine and Spirit Education Trust (WSET3 and dipWSET) (Sierra-Padilla et al., 2021), the Master Sommelier (MS) certification (Zhao et al., 2016) and its associated materials (Mujahid et al., 2017), as well as the certifications provided by the Society of Wine Educators (CSW and CWE) (Zhao et al., 2018; Gao et al., 2016). While panel tests are valuable tools in assessing wine aromas, several issues and challenges are associated with

this approach. One of the main limitations is the interpanel Variability. Hence, different panels may yield varied assessments, as panelists may interpret and describe aromas differently. This interpanel variability can pose challenges in achieving standardized and reproducible results (Okur et al., 2021). The effectiveness of panel tests is highly dependent on the training and experience of the panelists. Inadequate training or lack of experience may result in less accurate and reliable evaluations (Okur et al., 2020). Continuous exposure to aromas during a tasting session can lead to sensory fatigue among panelists, affecting their ability to detect and differentiate aromas accurately over time (Li et al., 2017; Shuba et al., 2022). Moreover, a limited vocabulary can often represent a problem. Describing aromas can be challenging, and panelists may have a limited vocabulary to express their sensory experiences (Ruangchaiwat et al., 2016). Lastly, panel tests are more qualitative than quantitative, making difficult to precisely quantify the concentration of specific aroma compounds in a wine (Zhang et al., 2017). Organizing and conducting panel tests can be resource intensive in terms of both time and cost. Training, maintaining, and coordinating a panel require significant investments (Na Songkhla et al., 2021). Opposed to sensory analysis for characterizing the aromatic components of wines are wet gas chromatography (GC) analyses. GC is an analytical technique used to separate, identify, and quantify volatile components present in a wine (Satapathy et al., 2023). This technique exploits the ability of compounds to migrate through a stationary phase within a separating column in response to a flow of carrier gas (Paolesse et al., 2017). However, like any destructive analytical method, GC presents its limitations that must be considered in the context of wine analysis:

- Volatile Compound Selectivity: GC is highly effective in analyzing volatile compounds, but it may not capture all non volatile or semi volatile components in wine. This limitation can lead to an incomplete representation of the wine chemical composition (Yang et al., 2019).
- Matrix Effects: The complex matrix of wine, including various organic and inorganic components, can influence the separation and detection of compounds in GC (Ling et al., 2021). Coelution of compounds and interference from matrix components may occur, affecting the accuracy and specificity of the results.
- Quantification Challenges: While GC provides excellent qualitative information, quantifying compounds can be a challenge without the use of appropriate internal or external standards. Variability in detector response can also offer quantification difficulties (Timmins et al., 2020).
- Need for Complementary Techniques: To achieve a comprehensive understanding of wine composition, GC is often coupled with other analytical methods such as Mass Spectrometry (GC-

MS) (Capone et al., 2018) or Flame Ionization Detection (GC-FID) (Wang et al., 2019). This integration adds complexity and may increase the cost of the analysis.

- Time-Consuming Sample Preparation: The preparation of wine samples for GC analysis involves extraction and concentration steps, which can be time-consuming. Delicate handling of samples is essential to prevent changes in composition during preparation (Alarcon et al., 2021).

- Instrumentation and Maintenance: GC instruments require regular maintenance, and the quality of results is contingent on the proper functioning of the equipment (Herderich et al., 2015; Alem et al., 2019). The need for skilled personnel and the associated costs of maintenance can be limiting factors (Van Leewen et al., 2018).

- Cost Implications: While GC analysis provides valuable insights, the initial investment in equipment, consumables, can be relatively high (Alem et al., 2019). This cost may be a limiting factor for smaller wineries or research facilities with budget constraints (Chambers et al., 2013).

1.3.1 Application of electronic noses in oenology: Principles, use and main issues.

Sensory analysis was commonly employed to discern the various aromatic characteristics of numerous food products and beverages (including wine). However, this method often proved to be imprecise, lacking in reproducibility, and highly subjective. It was recognized that human senses, including the sense of smell, can be influenced by both physical and mental conditions, as well as external factors. Consequently, sensory analysis began to be paired and compared with analytical instruments, such as gas chromatography, to mitigate these issues. Nevertheless, utilizing such analytical tools entailed significant time, expertise, and costly equipment. Additionally, this approach frequently necessitates intricate sample preparation procedures, which are time-consuming and not always feasible for the modern wine industry need for swift and straightforward quality assessment methods. To address these challenges, in recent years, there has been a notable shift towards utilizing tools that emulate the bio-logical sense of smell, known as E-noses. These devices have emerged as some of the most interesting and new instruments in the wine industry, offering a promising solution to the limitations associated with traditional sensory analysis and analytical techniques. Typically, owing to the advanced sensing performances of its sensor array, the E-nose has the aptitude to convert volatile compounds found within wine matrix into detectable electric signals, often in digital form. As a consequence, these signals are thoroughly analyzed, primarily during post-processing, to derive a potentially meaningful pattern relevant to the specific analysis at hand. It is evident, therefore, that the primary component of an E-nose tool,

which can be tailored to suit various applications, lies in its sensor array. The most part of E-nose systems relied on conducting polymers (CP), metal oxide semiconductors (MOS), metal oxide semiconductor field-effect transistors (MOSFET) and mass-sensitive (such as quartz microbalance), acoustic and optic sensors (Chambers et al., 2013). The composition and functioning of the different sensors are described below.

Metal-oxide Semiconductor (MOS) Sensors:

- Composition: Metal-oxide semiconducting film (commonly SnO₂) on a ceramic substrate (Petretto et al., 2021; Lesschaeve et al., 2022; Hove et al., 2016).
- Operation: In the presence of air, oxygen is adsorbed on the semiconductor surface, creating an electron-depleted region and high resistance contacts. Exposure to a reducing gas leads to a release of electrons, causing an exponential change in resistance (Wang et al., 2022; Njoman et al., 2017; Sipos et al., 2021)

Conductive Polymer (CP) Sensors:

- Composition: Made of organic aromatic or heteroaromatic materials, such as pyrrole, aniline, thiophene, and acetylene, deposited onto a ceramic substrate with gold-plated electrodes (Fizman et al., 2015; Coulon-Leroy et al., 2017; Larssen et al., 2018; Sharif et al., 2017; Robinson et al., 2016; Zrally et al., 2016; MacNeil et al., 2017).
- Operation: Conductivity is primarily due to electron movement along the extended π system. Exposure to vapor induces a change in electron density, resulting in a measurable change in conductivity (Wang et al., 2022; Zrally et al., 2016).
- Mass-based sensors:
 - Types: Divided into bulk acoustic wave (BAW) or quartz crystal microbalance (QCM), and surface acoustic wave (SAW) detectors (Nickles et al., 2017; Ruiz et al., 2019; de-la-Fuente-Blanco et al., 2020; Hage et al., 2018; Nolvachai et al., 2017; Ferreira et al., 2015; Pati et al., 2021).
 - Composition: Piezoelectric material (usually a single quartz crystal) coated with a sorbent membrane (Ferreira et al., 2015; Zhang et al., 2023; Karine et al., 2018; San Romàan et al., 2020).
 - Operation: An alternating current generates a resonant wave, and when vapor permeates the sorbent layer, the total mass of the film increases, leading to a proportional change in resonance

frequency (Zraly et al., 2016; Parrilla Vázquez et al., 2016; Chu et al., 2023; Lozano et al., 2015).

Graphene Nanofiber Sensors:

Graphene nanofiber sensors have demonstrated remarkable potential in detecting volatile organic compounds (VOCs) due to their high sensitivity.

- **Composition:** Nanocomposites formed by combining graphene with metal oxides or other nanomaterials can significantly enhance sensor performance. For example, hybrid structures of pure graphene (pG) and metal-organic frameworks (MOFs) have shown increased sensitivity and selectivity for VOC biomarkers such as methanol and chloroform (Tung et al., 2020). Three-dimensional structures utilizing graphene-based frameworks can further improve gas sensitivity, reducing detection limits from ppm to ppb (Dong et al., 2021). Sensors fabricated using electrospinning, a technique employed to create nanofibers and nanoribbons, are subsequently doped with graphene to enhance their detection capabilities (Ibrahim et al., 2020).

Operation: The molecules of volatile organic compounds present in the environment diffuse onto the sensor surface and are absorbed by the active material (Kimura et al., 2012; do Nascimento et al., 2006). Graphene, due to its high surface area and highly conductive nature, facilitates the interaction with VOCs (Tung et al., 2020), while MOFs or other porous materials improve selectivity towards specific target molecules (Shen et al., 2022). In resistive sensors, the interaction between VOC molecules and graphene or metal oxides alters the distribution of electrons in the sensitive material, modifying its electrical resistance (Gakhar et al., 2022; Hassan et al., 2021; Maqbool et al., 2023). Alternatively, graphene can be applied to a piezoelectric material, leveraging the frequency characteristics of the substrate (Gupta et al., 2020).

Commonly, MOS sensors are likely the most employed for wine analysis thanks to their cost-effectiveness, reliable performance, minimal drift tendencies, and sensitivity to many volatile compounds. Nonetheless, they exhibit poor selectivity and susceptibility. Regarding CP sensors, they are highly sensitive and resistant to poisoning effects compared to MOS devices. However, they exhibit limited reproducibility. In contrast, MOSFET sensors operate on the variations of electrostatic potential. They are generally robust in different environmental conditions, although precise temperature control is advantageous for accurate data interpretation. Finally, mass-sensitive devices, such as piezoelectric sensors, utilize piezoelectricity to convert mechanical variations caused by ligand mass into changes in resonance frequency. Despite their high

selectivity, they demonstrate limited stability to fluctuations in temperature and humidity (Chambers et al., 2013).

A key point of the E-nose use is that the data derived from the sensor detections require accurate manipulation and interpretation, which are normally performed under the Machine Learning and chemometrics principles. Among the most commonly used statistical approaches there are Support Vector Machines (SVM), clustering algorithms like k-means, multivariate statistical analysis, Artificial Neural Networks (ANN), and, in some cases, more complex algorithms such as those applied in Deep Learning (DL) computation. These are considered among the most popular when used within E-nose systems (Chambers et al., 2013). The E-nose presents several notable advantages over traditional sensory analysis methods. In particular, it stands out for greater precision and reliability, proving to be more time-efficient and less susceptible to environmental factors (Li et al., 2017; Shuba et al., 2022). Unlike analytical methods like gas chromatography, the E-nose is distinguished by its user-friendly nature, as it does not require extensive expertise for operation and interpretation (Chambers et al., 2013). Hence, E-nose sensors are extensively studied and utilized in the wine industry, especially considering that the aromatic profile of different wines provides a range of information regarding attributes, winemaking methods, geographical origin, wine aging, and potential deviations that wines may exhibit.

As clearly observable, E-nose equipped with MOS sensors are commonly used for detecting wine spoilage. These sensors detect volatile compounds associated with defects like trichloroanisole (TCA) and corky off-flavors (Santos et al., 2022; Meléndez et al., 2022; Gamboa et al., 2019). They are also employed for rapid detection and non-destructive analysis of wine defects, such as those caused by *Brettanomyces* spp (Franceschi et al., 2016). E-nose has also been used for the evaluation of wine and grape quality: E-noses are used to differentiate between grape musts with different degrees of ripeness and different varieties. This can be of extreme importance, being able to decide when to harvest a grape with a pronounced aromatic maturity (Baietto et al., 2015; Fan et al., 2023; Wotner et al., 2023) and improve the aromatic characteristics of the finished wine. Later, electronic noses could also be used to decipher the different grape varieties used to make wines (Yu et al., 2019; Han et al., 2020) or perhaps used to identify the wines themselves (Franceschi et al., 2017; Han et al., 2020; Gonzalez Viejo et al., 2022; Aleixandre et al., 2018). A further application of great interest is related to the possibility of defect detection in wine, such as the possibility of detecting the presence or absence of reduction (Ma et al., 2023), monitoring the development of acetic bacteria (Aleixandre et al.,

2018) or even for any other process deviations that may occur (Ma et al., 2023; Franceschi et al., 2016). Wine ageing is another of those production stages for which the application of electronic noses can be exploited, whether in sparkling wines (Martínez-García et al., 2021), liqueur wines (Petrozziello et al., 2022) or any other wine (Liu et al., 2020). There is also great interest in the use of e-nose to select different winemaking practices (Zhang et al., 2021; Aleixandre et al., 2015; Han et al., 2020), or to assess the activity of added enzymes on the aroma pattern (Zhang et al., 2021), or to monitor carbonic maceration in certain types of wine (Bianchi et al., 2023), to detect the development of noble rot (*Botrytis cinerea*) (Modesti et al., 2024) or to monitor the aromatic differences between a Charmat or traditional method re-fermented wine (Muñoz-Castells et al., 2024). The electronic nose can also be used to monitor how defoliation affects the aromatic component of the grapes (Georgiadou et al., 2023). The application of these tools in being able to compare different wines of different vintages and identify quality wines seems to be a widely studied aptitude (Harris et al., 2023; Viejo et al., 2023; Paknahad et al., 2017). Applications to monitor the varietal characteristics of certain varieties, such as Petit Manseng and Syrah, have also been studied (Harris et al., 2023; Gardner et al., 2017). These applications utilize various sensors and chemometric analyses, often in conjunction with wet chemistry techniques (Giungato et al., 2018). Additionally, they are used for the evaluation of differences in volatile profiles of sweet wines (Baniță et al., 2023), authentication, and quantitation of red wines (Cabellos et al., 2018; Celdrán et al., 2022; Liu et al., 2023; Wang et al., 2022; Paredes-Doig et al., 2021), and modification with zeolite for enhanced detection of wine aroma compounds (Paredes-Doig et al., 2019). Overall, electronic noses play a crucial role in various aspects of enology, offering rapid, non-destructive, and cost-effective methods for quality assessment, defect detection, and process optimization in winemaking and grape cultivation.

In this overview, however, it has to be recognized that the E-nose is a non-specific sensor-based device whose discriminative ability enables it to perceive a pattern or aroma profile much more like the human nose than GC-MS identification (Chambers et al., 2013). This leads to the fact that very often the results of E-nose measurements differ considerably from those of VOCs detected by traditional analytics, an expected observation considering that, in general, VOCs are derived from a quantitative GC-MS analysis of molecules that are definitely present in the analyzed matrix; whereas in the case of tasting or E-nose, not all VOCs are certainly detected (Wotner et al., 2023). This is because volatile molecules have different perception thresholds and are subject to chemical interactions, combinations, cover-ups, and synergies that can strongly influence their aromatic perception by the human nose, or E-nose (Buck et al., 2004; Bucak et al., 2009). It is known that the aroma, or better the smell, of grape juice is, in most cases, very

similar in many grape varieties due to the absence of fermentation processes and enzymatic or chemical reactions, even though, after performing GC-MS analyses, a significant difference in VOCs is appreciated.

1.4 Interpretation of non-destructive data

The final step in the use of a non-destructive instrument is the data acquisition and processing system, which converts the electrical signal produced by the detector into a digital format and analyzes it using a computer. A software processes the data to generate a signal and calculate parameters of interest (e.g., absorbance or Δf). Over the years, chemometric approaches have been increasingly employed to better interpret the data. This branch of chemistry applies mathematical and statistical methods to optimize data acquisition and improve interpretability, proving essential for handling the vast amount of data generated (Lavine et al., 2013).

Among the various applications of chemometrics, one involves exploring data to identify hidden groups or trends using some of the following analyses:

- Principal Component Analysis (PCA) is a widely used technique for dimensionality reduction, helping to visualize relationships between samples by capturing the most significant variance in the data with a reduced number of components (Baytas et al., 2016; Zhang et al., 2010; Vidal et al., 2011). PCA transforms the original variables into a new set of uncorrelated variables (principal components) that retain most of the data variance.

- Cluster Analysis (K-means, Hierarchical Clustering). The K-means algorithm partitions data into K clusters by minimizing the variance within each group (Song et al., 2009; Ruwet et al., 2011). It is widely used for its simplicity and efficiency but may struggle with non-convex shapes and is sensitive to initial centroid placement (Mamu et al., 2023). Hierarchical clustering, on the other hand, constructs a hierarchy of clusters by aggregating (bottom-up approach) or dividing (top-down approach) the data (Vijayakumar et al., 2011). While computationally more intensive, it can better handle clusters of different shapes and sizes than K-means (Abdullah et al., 2021).

- Linear Discriminant Analysis (LDA) and Support Vector Machines (SVM) are useful for classification between known groups. LDA finds a linear combination of features that best separates two or more classes. It is effective for high-dimensional data but assumes normality and homoscedasticity (equal covariance among classes) (Xu et al., 2009). SVMs are a powerful classification method that identifies the hyperplane maximizing the margin between different classes (Xu et al., 2009; Ksantini et al., 2010; Colin et al., 2010; Shashoa et al., 2016). They are particularly effective for binary classification but can be extended to multiclass problems using

various strategies (Murty et al., 2016; Shieh et al., 2006; Qian et al., 2012). SVMs are known for their robustness and high performance in applications such as pattern recognition and data mining (Nayak et al., 2015).

Another application of chemometrics in non-destructive techniques is the quantification of chemical compound concentrations, enabling the development of accurate predictive models.

Some of the most commonly used approaches include:

- Partial Least Squares Regression (PLS), a standard method for correlating spectral data with known concentration values. It is particularly effective in handling multicollinearity and overfitting issues by summarizing explanatory variables into latent variables that maximize covariance with the response variable (Silalahi et al., 2021; Mishra et al., 2022).

- Multiple Linear Regression (MLR) uses multiple wavelengths to predict quantitative values. It is effective when the number of explanatory variables is low, and multicollinearity is minimal (Baird et al., 2016; Bansal et al., 2023). However, MLR can suffer from reliability issues due to multicollinearity and overfitting when applied to datasets with numerous correlated variables (Huang et al., 2022).

- Artificial Neural Networks (ANNs), more advanced models based on human neural networks to enhance predictive capabilities in complex cases. ANNs consist of interconnected processing elements called neurons, organized into layers. These neurons process information by dynamically responding to external inputs (Wang et al., 2022; Chen et al., 2019). Each neuron receives inputs, processes them, and transmits the output to other neurons. ANNs can learn from data through a process called training, during which the network adjusts connection weights based on the input-output relationships present in the training data (Galimberti et al., 2023; Lange et al., 2016; Jayara et al., 2016). In many fields, spectroscopy combined with chemometrics is used to identify and classify samples, such as Discriminant Analysis (PLS-DA, LDA), which allows classification into predefined categories (e.g., authentic vs. counterfeit).

The integration of non-destructive techniques into the winemaking production process presents several challenges, despite the existence of these chemometric approaches, particularly in data processing. One of the most critical aspects concerns data collection and accuracy. Techniques such as spectroscopy require precise data acquisition, but operating under variable field conditions can complicate the process. For instance, grape cluster counting using deep learning models must account for factors such as occlusions and lighting variations, which can affect the reliability of yield estimates (Íñiguez et al., 2024). Additionally, the significant variability in vineyard conditions, ranging from different grapevine varieties to various phenological stages,

further complicates the uniform collection and processing of data (Victorino et al., 2022). For this reason, it is essential to develop robust models capable of adapting to diverse scenarios without losing precision.

Data processing and analysis also pose significant challenges. Non-destructive techniques generate vast amounts of information, requiring substantial computational resources and advanced algorithms for effective processing (Harrap et al., 2018). Artificial intelligence and machine learning play a key role in transforming raw data into valuable insights, but their effective application requires specialized expertise and adequate computational power (Tardaguila et al., 2021). An additional level of complexity arises when developing accurate predictive models, such as those for vineyard yield estimation or smoke taint detection in wines (Fuentes et al., 2023). These models require complex data processing and the integration of multiple information sources, making the process challenging to manage and optimize.

Furthermore, continuous monitoring requirements and the risk of instrument malfunctions present significant obstacles that must be addressed to ensure consistent data quality and system stability (Ferrè et al., 2019). Another challenge lies in transitioning from experimental applications to large-scale adoption in commercial vineyards and wineries. For these technologies to be effectively integrated into the industry, they must be intuitive, economically viable, and easily implementable across extensive cultivated areas. Proper planning and adaptation are necessary to ensure that these solutions align with existing vineyard and winery management practices without introducing operational complications.

The environmental impact and operational variability in vineyards and wineries add further challenges to the use of non-destructive techniques. These technologies must be flexible enough to adapt to different environmental conditions and operational practices, which can vary significantly from one winery to another (Ramirez et al., 2023; Mishra et al., 2021; Alves et al., 2021). Moreover, the variability in grapevine phenotypes and the need to adopt sustainable practices further emphasize the necessity for scalable technologies that can be adapted to different viticultural environments, from small-scale operations to large enterprises (Costa et al., 2024).

1.5 Aim of the Research

In light of the recent challenges faced by the wine industry, including climate-related changes, evolving consumer preferences, and the need to enhance environmental and economic

sustainability, this doctoral research focused on exploring innovative, non-destructive approaches for wine analysis and wine inspection. The growing interest in precision-oriented and low-impact technologies has highlighted the need for analytical tools that are fast, affordable, sustainable, and accessible even to small- and medium-sized producers. In this context, the study investigated the application of non-destructive technologies namely low-cost spectroscopic prototypes (both VIS and NIR), electronic noses (E-noses), and quartz crystal microbalances (QCM-D) functionalized with different materials. These technologies were tested with the aim of evaluating their potential for real-time process monitoring and quality assessment during key winemaking stages.

Spectroscopic prototypes were explored as tools mean to track the evolution of phenolic compounds during fermentation, The QCM-D sensor, on the other hand, was employed to investigate specific classes of phenolic compounds through selective functionalization. Altogether, these tools represent promising strategies for improving decision-making processes in wineries, reducing environmental impact, and responding to current market demands for transparency, quality, and sustainability.

1.6 Thesis Outline

Chapter 2 describes the development and application of a low-cost VIS prototype spectrophotometer for monitoring phenolic extraction during maceration on skins in red wine production. The system was tested on three grape varieties under different fermentation conditions (destemmed and whole-cluster). Destructive reference analyses were carried out at selected time points to validate and correlate the spectral data obtained from the prototype.

Chapter 3 presents the advancement of the previous approach through the implementation of a low-cost NIR prototype spectrometer. The same phenolic parameters were monitored with the aim of improving the performance, sensitivity, and applicability of the technology compared to the VIS-based prototype.

Chapter 4 focuses on the use of a QCM-D prototype sensor functionalized with different peptides, designed to selectively monitor specific phenolic families in wine. This approach aims to increase molecular specificity while maintaining a non-destructive and label-free analysis method.

Chapter 5 provides general conclusions and discusses possible future developments for the integration and optimization of the non-destructive technologies investigated throughout the research.

REFERENCES

1. Buck, L. B. (2004). Olfactory receptors and odor coding in mammals. *Nutrition reviews*, 62(suppl_3), S184-S188.
2. Bucak, İ. Ö., & Karlık, B. (2009). Hazardous odor recognition by CMAC based neural networks. *Sensors*, 9(9), 7308-7319.
3. McGann JP (2017) Poor human olfaction is a 19th-century myth, *Science* 356:eaam7263.
4. Gauglitz G. Ultraviolet and visible spectroscopy. In: Gunzler H, Williams A, editors. *Handbook of analytical techniques*. Vol. 1. Weinheim: Wiley-VCH, 2001.
5. Picollo, M., Aceto, M., & Vitorino, T. (2019). UV-Vis spectroscopy. *Physical sciences reviews*, 4(4), 20180008.
6. Nassau K. *The physics and chemistry of color, the fifteen causes of color*. New York: Wiley & Sons; 1983.
7. Castañeda-Ovando, A., de Lourdes Pacheco-Hernández, M., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food chemistry*, 113(4), 859-871.
8. Schmid, F.X. Biological macromolecules: UV-visible spectrophotometry. In *Encyclopedia of Life Sciences*; Robinson, S., Ayres, E., Eds.; Macmillan Publishers: London, UK, 2001; pp. 1–4. [[Google Scholar](#)]
9. Esfandiary, R.; Middaugh, C.R. Ultraviolet absorption spectroscopy. In *Analysis of Aggregates and Particles in Protein Pharmaceuticals*; Mahler, H.C., Jiskoot, W., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2012; pp. 171–200. [[Google Scholar](#)]
10. Hofmann, A. (2010). 12 Spectroscopic techniques: I Spectrophotometric techniques. *Principles and techniques of biochemistry and molecular biology*, 477-521.
11. Wang, X., Zhao, C., Li, F., Li, B., Li, K., Yue, Q., & Zhao, L. (2022, December). Near-infrared spectroscopy in biomedical applications. In *International Conference on Biomedical and Intelligent Systems (IC-BIS 2022)* (Vol. 12458, pp. 630-632). SPIE.
12. Zapata, F., Ferreiro-González, M., & García-Ruiz, C. (2018). Interpreting the near infrared region of explosives. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 204, 81-87

13. Nishida, J., Shigeto, S., Yabumoto, S., & Hamaguchi, H. O. (2012). Anharmonic coupling of the CH-stretch and CH-bend vibrations of chloroform as studied by near-infrared electroabsorption spectroscopy. *The Journal of Chemical Physics*, 137(23).
14. Han, L., Sun, Y., Cai, W., & Shao, X. (2023). Seeking the structure of water from the combination of bending and stretching vibrations in near infrared spectra. *Journal of Near Infrared Spectroscopy*, 31(4), 204-210.
15. (Koohafkan, P., & Altieri, M. A. (2011). Globally Important Agricultural Heritage Systems: A Legacy for the Future (pp. 1-47). Rome: Food and Agriculture Organization of the United Nations).
16. (Tripp, R. (2001). Agricultural Technology Policies for Rural Development. *Development Policy Review*, 19(4), 479-489).
17. (Banks, G., & Overton, J. (2010). Old World, New World, Third World? Reconceptualising the Worlds of Wine. *Journal of Wine Research*, 21(1), 57-75).
18. (OIV (International Organization of Vine and Wine). *State of the World Vine and Wine Sector in 2023*. 2024. Available online: https://www.oiv.int/sites/default/files/202404/OIV_STATE_OF_THE_WORLD_VINE_AND_WINE_SECTO_IN_2023.pdf (accessed on April 5, 2024));
19. Galati, A., Schifani, G., Crescimanno, M., & Migliore, G. (2019). “Natural wine” consumers and interest in label information: An analysis of willingness to pay in a new Italian wine market segment. *Journal of Cleaner Production*, 227, 405-413.
20. Scozzafava, G., Gerini, F., Boncinelli, F., Contini, C., & Casini, L. (2021). How much is a bottle of conventional, organic or biodynamic wine worth? Results of an experimental auction. *Food Quality and Preference*, 93, 104259.
21. Pasvanka, K., Tzachristas, A., & Proestos, C. (2019). Quality tools in wine traceability and authenticity. In *Quality control in the beverage industry* (pp. 289-334). Academic Press.
22. Schäufele, I., & Hamm, U. (2017). Consumers’ perceptions, preferences and willingness-to-pay for wine with sustainability characteristics: A review. *Journal of Cleaner production*, 147, 379-394.
23. Sellers, R. (2016). Would you pay a price premium for a sustainable wine? The voice of the Spanish consumer. *Agriculture and agricultural science procedia*, 8, 10-16.
24. Gallenti, G., Troiano, S., Marangon, F., Bogoni, P., Campisi, B., & Cosmina, M. (2019). Environmentally sustainable versus aesthetic values motivating millennials’

- 910 preferences for wine purchasing: evidence from an experimental analysis in
911 Italy. *Agricultural and Food Economics*, 7, 1-16.
- 912 25. Pomarici, E., & Vecchio, R. (2014). Millennial generation attitudes to sustainable wine:
913 An exploratory study on Italian consumers. *Journal of Cleaner Production*, 66, 537-
914 545.
- 915 26. Sykalia, D.; Chrisostomidou, Y.; Karabagias, I.K. (2023). *An Exploratory Research*
916 *Regarding Greek Consumers' Behavior on Wine and Wineries' Character*. *Beverages*,
917 9, 43. <https://doi.org/10.3390/beverages9020043>;
- 918 27. Aylward, D., & Zanko, M. (2008). Reconfigured domains: alternative pathways for the
919 international wine industry. *International journal of technology, policy and*
920 *management*, 8(2), 148-166.
- 921 28. Overton, J., Murray, W. E., & Banks, G. (2012). The race to the bottom of the glass?
922 Wine, geography, and globalization. *Globalizations*, 9(2), 273-287.
- 923 29. Del Rey, R., & Loose, S. (2023). State of the International Wine Market in 2022: New
924 market trends for wines require new strategies. *Wine Economics and Policy*, 12(1), 3-
925 18.
- 926 30. Duarte Alonso, A., Bressan, A., O'Shea, M., & Krajsic, V. (2014). Exporting wine in
927 complex times: A study among small and medium wineries. *Journal of Small Business*
928 *and Enterprise Development*, 21(2), 345-364.
- 929 31. Tsiakis, T.; Anagnostou, E.; Granata, G.; Manakou, V. (2022). *Communicating Terroir*
930 *through Wine Label Toponymy: Greek Wineries' Practice*. *Sustainability*, 14, 6067)
- 931 32. Futami, Y., Ozaki, Y., & Ozaki, Y. (2016). Absorption intensity changes and
932 frequency shifts of fundamental and first overtone bands for OH stretching vibration
933 of methanol upon methanol-pyridine complex formation in CCl₄: analysis by
934 NIR/IR spectroscopy and DFT calculations. *Physical Chemistry Chemical*
935 *Physics*, 18(7), 5580-5586.
- 936 33. Shenk, J. S., & Westerhaus, M. O. (1994). The application of near infrared reflectance
937 spectroscopy (NIRS) to forage analysis. *Forage quality, evaluation, and utilization*,
938 406-449.
- 939 34. Hofmann, A. (2010). 13 Spectroscopic techniques: II Structure and interactions.
- 940
- 941 35. Wen, J., Xu, G., Zhang, A., Ma, W., & Jin, G. (2024). Emerging technologies for
942 rapid non-destructive testing of grape quality: A review. *Journal of food composition*
943 *and analysis*, 106446.

36. Bellincontro, A., Valentini, M., Forniti, R., & Mencarelli, F. (2015). Innovative application of NIR-AOTF and MRI to study water behaviour in cut flowers. *Advances in horticultural science [rivista dell'ortoflorofrutticoltura italiana]*: 29, 1, 2015, 30-36.
37. Chapman, J., Gangadoo, S., Truong, V. K., & Cozzolino, D. (2019). Spectroscopic approaches for rapid beer and wine analysis. *Current Opinion in Food Science*, 28, 67-73.
38. Sigeru Omatu, Mitsuaki Yano, and Yoshinori Ikeda. 2015. Smell classification of wines by the learning vector quantization method. In *Proceedings of the 30th Annual ACM Symposium on Applied Computing (SAC '15)*. Association for Computing Machinery, New York, NY, USA, 195–200.
39. Maxwell, J. C. (1873). *A treatise on electricity and magnetism* (Vol. 1). Clarendon press.
40. Hofmann, A. (2010). 12 Spectroscopic techniques: I Spectrophotometric
41. Pérez-Juste, I., & Nieto Faza, O. (2015). Interaction of radiation with matter. *Structure Elucidation in Organic Chemistry: The Search for the Right Tools*, 1-26.
42. Svanberg, S., & Svanberg, S. (2001). *Atomic and molecular spectroscopy*. Heidelberg: Springer-Verlag.
43. Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2015). *Introduction to spectroscopy*.
44. Vitha, M. F. (2018). *Spectroscopy: Principles and instrumentation*. John Wiley & Sons
45. J.-K. Han, M. Kang, J. Jeong, I. Cho, J.-M. Yu, K.-J. Yoon, I. Park, Y.-K. Choi, Artificial Olfactory Neuron for an In-Sensor Neuromorphic Nose. *Adv. Sci.* 2022, 9, 2106017.
46. A Guangyu Zhou, Gregory Lane, Shiloh L Cooper, Thorsten Kahnt, Christina Zelano (2019) Characterizing functional pathways of the human olfactory system *eLife* 8:e47177.
47. Devanand DP, Lee S, Manly J, Andrews H, Schupf N, Masurkar A, Stern Y, Mayeux R, Doty RL (2015) Olfactory identification deficits and increased mortality in the community, *Annals of Neurology* 78:401–411.
48. Borowik, P.; Adamowicz, L.; Tarakowski, R.; Siwek, K.; Grzywacz, T. Odor Detection Using an E-Nose With a Reduced Sensor Array. *Sensors* 2020, 20, 3542.

- 977 49. Zhang, D.; Guo, D.; Yan, K. *Breath Analysis for Medical Applications*; Springer:
978 Singapore, 2017.
- 979 50. Dragonieri, S., Pennazza, G., Carratu, P. et al. Electronic Nose Technology in
980 Respiratory Diseases. *Lung* 195, 157–165 (2017).
- 981 51. Wilson, A.D. Application of Electronic-Nose Technologies and VOC-Biomarkers for
982 the Noninvasive Early Diagnosis of Gastrointestinal Diseases. *Sensors* 2018, 18,
983 2613.
- 984 52. Wilson, A.D. Developments of Recent Applications for Early Diagnosis of Diseases
985 Using Electronic-Nose and Other VOC-Detection Devices. *Sensors* 2023, 23, 7885.
- 986 53. Zarra T., Cimattorus C., Naddeo V., Reiser M., Belgiorio V. and Kranert M. (2018),
987 Environmental odour monitoring by electronic nose, *Global NEST Journal*, 20(XX),
988 XX-XX
- 989 54. Prasad, P., Raut, P., Goel, S. et al. Electronic nose and wireless sensor network for
990 environmental monitoring application in pulp and paper industry: a review. *Environ*
991 *Monit Assess* 194, 855 (2022).
- 992 55. Sayago, I.; Aleixandre, M.; Santos, J.P. Development of Tin Oxide-Based
993 Nanosensors for Electronic Nose Environmental Applications. *Biosensors* 2019, 9,
994 21.
- 995 56. E. Olenova, T. Kuchmenko, E. Drozdova, A. Legin, D. Kirsanov, Identification of
996 plastic toys contaminated with volatile organic compounds using QCM gas sensor
997 array, *Talanta*, Volume 211, 2020, 120701, ISSN 0039-9140.
- 998 57. Valeriy Zaytsev, Fedor S. Fedorov, Boris Goikhman, Alexander Maslennikov, Vasili
999 Mashukov, Nikolay P. Simonenko, Tatiana L. Simonenko, Dinara Gabdullina, Olga
1000 Kovalenko, Elizaveta P. Simonenko, Polina Kvitko, Olga Penkova, Dina Satybaldina,
1001 Shakhmaran Seilov, Tatiana S. Dubinina, Dmitry A. Gorin, Albert G. Nasibulin,
1002 Rapid and accurate quality assessment method of recycled food plastics VOCs by
1003 electronic nose based on Al-doped zinc oxide, *Journal of Cleaner Production*, Volume
1004 418, 2023, 138042, ISSN 0959-6526
- 1005 58. H. G. J. Voss, R. A. Ayub and S. L. Stevan, "E-nose Prototype to Monitoring the
1006 Growth and Maturation of Peaches in the Orchard," in *IEEE Sensors Journal*, vol. 20,
1007 no. 20, pp. 11741-11750, 15 Oct.15, 2020,
- 1008 59. Emre Yavuzer, Determination of fish quality parameters with low cost electronic
1009 nose, *Food Bioscience*, Volume 41, 2021, 100948, ISSN 2212-4292

- 1010 60. Rafaela S. Andre, Murilo H.M. Facure, Luiza A. Mercante, Daniel S. Correa,
1011 Electronic nose based on hybrid free-standing nanofibrous mats for meat spoilage
1012 monitoring, *Sensors and Actuators B: Chemical*, Volume 353, 2022, 131114, ISSN
1013 0925-4005.
- 1014 61. Cascos, G.; Barea-Ramos, J.D.; Montero-Fernández, I.; Ruiz-Canales, A.; Lozano, J.;
1015 Martín-Vertedor, D. Burn Defect and Phenol Prediction for Flavoured Californian-
1016 Style Black Olives Using Digital Sensors. *Foods* 2023, 12, 1377.
- 1017 62. Sánchez, R.; Martín-Tornero, E.; Lozano, J.; Boselli, E.; Arroyo, P.; Meléndez, F.;
1018 Martín-Vertedor, D. E-Nose Discrimination of Abnormal Fermentations in Spanish-
1019 Style Green Olives. *Molecules* 2021, 26, 5353.
- 1020 63. Fujioka, K. Comparison of Cheese Aroma Intensity Measured Using an Electronic
1021 Nose (E-Nose) Non-Destructively with the Aroma Intensity Scores of a Sensory
1022 Evaluation: A Pilot Study. *Sensors* 2021, 21,
- 1023 64. Juan C. Rodriguez Gamboa, Eva Susana Albarracin E, Adenilton J. da Silva, Luciana
1024 L. de Andrade Lima, Tiago A. E. Ferreira, Wine quality rapid detection using a
1025 compact electronic nose system: Application focused on spoilage thresholds by acetic
1026 acid, *LWT*, Volume 108, 2019, Pages 377-384, ISSN 0023-6438
- 1027 65. Balda, P.; de Toda, F.M. Variedades Minoritarias de vid en La Rioja, 1st ed.;
1028 Consejería de Agricultura; Ganadería y Medio Ambiente: Logroño, Spain, 2017
- 1029 66. Naranjo, A.; Martínez-Lapuente, L.; Ayestarán, B.; Guadalupe, Z.; Pérez, I.; Canals,
1030 C.; Adell, E. Aromatic and Sensory Characterization of Maturana Blanca Wines Made
1031 with Different Technologies. *Beverages* 2021, 7, 10.
- 1032 67. P. Ribereau-Gayon, et al., *Handbook of Enology*, Volume 2: The Chemistry of Wine
1033 Stabilization and Treatments, third ed., John Wiley & Sons, Chinchester, 2021, p. 560
- 1034 68. Swain, K.K., Mishra, P.M. and Behera, B.K. (2023). Semiconductor Metal Oxide
1035 Thin Films. In *Metal Oxide Nanocomposite Thin Films for Optoelectronic Device*
1036 *Applications*, R.A. Zargar (Ed.).
- 1037 69. Xin Tian, Zongxin Hu, Chen Jia, Hairong Wang, Xueyong Wei, A review of advanced
1038 gas sensor based on sputtering SnO₂ thin film— challenges and opportunities,
1039 *Journal of Environmental Chemical Engineering*, Volume 11, Issue 6, 2023, 111516,
1040 ISSN 2213-3437
- 1041 70. M. Boomashri, P. Perumal, Aslam Khan, Ahmed M. El-Toni, Anees A Ansari,
1042 Ravindra K. Gupta, Prashantha Murahari, K. Deva Arun Kumar, Zinc influence on

nanostructured tin oxide (SnO₂) films as ammonia sensor at room temperature,
Surfaces and Interfaces, Volume 25, 2021, 101195, ISSN 2468-0230

71. Ambra Rita Di Rosa, Francesco Leone, Federica Cheli, Vincenzo Chiofalo, Fusion of electronic nose, electronic tongue and computer vision for animal source food authentication and quality assessment – A review, Journal of Food Engineering, Volume 210, 2017, Pages 62-75, ISSN 0260-8774
72. Robbiani, S.; Lotesoriere, B.J.; Dellacà, R.L.; Capelli, L. Physical Confounding Factors Affecting Gas Sensors Response: A Review on Effects and Compensation Strategies for Electronic Nose Applications. Chemosensors 2023, 11, 514.
73. Dey, A. Semiconductor metal oxide gas sensors: A review. Mater. Sci. Eng. B Solid-State Mater. Adv. Technol. 2018, 229, 206–217.
74. F. Miramirkhani and A. H. Navarchian, "Morphology, Structure, and Gas Sensing Performance of Conductive Polymers and Polymer/Carbon Black Composites Used for Volatile Compounds Detection," in IEEE Sensors Journal, vol. 17, no. 10, pp. 2992-3000, 15 May15, 2017,
75. Sorvin Michail, Belyakova Svetlana, Stoikov Ivan, Shamagsumova Rezeda, Evtugyn Gennady, Solid-Contact Potentiometric Sensors and Multisensors Based on Polyaniline and Thiocalixarene Receptors for the Analysis of Some Beverages and Alcoholic Drinks,Frontiers in Chemistry, 6, 2018.
76. Graboski, A. M., Galvagni, E., Manzoli, A., Shimizu, F. M., Zakrzewski, C. A., Weschenfelder, T. A., ... & Steffens, C. (2018). Lab-made electronic-nose with polyaniline sensor array used in classification of different aromas in gummy candies. *Food Research International*, 113, 309-315.
77. C. Henrique, A. Esteves, B.A. Iglesias, T. Ogawa, K. Araki, L. Hoehne, J. Gruber, Identification of tobacco types and cigarette brands using an electronic nose based on conductive polymer/porphyrin composite sensors, ACS Omega 3 (2018) 6476–6482.
78. Zhijie Wei, Qu Zhou, Zhaorui Lu, Lingna Xu, Yingang Gui, Chao Tang, Morphology controllable synthesis of hierarchical WO₃ nanostructures and C₂H₂ sensing properties, Physica E: Low-dimensional Systems and Nanostructures, Volume 109, 2019, Pages 253-260, ISSN 1386-9477
79. Ambra Rita Di Rosa, Francesco Leone, Vincenzo Chiofalo, 7 - Electronic noses and tongues, Editor(s): Yolanda Pico, Chemical Analysis of Food (Second Edition), Academic Press, 2020, Pages 353-389, ISBN 9780128132661

- 1076 80. Sierra-Padilla, A.; García-Guzmán, J.J.; López-Iglesias, D.; Palacios-Santander, J.M.;
1077 Cubillana-Aguilera, L. E-Tongues/Noses Based on Conducting Polymers and
1078 Composite Materials: Expanding the Possibilities in Complex Analytical Sensing.
1079 Sensors 2021, 21, 4976.
- 1080 81. Y. Zhao et al., "Detection of volatile organic compounds by high-Q piezotransduced
1081 single-crystal silicon bulk acoustic resonator arrays," 2016 IEEE SENSORS, Orlando,
1082 FL, USA, 2016, pp. 1-3,
- 1083 82. Mujahid, A.; Dickert, F.L. Surface Acoustic Wave (SAW) for Chemical Sensing
1084 Applications of Recognition Layers. Sensors 2017, 17, 2716.
- 1085 83. Yuan Zhao, Qingrui Yang, Ye Chang, Hemi Qu, Wei Pang, Hao Zhang, Xuexin Duan,
1086 Detection and discrimination of volatile organic compounds using a single multi-
1087 resonance mode piezotransduced silicon bulk acoustic wave resonator (PSBAR) as
1088 virtual sensor array, Sensors and Actuators B: Chemical, Volume 254, 2018, Pages
1089 1191-1199, ISSN 0925-4005
- 1090 84. Gao, Junning and Liu, Guorong and Li, Jie and Li, Guoqiang, Recent developments
1091 of film bulk acoustic resonators, Functional Materials Letters, 09, 03, 1630002, 2016.
- 1092 85. Okur, S.; Sarheed, M.; Huber, R.; Zhang, Z.; Heinke, L.; Kanbar, A.; Wöll, C.; Nick,
1093 P.; Lemmer, U. Identification of Mint Scents Using a QCM Based E-Nose.
1094 Chemosensors 2021, 9, 31.
- 1095 86. Salih Okur, Zejun Zhang, Mohammed Sarheed, Peter Nick, Uli Lemmer, Lars Heinke,
1096 Towards a MOF e-Nose: A SURMOF sensor array for detection and discrimination of
1097 plant oil scents and their mixtures, Sensors and Actuators B: Chemical, Volume 306,
1098 2020, 127502.
- 1099 87. Q. Li, Y. Gu and N. -f. Wang, "Application of Random Forest Classifier by Means of
1100 a QCM-Based E-Nose in the Identification of Chinese Liquor Flavors," in IEEE
1101 Sensors Journal, vol. 17, no. 6, pp. 1788-1794, 15 March15, 2017.
- 1102 88. Shuba, A.; Kuchmenko, T.; Umarkhanov, R. Piezoelectric Gas Sensors with
1103 Polycomposite Coatings in Biomedical Application. Sensors 2022, 22, 8529.
- 1104 89. Ruangchaiwat, Amnat, et al. "Simple laboratory-made piezoelectric sensors for
1105 detection of selected gaseous and/or vapor sample." High temperature 6 (2016): 7.
- 1106 90. Kaihuan Zhang, Ruifen Hu, Guokang Fan, Guang Li, Graphene oxide/chitosan
1107 nanocomposite coated quartz crystal microbalance sensor for detection of amine
1108 vapors, Sensors and Actuators B: Chemical, Volume 243, 2017, Pages 721-730, ISSN
1109 0925-4005.

- 1110 91. Na Songkhla, S.; Nakamoto, T. Overview of Quartz Crystal Microbalance Behavior
1111 Analysis and Measurement. *Chemosensors* 2021, 9, 350.
- 1112 92. Satapathy, A., Mahimkar, K., Mondal, S. et al. Attenuation of electromagnetic waves
1113 in polymeric terahertz imbibers: review. *J Mater Sci: Mater Electron* 34, 516 (2023).
- 1114 93. Paolesse, Roberto and Nardis, Sara and Monti, Donato and Stefanelli, Manuela and
1115 Di Natale, Corrado, Porphyrinoids for Chemical Sensor Applications, *Chemical*
1116 *Reviews*, 117, 4, 2517-2583, 2017.
- 1117 94. Yu Yang, Guo-Jie Jin, Xing-Jie Wang, Cai-Lin Kong, JiBin Liu, Yong-Sheng Tao,
1118 Chemical profiles and aroma contribution of terpene compounds in Meili (*Vitis*
1119 *vinifera* L.) grape and wine, *Food Chemistry*, Volume 284, 2019, Pages 155-161
- 1120 95. Ling, M.; Zhou, Y.; Lan, Y.; Cheng, C.; Wu, G.; Duan, C.; Shi, Y. Modification of
1121 Sensory Expression of 3-Isobutyl-2-methoxypyrazine in Wines through Blending
1122 Technique. *Molecules* 2021, 26, 3172.
- 1123 96. Timmins, J.J.B.; Kroukamp, H.; Paulsen, I.T.; Pretorius, I.S. The Sensory
1124 Significance of Apocarotenoids in Wine: Importance of Carotenoid Cleavage
1125 Dioxygenase 1 (CCD1) in the Production of β -Ionone. *Molecules* 2020.
- 1126 97. Capone, D.L., Barker, A., Williamson, P.O. and Francis, I.L. (2018), The role of
1127 potent thiols in Chardonnay wine aroma. *Australian Journal of Grape and Wine*
1128 *Research*, 24: 38-50.
- 1129 98. Junfang Wang, Tyler Abbey, Bartosz Kozak, Lufiani Lina Madilao, Ricco Tindjau,
1130 Jeff Del Nin, Simone Diego Castellarin, Evolution over the growing season of volatile
1131 organic compounds in Viognier (*Vitis vinifera* L.) grapes under three irrigation
1132 regimes, *Food Research International*, Volume 125, 2019, 108512.
- 1133 99. Marina Alarcón, M. Soledad Pérez-Coello, M. Consuelo Díaz-Maroto, M. Elena
1134 Alañón, Almudena Soriano, Effect of winery by-product extracts on oxidative
1135 stability, volatile organic compounds and aroma profile of cooked pork model
1136 systems during chilled storage, *LWT*, Volume 152, 2021, 112260, ISSN 0023-6438
- 1137 100. Herderich, M.; Barter, S.; Black, C.A.; Bramley, R.; Capone, D.; Dry, P.; Siebert, T.;
1138 Zhang, P. Terroir Effects on Grape and Wine Aroma Compounds. In *Advances in*
1139 *Wine Research*; American Chemical Society: Washington, DC, USA, 2015; Volume
1140 1203, pp. 131–146.
- 1141 101. Alem, H.; Rigou, P.; Schneider, R.; Ojeda, H.; Torregrosa, L. Impact of agronomic
1142 practices on grape aroma composition: A review. *J. Agric. Food Chem.* 2019, 99,
1143 975–985.

- 1144 102. Van Leewen, C.; Roby, J.; de Resseguier, L. Soil-Related terroir factors: A review.
1145 OENO One 2018, 52, 173–188.
- 1146 103. Chambers, E., IV; Koppel, K. Association of Volatile Compounds with Sensory
1147 Aroma and Flavor: The Complex Nature of Flavor. *Molecules* 2013, 18, 4887–4905.
- 1148 104. Petretto, G.L.; Mercenaro, L.; Urgeghe, P.P.; Fadda, C.; Valentoni, A.; Del Caro, A.
1149 Grape and Wine Composition in *Vitis vinifera* L. cv. Cannonau Explored by GC-MS
1150 and Sensory Analysis. *Foods* 2021, 10, 101.
- 1151 105. Isabelle Lesschaeve, Ann C. Noble, 7 - Sensory analysis of wine, Editor(s): Andrew
1152 G. Reynolds, In *Woodhead Publishing Series in Food Science, Technology and*
1153 *Nutrition, Managing Wine Quality (Second Edition)*, Woodhead Publishing, 2022,
1154 Pages 243-277, ISBN 9780081020678,
- 1155 106. Hove NCY, van Langenove H, Van Weyenberg S, Demeyer P (2016) Comparative
1156 odour measurements according to EN 13725 using pig house odour and n-butanol
1157 reference gas. *Biosyst Eng* 143: 119–127
- 1158 107. Hongwei Wang, Xiya Feng, Huayi Suo, Xing Yuan, Shengmin Zhou, Huijing Ren,
1159 Yuanrong Jiang, Jianquan Kan, Comparison of the performance of the same panel
1160 with different training levels: Flash profile versus descriptive analysis, *Food Quality*
1161 *and Preference*, Volume 99, 2022, 104582
- 1162 108. Njoman, M.F., Nugroho, G., Chandra, S.D.P., Permana, Y., Suhadi, S., Mujiono, M.,
1163 Hermawan, A.D. and Sugiono, S. (2017), "The vulnerability of human sensory
1164 evaluation and the promising senses instrumentation", *British Food Journal*, Vol. 119
1165 No. 10, pp. 2145-2160.
- 1166 109. Sipos, L.; Nyitrai, Á.; Hitka, G.; Friedrich, L.F.; Kókai, Z. Sensory Panel
1167 Performance Evaluation—Comprehensive Review of Practical Approaches. *Appl.*
1168 *Sci.* 2021, 11, 11977.
- 1169 110. Susana Fiszman, Natalia Salgado, Carlos E. Orrego, Gastón Ares, Comparison of
1170 methods for generating sensory vocabulary with consumers: A case study with two
1171 types of satiating foods, *Food Quality and Preference*, Volume 44, 2015, Pages 111-
1172 118, ISSN 0950-3293
- 1173 111. C. Coulon-Leroy, R. Symoneaux, G. Lawrence, E. Mehinagic, I. Maitre, Mixed
1174 Profiling: A new tool of sensory analysis in a professional context. Application to
1175 wines, *Food Quality and Preference*, Volume 57, 2017, Pages 8-16, ISSN 0950-3293

112. W.E. Larssen, E. Monteleone, M. Hersleth, Sensory description of marine oils through development of a sensory wheel and vocabulary, Food Research International, Volume 106, 2018, Pages 45-53, ISSN 0963-9969
113. Sharif, Mian and Butt, Masood and Sharif, Hafiz and Nasir, Muhammad, 2017,10,362-386,Sensory Evaluation and Consumer Acceptability
114. Robinson J, Harris I, King N (2016) Understanding Wines: Explaining Style and Quality. Wine & Spirit Education Trust, London.
115. Zraly K (2016) Windows on the world complete wine course. Sterling Publishing Company, New York.
116. MacNeil K (2015) The wine bible. Workman Publishing, New York.
117. Nickles J (2017) Certified Wine Educator Manual for Candidates. Create Space Independent Publishing Platform
118. Ruiz, J., Kiene, F., Belda, I. et al. Effects on varietal aromas during wine making: a review of the impact of varietal aromas on the flavor of wine. Appl Microbiol Biotechnol 103, 7425–7450 (2019).
119. de-la-Fuente-Blanco, A.; Ferreira, V. Gas Chromatography Olfactometry (GC-O) for the (Semi)Quantitative Screening of Wine Aroma. Foods 2020, 9, 1892.
120. David S. Hage, 1 - Chromatography, Editor(s): Nader Rifai, Andrea Rita Horvath, Carl T. Wittwer, Principles and Applications of Clinical Mass Spectrometry, Elsevier, 2018, Pages 1-32, ISBN 9780128160633
121. Yada Nolvachai, Chadin Kulsing, Philip J. Marriott, Multidimensional gas chromatography in food analysis, TrAC Trends in Analytical Chemistry, Volume 96, 2017, Pages 124-137, ISSN 0165-9936,
122. Vicente Ferreira, Paula Herrero, Julián Zapata, Ana Escudero, Coping with matrix effects in headspace solid phase microextraction gas chromatography using multivariate calibration strategies, Journal of Chromatography A, Volume 1407, 2015, Pages 30-41, ISSN 0021-9673
123. Pati, S.; Tufariello, M.; Crupi, P.; Coletta, A.; Grieco, F.; Losito, I. Quantification of Volatile Compounds in Wines by HS-SPME-GC/MS: Critical Issues and Use of Multivariate Statistics in Method Optimization. Processes 2021, 9, 662.
124. Zhang, P.; Piergiovanni, M.; Franceschi, P.; Mattivi, F.; Vrhovsek, U.; Carlin, S. Application of Comprehensive 2D Gas Chromatography Coupled with Mass Spectrometry in Beer and Wine VOC Analysis. Analytica 2023, 4, 347-373.

- 1209 125. Karine P. Nicolli, Aline C.T. Biasoto, Érica A. Souza-Silva, Celito C. Guerra,
1210 Henrique P. dos Santos, Juliane E. Welke, Cláudia A. Zini,
1211 126. Sensory, olfactometry and comprehensive two-dimensional gas chromatography
1212 analyses as appropriate tools to characterize the effects of vine management on wine
1213 aroma, *Food Chemistry*, Volume 243, 2018, Pages 103-117, ISSN 0308-8146.
1214 127. Sandra Marín-San Román, Pilar Rubio-Bretón, Eva P. Pérez-Álvarez, Teresa Garde-
1215 Cerdán Advancement in analytical techniques for the extraction of grape and wine
1216 volatile compounds, *Food Research International*, Volume 137, 2020, 109712, ISSN
1217 0963-9969.
1218 128. P. Parrilla Vázquez, E. Hakme, S. Uclés, V. Cutillas, M. Martínez Galera, A.R.
1219 Mughari, A.R. Fernández-Alba, Large multiresidue analysis of pesticides in edible
1220 vegetable oils by using efficient solid-phase extraction sorbents based on quick, easy,
1221 cheap, effective, rugged and safe methodology followed by gas chromatography–
1222 tandem mass spectrometry, *Journal of Chromatography A*, Volume 1463, 2016, Pages
1223 20-31, ISSN 0021-9673
1224 129. Chu, H.; Jo, J.; Son, Y.; Lee, J.Y.; Ahn, Y.G. Developing an Improved Strategy for the
1225 Analysis of Polychlorinated Dibenzo-p-Dioxins/Furans and Dioxin-like
1226 Polychlorinated Biphenyls in Contaminated Soils Using a Combination of a One-Step
1227 Cleanup Method and Gas Chromatography with Triple Quadrupole Mass
1228 Spectrometry. *Toxics* 2023, 11, 738.
1229 130. Jesús Lozano, José Pedro Santos, José Ignacio Suárez, Mariano Cabellos, Teresa
1230 Arroyo, Carmen Horrillo, Restricted accessAutomatic Sensor System for the
1231 Continuous Analysis of the Evolution of Wine, *Am J Enol Vitic.* 2015 66:148-155
1232 131. Rafael Martínez-García, Juan Moreno, Andrea Bellincontro, Luna Centioni, Anna
1233 Puig-Pujol, Rafael A. Peinado, Juan Carlos Mauricio, Teresa García-Martínez, Using
1234 an electronic nose and volatilome analysis to differentiate sparkling wines obtained
1235 under different conditions of temperature, ageing time and yeast formats, *Food*
1236 *Chemistry*, Volume 334, 2021, 127574, ISSN 0308-8146
1237 132. Petrozziello, M.; Rosso, L.; Portesi, C.; Asproudi, A.; Bonello, F.; Nardi, T.; Rossi,
1238 A.M.; Schiavone, C.; Scuppa, S.; Cantamessa, S.; et al. Characterisation of Refined
1239 Marc Distillates with Alternative Oak Products Using Different Analytical
1240 Approaches. *Appl. Sci.* 2022, 12, 8444.

- 1241 133. Juan C. Rodriguez Gamboa, Eva Susana Albarracin E., Adenilton J. da Silva, Tiago
1242 A. E. Ferreira, Electronic nose dataset for detection of wine spoilage thresholds, Data
1243 in Brief, Volume 25, 2019, 104202, ISSN 2352-3409
- 1244 134. Juan C. Rodriguez Gamboa, Adenilton J. da Silva, Ismael C. S. Araujo, Eva Susana
1245 Albarracin E., Cristhian M. Duran A., Validation of the rapid detection approach for
1246 enhancing the electronic nose systems performance, using different deep learning
1247 models and support vector machines, Sensors and Actuators B: Chemical, Volume
1248 327, 2021, 128921, ISSN 0925-4005
- 1249 135. Ma, Y.; Yu, K.; Chen, X.; Wu, H.; Xiao, X.; Xie, L.; Wei, Z.; Xiong, R.; Zhou, X.
1250 Effects of Plant-Derived Polyphenols on the Antioxidant Activity and Aroma of
1251 Sulfur-Dioxide-Free Red Wine. Molecules 2023, 28, 5255.
- 1252 136. Franceschi, Deborah; Cavalet, Elisabetta; Boatto, Vasco; Conte, Gianni; Bravi,
1253 Marco, Artificial diagnosis of sensory taints due to *brettanomyces* spp. contamination
1254 in Valpolicella wines, In: CHEMICAL ENGINEERING TRANSACTIONS. - ISSN
1255 2283-9216. - 54:(2016), pp. 343-348. [10.3303/CET1654058]
- 1256 137. F. Meléndez et al., "Fast Detection of TCA in Cork Stoppers by Means of Electronic
1257 Noses," 2020 IEEE International Symposium on Circuits and Systems (ISCAS),
1258 Seville, Spain, 2020, pp. 1-4.
- 1259 138. Santos, J.P.; Sayago, I.; Sanjurjo, J.L.; Perez-Coello, M.S.; Díaz-Maroto, M.C. Rapid
1260 and Non-Destructive Analysis of Corky Off-Flavors in Natural Cork Stoppers by a
1261 Wireless and Portable Electronic Nose. Sensors 2022, 22, 4687.
- 1262 139. Meléndez, F.; Arroyo, P.; Gómez-Suárez, J.; Palomeque-Mangut, S.; Suárez, J.I.;
1263 Lozano, J. Portable Electronic Nose Based on Digital and Analog Chemical Sensors
1264 for 2,4,6-Trichloroanisole Discrimination. Sensors 2022, 22, 3453.
- 1265 140. Juan C. Rodriguez Gamboa, Eva Susana Albarracin E, Adenilton J. da Silva, Luciana
1266 L. de Andrade Lima, Tiago A. E. Ferreira, Wine quality rapid detection using a
1267 compact electronic nose system: Application focused on spoilage thresholds by acetic
1268 acid, LWT, Volume 108, 2019, Pages 377-384, ISSN 0023-6438
- 1269 141. Aleixandre, M.; Santos, J.P.; Sayago, I.; Cabellos, J.M.; Arroyo, T.; Horrillo, M.C. A
1270 Wireless and Portable Electronic Nose to Differentiate Musts of Different Ripeness
1271 Degree and Grape Varieties. Sensors 2015, 15, 8429-8443
- 1272 142. Baietto, M.; Wilson, A.D. Electronic-Nose Applications for Fruit Identification,
1273 Ripeness and Quality Grading. Sensors 2015, 15, 899-931.

- 1274 143. Fan X, Pan L, Chen R. 2023. Characterization of flavor frame in grape wines detected
1275 by HS-SPME-GC-MS coupled with HPLC, electronic nose, and electronic tongue.
1276 Food Materials Research 3:9.
- 1277 144. Natalie Harris, Claudia Gonzalez Viejo, Christopher Barnes, Alexis Pang, Sigfredo
1278 Fuentes, Wine quality assessment for Shiraz vertical vintages based on digital
1279 technologies and machine learning modeling., Food Bioscience, Volume 56, 2023,
1280 103354, ISSN 2212-4292
- 1281 145. Gonzalez Viejo, Claudia and Harris, Natalie and Pang, Alexis and Barnes,
1282 Christopher and Fuentes, Sigfredo, Quality Assessment of Wine Vertical Vintages
1283 Based on Electronic Nose Coupled with Machine Learning Modelling. 15TH
1284 PANGBORN SENSORY SCIENCE SYMPOSIUM - MEETING NEW
1285 CHALLENGES IN A CHANGING WORLD (PSSS 2023):E02.
- 1286 146. M. Paknahad, A. Ahmadi, J. Rousseau, H. R. Nejad and M. Hoorfar, "On-Chip
1287 Electronic Nose For Wine Tasting: A Digital Microfluidic Approach," in IEEE
1288 Sensors Journal, vol. 17, no. 14, pp. 4322-4329, 15 July15, 2017.
- 1289 147. Georgiadou, E.C., Mina, M., Neoptoleμου, V., Koundouras, S., D'Onofrio, C.,
1290 Bellincontro, A., Mencarelli, F., Fotopoulos, V. and Manganaris, G.A. (2023), The
1291 beneficial effect of leaf removal during fruit set on physiological, biochemical, and
1292 qualitative indices and volatile organic compound profile of the Cypriot reference
1293 cultivar 'Xynisteri'. J Sci Food Agric, 103: 3776-3786.
- 1294 148. Antoce, A.O.; Cojocar, G.A. Evaluation by Flash GC Electronic Nose of the Effect
1295 of Combinations of Yeasts and Nutrients on the Aromatic Profiles of Feteasca Regala
1296 Wines after Two Years of Storage. Fermentation 2021, 7, 223.
- 1297 149. Antoce, A.O.; Cojocar, G.A. The use of GC-Electronic nose for the selection of a
1298 winemaking protocol leading to an enhanced volatile profile in wines from aromatic
1299 grape varieties, Scientific Papers. Series B, Horticulture. Vol. LXI, 2017, Print ISSN
1300 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.
- 1301 150. Jie Zhang, Tieru Wang, Ning Zhao, Junnan Xu, Yiman Qi, Xinyuan Wei, Mingtao
1302 Fan, Performance of a novel β -glucosidase BGL0224 for aroma enhancement of
1303 Cabernet Sauvignon wines, LWT, Volume 144, 2021, 111244, ISSN 0023-6438.
- 1304 151. Alessandro Bianchi, Gregorio Santini, Paola Piombino, Elisabetta Pittari, Chiara
1305 Sanmartin, Luigi Moio, Margherita Modesti, Andrea Bellincontro, Fabio Mencarelli,
1306 Nitrogen maceration of wine grape: An alternative and sustainable technique to

1307 carbonic maceration, Food Chemistry, Volume 404, Part A, 2023, 134138, ISSN
1308 0308-8146

1309 152. Muñoz-Castells, R., Modesti, M., Moreno-García, J., Rodríguez-Moreno, M., Catini,
1310 A., Capuano, R., Di Natale, C., Bellincontro, A. and Moreno, J. (2024),
1311 Differentiation through E-nose and GC-FID data modeling of rosé sparkling wines
1312 elaborated via traditional and Charmat methods. J Sci Food Agric.

1313 153. Modesti, Margherita and Alfieri, Gianmarco and Chieffo, Chiara and Mencarelli,
1314 Fabio and Vannini, Andrea and Catalani, Alessia and Chilosì, Gabriele and
1315 Bellincontro, Andrea, Destructive and non-destructive early detection of postharvest
1316 noble rot (*Botrytis cinerea*) in wine grapes aimed at producing high-quality
1317 wine, 2023, Journal of the Science of Food and Agriculture.

1318 154. Cojocaru, G.A.; Antocea, A.O. Influence of Glutathione and Ascorbic Acid Treatments
1319 during Vinification of Feteasca Regala Variety and Their Antioxidant Effect on
1320 Volatile Profile. Biosensors 2019,

1321 155. M. Aleixandre, J. P. Santos, I. Sayago, M. C. Horrillo, J. M. Cabellos and T. Arroyo,
1322 "Use of an electronic nose as a tool to differentiate winemaking techniques," 2015
1323 10th Spanish Conference on Electron Devices (CDE), Aranjuez - Madrid, Spain,
1324 2015, pp. 1-4,

1325 156. Han F, Zhang D, Aheto JH, Feng F, Duan T. Integration of a low-cost electronic nose
1326 and a voltammetric electronic tongue for red wines identification. Food Sci Nutr.
1327 2020; 8: 4330–4339.

1328 157. Franceschi, Deborah; Vincenzi, Simone; Boatto, Vasco L.; Bravi, Marco. Can sensory
1329 analysis and e-noses support the assessment work behind DOC and DOCG wines? In:
1330 CHEMICAL ENGINEERING TRANSACTIONS. - ISSN 2283-9216. -
1331 ELETTRONICO. - 57:(2017), pp. 1759-1764.

1332 158. Khorramifar, A.; Karami, H.; Wilson, A.D.; Sayyah, A.H.A.; Shuba, A.; Lozano, J.
1333 Grape Cultivar Identification and Classification by Machine Olfaction Analysis of
1334 Leaf Volatiles. Chemosensors 2022, 10, 125.

1335 159. Arina Oana Antocea, Marinela Vicuța Stroe, George Adrian Cojocaru, Tentative
1336 Application of an Electronic Nose to the Study of the Parentage of Romanian Grape
1337 Varieties Sarba and Alb Aromat, Agriculture and Agricultural Science Procedia,
1338 Volume 6, 2015, Pages 110-117, ISSN 2210-7843

- 1339 160. Tarì, Umberto, et al. "Identifying wine grape aromatic maturity using e-nose and GC-
1340 MS: the case of Nerello Mascalese grapes from two contrade of the Etna area."
1341 OENO One 58.1 (2024).
- 1342 161. Discriminant analysis of volatile compounds in wines obtained from different
1343 managements of vineyards obtained by e-nose, Smart Agricultural
1344 Technology, 6, 100343, 2023, 2772-375.
- 1345 162. Wotner, Julius and Dorksen, Helene and Pein-Hackelbusch, Miriam, Key Indicators
1346 for the Discrimination of Wines by Electronic Noses, 2023, IEEE International
1347 Conference on Industrial Informatics (INDIN), 2023-July
- 1348 163. D. Yu, X. Wang, H. Liu and Y. Gu, "A Multitask Learning Framework for Multi-
1349 Property Detection of Wine," in IEEE Access, vol. 7, pp. 123151-123157, 2019,
- 1350 164. Han F, Zhang D, Aheto JH, Feng F, Duan T. Integration of a low-cost electronic nose
1351 and a voltammetric electronic tongue for red wines identification. Food Sci Nutr.
1352 2020; 8: 4330–4339.
- 1353 165. Gonzalez Viejo, C.; Fuentes, S. Digital Assessment and Classification of Wine Faults
1354 Using a Low-Cost Electronic Nose, Near-Infrared Spectroscopy and Machine
1355 Learning Modelling. Sensors 2022, 22, 2303.
- 1356 166. Aleixandre, Manuel and Cabellos, Juan M. and Arroyo, Teresa and Horrillo, M. C.,
1357 Quantification of Wine Mixtures with an Electronic Nose and a Human Panel,
1358 Frontiers in Bioengineering and Biotechnology, 6, 2018.
- 1359 167. Denise M. Gardner, Susan E. Duncan, Bruce W. , Aroma Characterization of Petit
1360 Manseng Wines Using Sensory Consensus Training, SPME GC-MS, and Electronic
1361 Nose Analysis, Am J Enol Vitic. 2017 68:112-119;
- 1362 168. Celdrán, A.C.; Oates, M.J.; Molina Cabrera, C.; Pangua, C.; Tardaguila, J.; Ruiz-
1363 Canales, A. Low-Cost Electronic Nose for Wine Variety Identification through
1364 Machine Learning Algorithms. Agronomy 2022, 12, 2627.
- 1365 169. Baniță, C.; Antocea, O.A.; Cojocaru, G.A. Evaluation by a GC Electronic Nose of the
1366 Differences in Volatile Profile Induced by Stopping Fermentation with Octanoic and
1367 Decanoic Acid to Produce Sweet Wines. Chemosensors 2023, 11, 98.
- 1368 170. L. Liu, N. Na, J. Yu, W. Zhao, Z. Wang, Y. Zhu, C. Hu, Sniffing Like a Wine Taster:
1369 Multiple Overlapping Sniffs (MOSS) Strategy Enhances Electronic Nose Odor
1370 Recognition Capability. Adv. Sci. 2023, 2305639.

171. Hernández, E.; Pelegrí-Sebastiá, J.; Sogorb, T.; Chilo, J. Evaluation of Red Wine Acidification Using an E-Nose System with Venturi Tool Sampling. *Sensors* 2023, 23, 2878.
172. Song Wang, Xiao-Zhen Hu, Yan-Yan Liu, Ning-Ping Tao, Ying Lu, Xi-Chang Wang, Wing Lam, Ling Lin, Chang-Hua Xu, Direct authentication and composition quantitation of red wines based on Tri-step infrared spectroscopy and multivariate data fusion, *Food Chemistry*, Volume 372, 2022, 131259, ISSN 0308-8146.
173. Paredes-Doig, A.L., Cárcamo, H., Hurtado Cotillo, M., Sun Kou, R., Doig-Camino, E., Picasso, G. & La Rosa-Toro Gómez, A. (2019). Gas Sensors Modified with Zeolite Y for Assessing Wine Aroma Compounds, *Journal of Chemistry*, 7.
174. Paredes-Doig, Ana Lucía, et al. "Use of statistical methods for the interpretation of measurement results of Peruvian wines with MOS E-noses." (2021).
175. Liu, H.; Li, Q.; Yan, B.; Zhang, L.; Gu, Y. Bionic Electronic Nose Based on MOS Sensors Array and Machine Learning Algorithms Used for Wine Properties Detection. *Sensors* 2019, 19, 45
176. Giungato, P., Di Gilio, A., Palmisani, J., Marzocca, A., Mazzone, A., Brattoli, M., Giua, R., & de Gennaro, G. (2018). Synergistic approaches for odor active compounds monitoring and identification: State of the art, integration, limits and potentialities of analytical and sensorial techniques. *TrAC Trends in Analytical Chemistry*, 107, 116–129. <https://doi.org/10.1016/j.trac.2018.07.019>
177. Mastrangelo, N., Bianchi, A., Pettinelli, S., Santini, G., Merlani, G., Bellincontro, A., Baris, F., Chinnici, F., & Mencarelli, F. (2023). Novelty of Italian Grape Ale (IGA) beer: Influence of the addition of Gamay macerated grape must or dehydrated Aleatico grape pomace on the aromatic profile. *Heliyon*, 9(10), e20422. <https://doi.org/10.1016/j.heliyon.2023.e20422>
178. Liu, J., Zhu, X.-L., Ullah, N., & Tao, Y.-S. (2017). Aroma Glycosides in Grapes and Wine. *Journal of Food Science*, 82(2), 248–259. <https://doi.org/10.1111/1750-3841.13598>
179. Gupta, M., Athirah, N., & Hawari, H. F. (2020). Graphene derivative coated QCM-based gas sensor for volatile organic compound (VOC) detection at room temperature. *Indones. J. Electr. Eng. Comput. Sci*, 18(3), 1279-1286.
180. Gakhar, T., Basu, S., & Hazra, A. (2022). Carbon-metal oxide nanocomposites for selective detection of toxic and hazardous volatile organic compounds (VOC)—A review. *Green Analytical Chemistry*, 1, 100005.

- 1405 181. Hassan, K., Hossain, R., Farzana, R., & Sahajwalla, V. (2021). Microrecycled zinc
1406 oxide nanoparticles (ZnO NP) recovered from spent Zn–C batteries for VOC
1407 detection using ZnO sensor. *Analytica Chimica Acta*, 1165, 338563.
- 1408 182. Maqbool, Q., Yigit, N., Stöger-Pollach, M., Ruello, M. L., Tittarelli, F., &
1409 Rupprechter, G. (2023). Operando monitoring of a room temperature nanocomposite
1410 methanol sensor. *Catalysis Science & Technology*, 13(3), 624-636.
- 1411 183. Shen, Y., Tissot, A., & Serre, C. (2022). Recent progress on MOF-based optical
1412 sensors for VOC sensing. *Chemical Science*, 13(47), 13978-14007.
- 1413 184. Liu, X., Li, T., Liu, Y., Sun, Y., Han, Y., Lee, T. C., ... & Dang, A. (2024). Hybrid
1414 plasmonic aerogel with tunable hierarchical pores for size-selective multiplexed
1415 detection of VOCs with ultrahigh sensitivity. *Journal of Hazardous Materials*, 469,
1416 133893.
- 1417 185. Tung, T. T., Tran, M. T., Feller, J. F., Castro, M., Van Ngo, T., Hassan, K., ... & Losic,
1418 D. (2020). Graphene and metal organic frameworks (MOFs) hybridization for tunable
1419 chemoresistive sensors for detection of volatile organic compounds (VOCs)
1420 biomarkers. *Carbon*, 159, 333-344.
- 1421 186. Gakhar, T., Basu, S., & Hazra, A. (2022). Carbon-metal oxide nanocomposites for
1422 selective detection of toxic and hazardous volatile organic compounds (VOC)—A
1423 review. *Green Analytical Chemistry*, 1, 100005.
- 1424 187. Kimura, M., Sakai, R., Sato, S., Fukawa, T., Ikehara, T., Maeda, R., & Mihara, T.
1425 (2012). Sensing of vaporous organic compounds by TiO₂ porous films covered with
1426 polythiophene layers. *Advanced Functional Materials*, 22(3), 469-476.
- 1427 188. do Nascimento, G. C., De Melo, C. P., & Souto-Maior, R. (2006). Sensors of volatile
1428 compounds based on the dielectric relaxation of organic molecules. *Sensors and*
1429 *Actuators B: Chemical*, 115(1), 542-546.
- 1430 189. Ibrahim, H. M., & Klingner, A. (2020). A review on electrospun polymeric
1431 nanofibers: Production parameters and potential applications. *Polymer Testing*, 90,
1432 106647.
- 1433 190. Dong, Q., Xiao, M., Chu, Z., Li, G., & Zhang, Y. (2021). Recent progress of toxic gas
1434 sensors based on 3d graphene frameworks. *Sensors*, 21(10), 3386.
- 1435 191. Tung, T. T., Tran, M. T., Feller, J. F., Castro, M., Van Ngo, T., Hassan, K., ... & Losic,
1436 D. (2020). Graphene and metal organic frameworks (MOFs) hybridization for tunable
1437 chemoresistive sensors for detection of volatile organic compounds (VOCs)
1438 biomarkers. *Carbon*, 159, 333-344.

- 1439 192. Íñiguez, R., Gutiérrez, S., Poblete-Echeverría, C., Hernández, I., Barrio, I., &
1440 Tardáguila, J. (2024). Deep learning modelling for non-invasive grape bunch
1441 detection under diverse occlusion conditions. *Computers and Electronics in*
1442 *Agriculture*, 226, 109421.
- 1443 193. Victorino, G., Braga, R., Santos-Victor, J., & Lopes, C. M. (2022). Overcoming the
1444 challenge of bunch occlusion by leaves for vineyard yield estimation using image
1445 analysis. *Oeno One*.
- 1446 194. Ramirez Cando, L. J., & Guerrini, L. (2023, April). Assessment of grape pressing
1447 environmental performance with device technical sheets. In *III International*
1448 *Symposium on Beverage Crops 1387* (pp. 159-166).
- 1449 195. Costa, J. M., Carvalho, L., Figueira, R., Shimono, H., & da Silva, J. M. (2024)
1450 Molecular, Morpho-Functional, and Agronomic Phenotyping Tools for Grapevine
1451 Selection and Breeding. In *Plant Speed Breeding and High-throughput*
1452 *Technologies* (pp. 294-317). CRC Press.
- 1453 196. Ferré, G., Rezzouki, M., Germain, C., & Toulon, N. (2019, November). WineNote: an
1454 IoT project to enhance the wine taste. In *2019 26th IEEE International Conference on*
1455 *Electronics, Circuits and Systems (ICECS)* (pp. 185-188). IEEE.
- 1456 197. Tardaguila, J., Stoll, M., Gutiérrez, S., Proffitt, T., & Diago, M. P. (2021). Smart
1457 applications and digital technologies in viticulture: A review. *Smart Agricultural*
1458 *Technology*, 1, 100005.
- 1459 198. Fuentes, S., Summerson, V., & Viejo, C. G. (2023). Novel digital technologies to
1460 assess smoke taint in berries and wines due to bushfires. In *BIO Web of*
1461 *Conferences* (Vol. 56, p. 01007). EDP Sciences.
- 1462 199. Harrap, N., Rheaume, R., & Gosselin, A. (2018, November). Artificial Intelligence
1463 Approaches as tools for auditing and improving data analysis of advanced ultrasound
1464 techniques in non-destructive testing. In *Abu Dhabi International Petroleum*
1465 *Exhibition and Conference* (p. D011S008R004). SPE.
- 1466 200. Alves, V. M., Auerbach, S. S., Kleinstreuer, N., Rooney, J. P., Muratov, E. N., Rusyn,
1467 I., ... & Schmitt, C. (2021). Curated data in—trustworthy in silico models out: the
1468 impact of data quality on the reliability of artificial intelligence models as alternatives
1469 to animal testing. *Alternatives to laboratory animals*, 49(3), 73-82.
- 1470 201. Mishra, P., Roger, J. M., Marini, F., Biancolillo, A., & Rutledge, D. N. (2021).
1471 FRUITNIR-GUI: A graphical user interface for correcting external influences in

- multi-batch near infrared experiments related to fruit quality prediction. *Postharvest Biology and Technology*, 175, 111-114.
202. Littarru, E., Modesti, M., Alfieri, G., Pettinelli, S., Floridia, G., Bellincontro, A., ... & Brizzolara, S. (2025). Optimizing the winemaking process: NIR spectroscopy and e-nose analysis for the online monitoring of fermentation. *Journal of the Science of Food and Agriculture*, 105(3), 1465-1475.
203. Gayda, G., Stasyuk, N., Klepach, H., Gonchar, M., & Nisnevitch, M. (2019). Promising bioanalytical approaches to wine analysis. In *Quality Control in the Beverage Industry* (pp. 419-457). Academic Press.
204. Sergeeva, Y. E., Pozhidaev, V. M., & Fedosov, D. Y. (2024). Study of the Capabilities of the Fluorescence Spectroscopy Method for the Identification of Wine Varietal. *Nanobiotechnology Reports*, 19(2), 311-316.
205. Buglass, A. J., & Caven-Quantrill, D. J. (2013). Instrumental assessment of the sensory quality of wine. In *Instrumental Assessment of Food Sensory Quality* (pp. 466-546). Woodhead Publishing.
206. Ranaweera, R. K., Capone, D. L., Bastian, S. E., Cozzolino, D., & Jeffery, D. W. (2021). A review of wine authentication using spectroscopic approaches in combination with chemometrics. *Molecules*, 26(14), 4334.
207. Comuzzo, P., Natolino, A., & Celotti, E. (2022). Sustainable approach to quality control of grape and wine. *Improving sustainable viticulture and winemaking practices*, 327-349.
208. Clodoveo, M. L., Dipalmo, T., Rizzello, C. G., Corbo, F., & Crupi, P. (2016). Emerging technology to develop novel red winemaking practices: An overview. *Innovative Food Science & Emerging Technologies*, 38, 41-56. (Clodoveo et al., 2016; Lambrecht et al., 2024)
209. Lambrecht, K., Diaz, V. F., Saeys, W., Louw, T., du Toit, W., & Aleixandre-Tudo, J. L. (2024). Forecasting wine phenolic composition from infrared spectra of grapes extracts and monitoring of fermentations with optimised time-specific prediction models. *Chemometrics and Intelligent Laboratory Systems*, 248, 105114.
210. Gonzalez Viejo, C., & Fuentes, S. (2022). Digital assessment and classification of wine faults using a low-cost electronic nose, near-infrared spectroscopy and machine learning modelling. *Sensors*, 22(6), 2303.

211. Rivera-Dominguez, M., Yahia, E. M., Wlodarchak, N., & Kushad, M. (2009, April). Identification and quantification of phenolic compounds in grapes. In VI International Postharvest Symposium 877 (pp. 1233-1240).
212. Garcia-Hernandez, C., Perez-Gonzalez, C., Martin-Pedrosa, F., Dias, L. G., Barajas-Tola, E., Rodriguez-Mendez, M. L., & Garcia-Cabazon, C. (2025). Looking for the optimal harvest time of red grapes with an enzymatic electrochemical multisensory system. *Journal of Agriculture and Food Research*, 19, 101554.
213. Basha, S. M., Musingo, M., & Colova, V. S. (2004). Compositional differences in the phenolics compounds of muscadine and bunch grape wines. *African journal of biotechnology*, 3(10), 523-528.
214. Jakobović, S., Jeromel, A., Maslov, L., & Jakobović, M. (2015). Influence of grape ripeness of Rhine Riesling on the composition of polyphenolic compounds in must and wine.
215. La Iacona, T., Sanna, M., Spanna, F., & Schubert, A. (2010, August). Berry polyphenols and climatic variability in 'Nebbiolo' grapes. In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on the 931 (pp. 287-298).
216. Rouxinol, M. I., Martins, M. R., Salgueiro, V., Costa, M. J., Barroso, J. M., & Rato, A. E. (2023). Climate effect on morphological traits and polyphenolic composition of red wine grapes of *Vitis vinifera*. *Beverages*, 9(1), 8.
217. Rouxinol, M. I., Martins, M. R., Barroso, J. M., & Rato, A. E. (2023). Wine grapes ripening: A review on climate effect and analytical approach to increase wine quality. *Applied Biosciences*, 2(3), 347-372.
218. Jasse, A., Berry, A., Aleixandre-Tudo, J. L., & Poblete-Echeverría, C. (2021). Intra-block spatial and temporal variability of plant water status and its effect on grape and wine parameters. *Agricultural Water Management*, 246, 106696.
219. Chidi, B. S., Bauer, F. F., & Rossouw, D. (2018). Organic acid metabolism and the impact of fermentation practices on wine acidity: A review. *South African Journal of Enology and Viticulture*, 39(2), 1-15.
220. Villangó, S., Pásti, G., Kállay, M., Leskó, A., Balga, I., Donkó, A., ... & Zsófi, Z. (2015). Enhancing phenolic maturity of Syrah with the application of a new foliar spray. *South African Journal of Enology and Viticulture*, 36(3), 304-315.

221. González-Rodríguez, J., Pérez-Juan, P., & de Castro, M. L. (2002). Method for the simultaneous determination of total polyphenol and anthocyan indexes in red wines using a flow injection approach. *Talanta*, 56(1), 53-59.
- 222.
223. Manley, M., van Zyl, A., and Wolf, E.E.H. (2001) The evaluation of the applicability of Fourier transform near-infrared (FT-NIR) spectroscopy in the measurement of analytical parameters in must and wine. *South African Journal for Enology & Viticulture*, 22: 93–100.
224. Barnaba, F.E., Bellicontro, A., and Mencarelli, F. (2014) Portable NIR-AOTF spectroscopy combined with winery FTIR spectroscopy to an easy, rapid in field monitoring of Sangiovese grape quality. *J. Sci. Food Agr.*, 94:1071–1077.
227. Cavaglia, J., Giussani, B., Mestres, M., Puxeu, M., Busto, O., Ferré, J., & Boqué, R. (2019). Early detection of undesirable deviations in must fermentation using a portable FTIR-ATR instrument and multivariate analysis. *Journal of Chemometrics*, 33(8), e3162.
228. Rienth, M., Vigneron, N., Darriet, P., Sweetman, C., Burbidge, C., Bonghi, C., ... & Castellarin, S. D. (2021). Grape berry secondary metabolites and their modulation by abiotic factors in a climate change scenario—a review. *Frontiers in Plant Science*, 12, 643258.
229. De Resseguier, L., Le Roux, R., Petitjean, T., Mary, S., Quénol, H., & Van Leeuwen, C. (2018). Variability of climate, water and nitrogen status and its influence on vine phenology and grape composition inside a small winegrowing estate. In *E3S Web of Conferences* (Vol. 50, No. 01016).
230. Baroň, M. (2011). Yeast assimilable nitrogen in South Moravian grape musts and its effect on acetic acid production during fermentation. *Czech Journal of Food Sciences*, 29(6), 603-609.
231. Casalta, E., Sablayrolles, J. M., & Salmon, J. M. (2013). Comparison of different methods for the determination of assimilable nitrogen in grape musts. *LWT-Food Science and Technology*, 54(1), 271-277.
232. Walker, H. V., Jones, J. E., Swarts, N. D., Rodemann, T., Kerslake, F., & Damberg, R. G. (2021). Predicting grapevine canopy nitrogen status using proximal sensors and

- near-infrared reflectance spectroscopy. *Journal of Plant Nutrition and Soil Science*, 184(2), 204-304.
233. Porep, J. U., Erdmann, M. E., Körzendörfer, A., Kammerer, D. R., & Carle, R. (2014). Rapid determination of ergosterol in grape mashes for grape rot indication and further quality assessment by means of an industrial near infrared/visible (NIR/VIS) spectrometer—A feasibility study. *Food Control*, 43, 142-149.
234. Hill, G. N., Evans, K. J., Beresford, R. M., & Dambergs, R. G. (2013). Near and mid-infrared spectroscopy for the quantification of botrytis bunch rot in white wine grapes. *Journal of Near Infrared Spectroscopy*, 21(6), 467-475.
235. Beghi, R., Giovenzana, V., Brancadoro, L., & Guidetti, R. (2017). Rapid evaluation of grape phytosanitary status directly at the check point station entering the winery by using visible/near infrared spectroscopy. *Journal of Food Engineering*, 204, 46-54.
236. Giovenzana, V., Beghi, R., Tugnolo, A., Brancadoro, L., & Guidetti, R. (2018). Comparison of two immersion probes coupled with visible/near infrared spectroscopy to assess the must infection at the grape receiving area. *Computers and electronics in agriculture*, 146, 86-92.
237. Cozzolino, D., Kwiatkowski, M. J., Waters, E. J., & Gishen, M. (2007). A feasibility study on the use of visible and short wavelengths in the near-infrared region for the non-destructive measurement of wine composition. *Analytical and bioanalytical chemistry*, 387(6), 2289-2295.
238. Urbano-Cuadrado, M., De Castro, M. L., Pérez-Juan, P. M., García-Olmo, J., & Gómez-Nieto, M. A. (2004). Near infrared reflectance spectroscopy and multivariate analysis in enology: Determination or screening of fifteen parameters in different types of wines. *Analytica Chimica Acta*, 527(1), 81-88.
239. Regmi, U., Palma, M., & Barroso, C. G. (2012). Direct determination of organic acids in wine and wine-derived products by Fourier transform infrared (FT-IR) spectroscopy and chemometric techniques. *Analytica Chimica Acta*, 732, 137-144.
240. Cornehl, L., Krause, J., Zheng, X., Gauweiler, P., Schwander, F., Töpfer, R., ... & Kicherer, A. (2023). Determination of sugars and acids in grape must using miniaturized near-infrared spectroscopy. *Sensors*, 23(11), 5287.
241. Manley, M., Van Zyl, A., & Wolf, E. E. H. (2001). The evaluation of the applicability of Fourier transform near-infrared (FT-NIR) spectroscopy in the measurement of analytical parameters in must and wine.

- 1602 242. Grassi, S., Vigentini, I., Sinelli, N., Foschino, R., & Casiraghi, E. (2012). Near infrared
1603 and mid infrared spectroscopy in oenology: determination of main components
1604 involved in malolactic transformation. *NIR news*, 23(3), 11-14.
- 1605 243. Cozzolino, D., McCarthy, J., & Bartowsky, E. (2012). Comparison of near infrared and
1606 mid infrared spectroscopy to discriminate between wines produced by different
1607 *Oenococcus Oeni* strains after malolactic fermentation: A feasibility study. *Food*
1608 *Control*, 26(1), 81-87.

1621 2. Chapter II- Feasibility assessment of a low-cost visible 1622 spectroscopy-based prototype for monitoring polyphenol 1623 extraction in fermenting musts.

1624
1625 The present chapter is based on the article entitled "*Feasibility assessment of a low-cost visible*
1626 *spectroscopy-based prototype for monitoring polyphenol extraction in fermenting musts*",
1627 published in *Journal of the Science of Food and Agriculture* in 2024. This work describes the
1628 development and validation of an initial prototypal spectrophotometer utilizing VIS technology
1629 for the non-destructive measurement of polyphenols during the winemaking process, with a
1630 particular focus on the predictive efficiency of phenolic compounds and comparison with a
1631 commercial VIS device.

1632 Compared to the published version, the text has been slightly modified to ensure consistency with
1633 the overall style of the thesis and to include additional methodological details. Furthermore,
1634 connections with other chapters have been added to highlight the continuity and coherence of the
1635 experimental approach adopted.

1636 This study represents the first of the four experimental works that constitute the main body of the
1637 thesis. Destructive results here presented find further application in Chapter III, where the
1638 experimental trial is continued in a second version of the study by employing other non-destructive
1639 technologies.

Abstract

Background: Polyphenols have long been employed to evaluate grape and wine quality, emphasizing the critical need for their measurement throughout various winemaking stages. Currently, polyphenols are predominantly assessed through analytical methods, which are characterized by time-consuming procedures, and environmentally unfriendly practices. Alternatively, non-destructive spectroscopy-based devices offer an option, yet they tend to be costly and not readily accessible for smaller wineries. This study introduces initial steps in employing a portable, user-friendly, and cost-effective visible (VIS) spectrophotometer prototype for direct polyphenol measurement during winemaking.

Results: Grapes (cv Syrah, Bobal, and Cabernet Sauvignon) at different maturation stages were fermented with or without stems. Throughout fermentation, parameters such as color intensity, total polyphenol index, total anthocyanins, and tannins were monitored. Concurrently, VIS spectra were acquired using both the prototype and a commercial instrument. Chemometric approaches were then applied to establish correlation models between spectra and destructive analyses. The prototype models demonstrated an acceptable level of confidence for only a few parameters, indicating its current lack of complete reliability at this stage.

Conclusions: While VIS spectroscopy is already utilized for polyphenol analysis in winemaking, the aspiration to automate the process in wineries, particularly with low-cost devices, remains unrealized. This study proposes the feasibility of a low-cost and user-friendly spectrophotometer. The results indicate that, in the early stages of prototype utilization, the goal is attainable but requires further development and in-depth assessments.

Keywords

Polyphenols; Visible (VIS) spectrophotometer; tannins; anthocyanins; non-destructive analysis.

Introduction

Polyphenols play one of the most important roles in the quality of red wine. On one hand, the phenolic composition of wines is responsible for their organoleptic properties (i.e. body, structure, and astringency). Polyphenols can also be used as a fingerprint for their differentiation, according to the geographical origin, variety, and vintage (Jorge Garrido et al., 2013). On the other hand, they are known for antioxidant properties which play an important role in wine longevity and human health (Buljeta et al., 2023; Giovinazzo et al., 2015). Hence, polyphenols exhibit beneficial effects on human health, such as anti-inflammatory, anticarcinogenic, and cardio-protective effects

(Buljeta et al.,2023; Giovinazzo et al., 2015; Jorge Garrido et al., 2013;Rodrigo et al.,2001). Moreover, polyphenols have been used for traceability and identification of grapes in certain quality wines, such as in Brunello di Montalcino (Rustioni et al., 2013).

In this context, monitoring polyphenols at different wine production stages becomes crucial for producing high-quality wines, given the variations in polyphenol concentration between different wines and the great impact of vinification processes (Visioli et al., 2020; Somers et al., 1977).

Monitoring the extraction of these compounds during the contact period between must, skins, and seeds for red wine production is strongly important (Baiano et al., 2014). To date, destructive and analytical approaches have been used for polyphenols analysis and identification. Traditional methods for quantifying polyphenols in wine include the Folin-Ciocalteu assay for total phenolic content, High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and various spectrophotometric and colorimetric assays targeting specific polyphenolic groups. However, these methods have limitations such as lack of specificity, being labor-intensive, destructive, generating chemical waste (such as heavy metals present in the Folin-Ciocalteu reagent, hydrochloric acid, aluminum chloride and so on) (Somers et al., 1977), requiring expensive equipment, and having limited throughput (Pekal et al., 2014) Additionally, analytical approaches require time, a laboratory, expensive instruments, and trained staff. In the last years, spectroscopy has been recognized as one of the most interesting tools for non- destructive analysis of quality parameters and polyphenols in wine (De Santis et al., 2021; Jia- Huan et al., 2015; Bellincontro et al., 2009; Cozzolino et al., 2012). Spectroscopy has firmly established itself as a vital analytical tool in the evaluation of wine. Within the winemaking industry, these techniques have been eagerly adopted for the routine examination of chemical constituents, encompassing crucial parameters such as pH, sugar content, and ethanol concentration (Chapman et al., 2019). The relentless progress in instrumentation has offered the wine industry a diverse array of accessible and adaptable measurement tools. Recent and pioneering applications of these spectroscopic techniques extend to the quantification of wine volatiles (Genisheva et al.,2018) phenolic compounds, and antioxidants (Aleixandre-Tudo et al., 2018; Martelo-Vidal et al., 2014a; Martelo-Vidal et al., 2014b; Grijalva-Verdugo et al., 2017), as well as the assessment of sensory attributes (Dufour et al., 2009). VIS-NIR technology has been used in oenology to measure color by means of color intensity(Pettinelli et al., 2022; Cozzolino et al.,2007), alcohol content in bottled wine (Martelo-Vidal et al., 2015), tartaric acid, acetic acid, malic acid and lactic acid (Liu et al., 2007), for sugar monitoring (Martelo-Vidal et al., 2014a), to measure polyphenol families and individual phenolics (Martelo-Vidal et al., 2014c) or to determine

the geographical origin of different wines (Acevedo et al., 2007; Yu et al., 2018; Bellincontro et al., 2012). VIS spectroscopy, as a non-destructive technology, must be supported by multivariate data analysis and chemometrics (Bellincontro et al., 2009; Bellincontro et al., 2011; Ribereau-Gayon et al., 1970).

Spectrophotometric based methods have gained popularity due to their perceived benefits, such as cost-effectiveness and straightforward experimental procedures but they also come with limitations. One major drawback is their lack of specificity and reproducibility (Lorrain et al., 2013). This is because the reagents used in the assays are not exclusively selective for the intended target substances, and other substances may absorb at the same wavelengths of interest, leading to potential interference. Moreover, it requires trained staff, has an initial high cost (directly linked to the instruments), as well as the impossibility of moving the instrument. As such, performing analysis directly in the cellar during fermentation becomes difficult. In this context, the study here proposed explores the potential of a low-cost and portable VIS spectrometer prototype, developed by Nature 4.0 (Viterbo, Italy), for monitoring polyphenol extraction during winemaking. This prototype's affordability, user-friendly design, rapid data acquisition, and minimal operating costs make it an attractive tool for wineries. The objective is to assess its performance compared to a bench-top VIS spectrophotometer, develop prediction calibrations, and explore its practical suitability in winemaking.

Materials and methods

Sampling and experimental design

The grapes were harvested from the 'Coloraos' experimental vineyard in the municipality of Requena, during the 2022 vintage. The grapes of three different varieties (*Vitis vinifera* L. cv Syrah, Bobal, and Cabernet Sauvignon) were harvested between the 15 September and the 29 September at three distinct sampling points, except for Cabernet Sauvignon that was sampled twice (Figure 1). Grapes were collected at different degrees of ripeness and separately fermented. For each fermentation, part of the grapes was crushed and destemmed ("a" series), and part of the grapes were crushed and fermented including stems ("b" series). To summarize, 6 different fermentations were carried out for Syrah and Bobal, and 4 different fermentations for Cabernet Sauvignon (Figure 1), presenting different polyphenol concentrations.

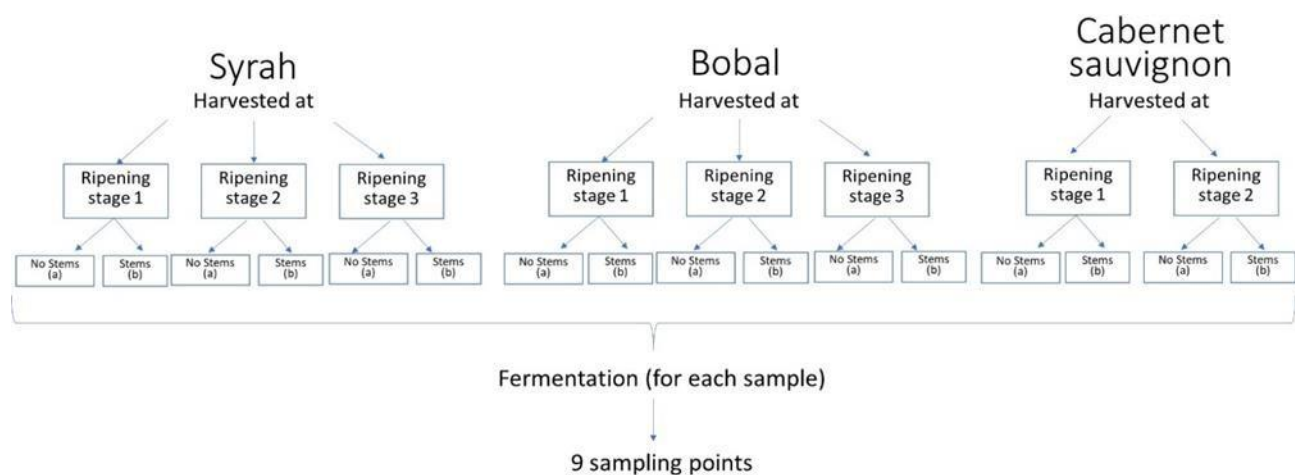


Figure 1. Experimental design.

Winemaking

Grape bunches (~5 kg per sample) were crushed and de-stemmed (no de-stemming was carried out for the “b” series) in 10 L glass jar and inoculated with 30 mg/L of commercial yeasts (Zymaflore RX60 red wine yeast, Renteria, Spain), also adding 0.03 g/kg of sulfur dioxide (added as an 8% solution of potassium metabisulphite). To ensure variability in terms of phenolic extraction, destemmed grapes were fermented on skins at controlled temperature and relative humidity (18 °C and 80% HR), while the fermentation with stems was performed at room temperature. Must/wine samples were taken daily for a total of 9 days, centrifuged, and filtered at 0.45 µm.

Analytical analyses

The collected fermenting musts were used to determine different phenolic parameters. Specifically, the Total Polyphenol Index (TPI) was obtained after diluting the wine 50 times with distilled water and by reading the absorbance at 280 nm with 10 mm quartz cuvettes (Ribereau-Gayon et al., 2006). Distilled water was used as a reference blank. Total and bleachable anthocyanins were determined using the sodium hydrogen sulfite bleaching method, even though only total anthocyanins were included in the model building-up (Glories et al., 2016) . To determine color intensity (CI), wine samples were spectrophotometric assayed at three wavelengths (420nm, 520nm and 620nm) and the results were added to compute the index values (Sarneckis et al., 2006). The determination of tannins was performed by using the methyl cellulose precipitation (MCP) method (Mercurio et al., 2007; taglieri et al., 2023). Three replicates were performed per sample of each analysis except for the measurement of colour intensity. For the measurement of the

different analytical parameters a commercial ultraviolet visible light spectrophotometer (Jasco 730 UV-VIS), working in the 200-705 nm range, with 2 nm wavelength increments and 1 mm quartz cuvette, was used.

Spectral acquisitions with the prototype device

The low-cost prototype (Figure 2) was developed in the laboratories of Tuscia University in cooperation with Nature 4.0 (Viterbo, Italy). The device is a similar model to the one described by Taglieri et al. (2023), but presenting a spectral acquisition based on 8 wavelengths between 460 nm and 680 nm with deviation in each wavelength of approximately 25-30 nm (Pascual et al., 2016).

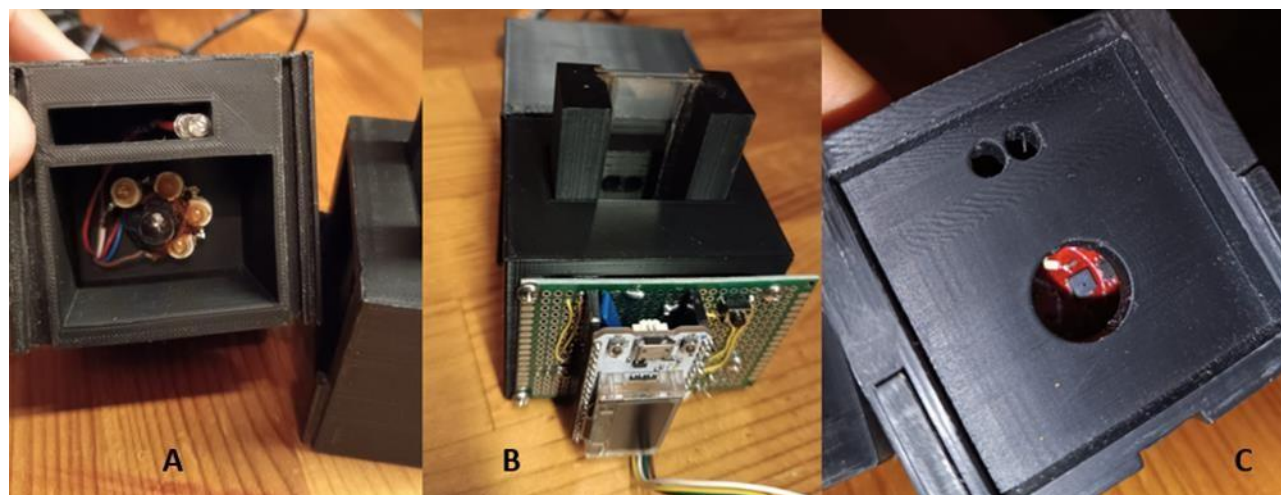


Figure 2. Low-cost VIS spectrophotometer components: (A) Low-cost lamp of the VIS spectrophotometer; (B) 1 mm cuvette housing and the esp32 microcontroller; (C) 3 sensors with optical filters.

The low-cost prototype was developed with three multi-channel sensors each with a specific wavelength segment. The 3 sensors have optical filters for a read-out with a resolution of approximately 20 nm. The light source is a low-cost halogen lamp implemented by two low-cost LEDs, while the structure of the instrument is molded in a rigid plastic case. The AS7265x chipset consists of three sensor devices i.e. AS72651 (with master capability), AS72652 and AS72653. The combination of these three sensors forms the multispectral sensor chip-set with different channels. The multispectral sensors can be used for spectral identification in a range from VIS to NIR. Every of the three sensor devices has independent on-device optical filters whose spectral response is defined in a range from 410 nm to 680 nm. Using the AS7265x chipset requires the use of firmware. The components AS72651, AS72652, and AS72653 are pre-calibrated with a specific light source. Each AS7265x device has two integrated LED drivers with programmable current and can be timed for electronic shutter applications. All three of these 6-channel devices utilize a conversion process implemented through two sets of photodiodes in each device. In the

first set (Bank 1), data is gathered from 4 out of the 6 photodiodes, with 2 registers zeroed. The second set (Bank 2) gathers data from a different set of 4 photodiodes, with 2 different registers zeroed. Spectral conversion necessitates setting the integration time (IT in milliseconds) to complete the process. If both photodiode banks are needed for the conversion, the second bank requires an additional integration time in milliseconds. The minimum integration time for a single bank conversion is 2.8 milliseconds. If data from all 6 photodiodes is necessary, the device must undergo 2 full conversions ($2 \times$ Integration Time). The device integrates Gaussian filters into standard CMOS silicon via nano-optic deposited interference filter technology in LGA packages that also provide built-in apertures to control the light entering the sensor array. All optical characteristics are designed for optimal performance under diffused light conditions. However, if a point light source or collimated light is used on the sensor, it is necessary to cover the sensor opening with a Lambertian diffuser that exhibits achromatic characteristics. The instrument is designed with 'Arduino' software interface and connected to a laptop. Spectra of must/wine samples were detected using a 1mm quartz cuvette. For each sample, the prototype performs 10 readings and provides an average spectrum.

Data analysis

The absorbance values obtained through both the commercial and prototype spectrophotometers were used for computing linear regression models using Principal Components Regression (PCR). VIS detections were set as dependent variables (response variables), while destructive analyses (i.e. color intensity, total polyphenol index, anthocyanins, and tannins) as independent ones (predictor variables). Both datasets were first auto-scaled and venetian blinds with blind thickness = 1 was used as cross-validation method. The multivariate analyses were performed by Matlab R2013a (MathWorks®, Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA).

Results

Monitoring of polyphenol extraction by analytical method

The analytical parameters (i.e. color intensity, total polyphenol index, anthocyanins and tannins) measured in the different wines are shown in table S1 (Syrah), S2 (Bobal) and S3 (Cabernet Sauvignon). Uniquely, the b-series samples (those vinified with stalks), of any grape variety showed a significant increase in tannin concentration. This was certainly due to the extraction of these compounds from the stalks following the increase of ethanol in the medium.

Tannins for Bobal in the absence of stalks rose from 1000 mg/L at the third fermentation day to 2458.51 mg/L at the last fermentation day, peaking at 2535.10 mg/L at the eighth fermentation day. For Bobal, both the color intensity and anthocyanin content are slightly higher in the series without stems. This can be attributed to the 'sponge' effect of the stems, which tend to color the stems and therefore subtract color from the must (Sarneckis et al., 2006). The concentration of anthocyanins rose in the a-series from 484.66 mg/L for sample 1, 400.71 mg/L for sample 2 and 554.97 mg/L for sample 3 on the first day of fermentation, to 354.99 mg/L, 490.53 mg/L and 458.19 mg/L respectively. The b-series, on the other hand, rose from 861.57 mg/L in sample 1, 751.84 mg/L in sample 2 and 897.44 mg/L for sample 3 on the first day of fermentation to 588.35 mg/L, 649.64 mg/L and 835.76 mg/L, respectively. The TPI gradually increased with higher increases in the last days of fermentation, obviously the b-series samples reached higher average TPI levels than the a-series, vinified without stalks. At the last fermentation day, however, a decrease in the total polyphenol value was observed in both sets of samples. The TPI followed an upward trend, rising in the a-series from 14.46 in sample 1, 5.93 in sample 2 and 23.79 in sample 3 on the first day of fermentation to 62.97, 47.12 and 80.3 respectively. The b-series, on the other hand, rose from 19.15 in sample 1, 27.9 in sample 2 and 30.77 in sample 3 on the first day of fermentation to 66.01, 71.13 and 96.66 in samples 1, 2 and 3 respectively.

As far as Syrah is concerned, the tannin concentration in destemmed vinification doubled from the first to the last day of fermentation, while it increased by about two and a half to three times for musts fermented with stems. The starting tannin contents for the a-series were 439.3 mg/L for sample 1, 390.4 mg/L for sample 2 and 309.2 mg/L for sample 3 on the first day of fermentation. At the end of the fermentation stood at 1010.2 mg/L, 1049.6 mg/L and 1289.8 mg/L respectively. The b-series, on the other hand, rose from 1098.8 mg/L in sample 1, 1177.6 mg/L in sample 2 and 766.4 mg/L for sample 3 on the first day of fermentation to 2419 mg/L, 3233.1 mg/L and 2530.4 mg/L, respectively. The color intensity between the first and last day of sampling remains very similar, although some peaks were observed at 2.74 for sampling 6 of series 2b and 2.70 for 3b, showing how incorrect oenological activities can take place and color intensity can be lost if this parameter is not monitored. Anthocyanins doubled its content in both series, with a slight overall decrease on last fermentation day (day 9). The TPI increased during fermentation and appears to be more stable than in Bobal.

For Cabernet Sauvignon, a large increase in tannin concentration was observed in both series. However, in the de-stemmed series the increase was higher, while for the b-series a smaller increase was observed, but the starting concentration was higher, again due to the presence of the

stalks. The starting tannins for the a-series were 230.1 mg/L for sample 1 and 558.2 mg/L for sample 2 on the first day of fermentation, and then stood at 1228.7 mg/L and 1295.8 mg/L respectively. The b series, on the other hand, rose from 573 mg/L in sample 1 and 1191.5 mg/L in sample 2 on the first day of fermentation to 1991.4 mg/L and 2370.2 mg/L respectively. Cabernet wines color intensity and anthocyanins were the least stable, reaching slightly higher values at the end of fermentation than those found on the first fermentation day. The TPI followed an upward trend throughout the test, rising in the a-series from 7.01 for sample 1 and 29.61 for sample 2 on the first day of fermentation, and then stood at 14.05 and 32.92 respectively. The b-series, on the other hand, rose from 61.4 in sample 1 and 63.26 in sample 2 on the first day of fermentation to 73.48 and 105.18, respectively in sample 1 and 2. The Cabernet Sauvignon grapes were the ones with the lowest visual ripeness than the other varieties.

Monitoring polyphenol extraction with a commercial VIS spectrophotometer

To compare the performance of the two spectrophotometers in predicting the different analytical parameters, PCR models were built using both instruments' VIS information. As far as the commercial instrument is concerned, 5 principal components were selected to reach a cumulative explained variability of 96 %. The prediction correlation coefficients (R^2) values were quite positive. Specifically, for TPI an R^2 of 0.709 and 0.683 was obtained in calibration and cross validation respectively (Figure 3.A). A good predicting aptitude was also observed for tannins (0.750 in calibration and 0.727 in cross validation) (Figure 3.B), and anthocyanins (0.732 in calibration and 0.715 in cross validation) (3.C). On the other hand, the worst performance was observed for the color density model (R^2 of 0.431 and 0.387 for calibration and cross validation respectively) (Figure 3.D). Model details are shown in table 1.

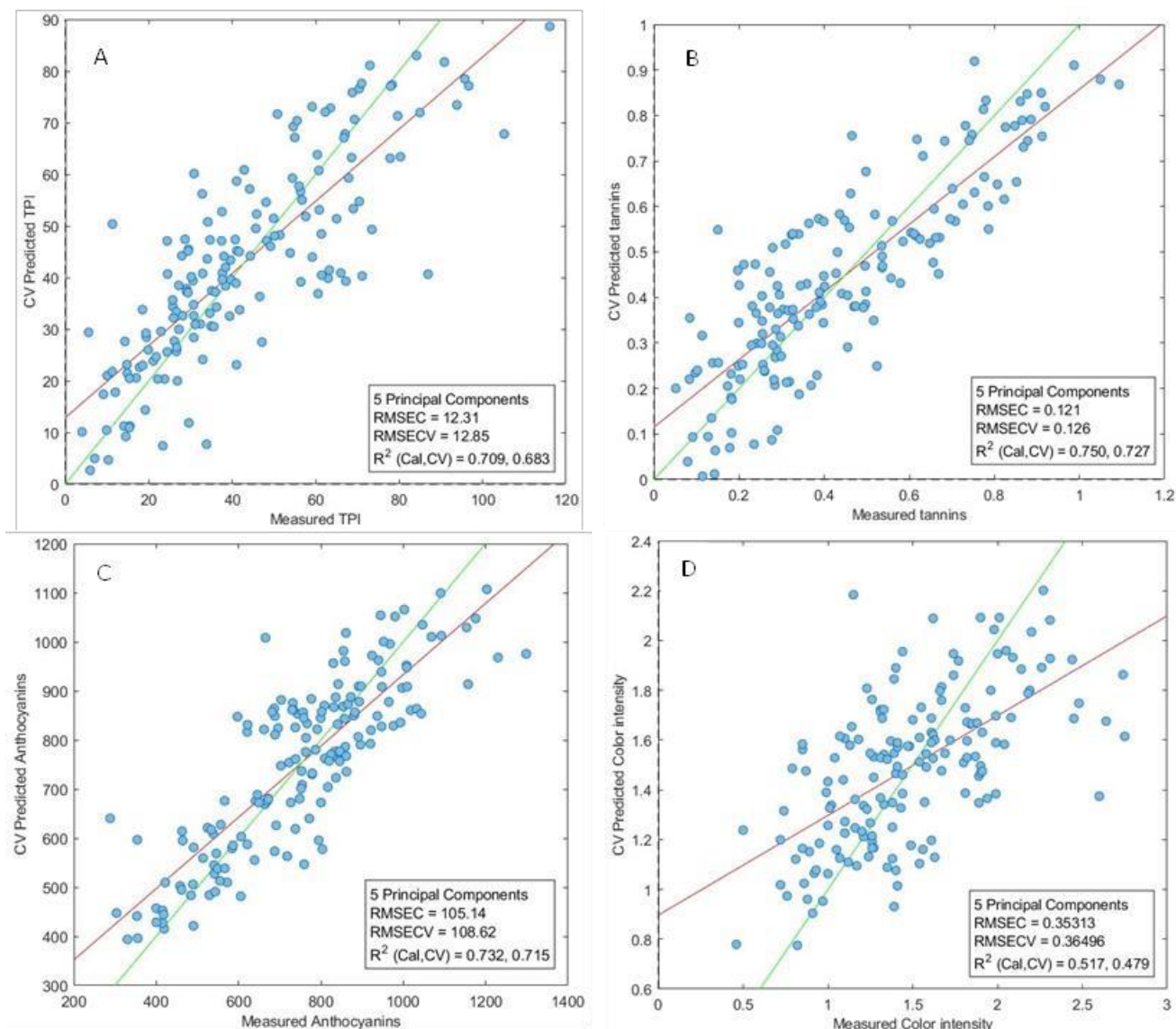


Figure 3. Observed versus predicted plots of the PCR models built with the commercial VIS spectrometer for total polyphenols index (A), tannins (B), anthocyanins (C) and color intensity (D). RMSEC = Root Mean Square Error in calibration. RMSECV = Root Mean Square Error in cross validation. R^2 =correlation coefficient in calibration. R^2 =correlation coefficient in cross validation.

Table 1 Summary table for the PCR models shown in figure 3 and 4. RMSEC = Root Mean Square Error in calibration. RMSECV = Root Mean Square Error in cross validation. RPD = Relative Percent Difference (Std/RMSEC). Error (%) = (RMSECV/mean)*100.

		TPI	Tannins	Anthocyanins	CI

Mean		42.5	0.4	750	1.4
Std		22.8	0.24	203	0.4
n		160	160	160	160
Min		4	0.05	288	0.4
max		116	1	1298	2.7
RMSEC	Commercial	12.31	0.12	105.1	0.35
	Prototype	13.88	0.15	114	0.36
RMSECV	Commercial	12.85	0.12	106.62	0.36
	Prototype	14.07	0.16	117.45	0.37
RPD	Commercial	1.85	2	1.93	1.31
	Prototype	1.64	1.6	1.78	1.27
Error (%)	Commercial	30.24	30.00	14.22	25.71
	Prototype	61.71	66.67	57.86	92.50

Monitoring polyphenol extraction by prototype VIS spectrophotometer

As previously mentioned, to verify the differences in term of performance in prediction of the different parameters, regressive models were built with the prototype device acquired spectra by using the same criteria of the PCR modeling carried out for the commercial device. In this case, 5 principal components explained 79.65% of the variability. The R^2 values in prediction are lower than the R^2 values relative to the models obtained from the commercial spectrophotometer. Specifically, an R^2 of 0.603 and 0.592 was obtained in calibration and cross validation for TPI (Figure 4.A), 0.550 and 0.535 for tannins (Figure 4.B), and 0.517 and 0.495 for anthocyanins (Figure 4.C). As observed for the commercial spectrophotometer, the prototype shows the worst performance in predicting the color intensity, with lower correlation coefficients (R^2 0.304 and 0.275, respectively). Model details are shown in table 1.

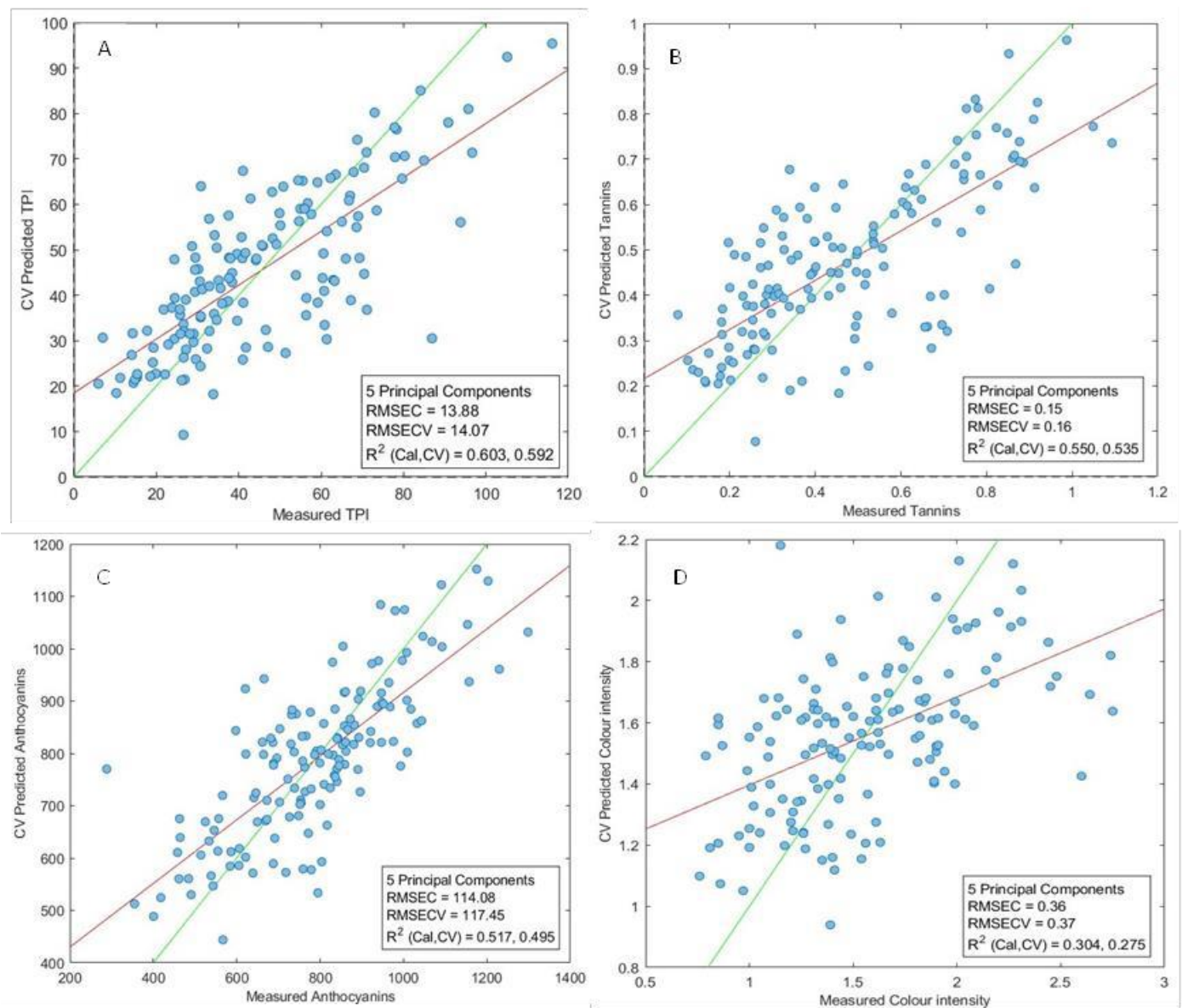


Figure 4 Observed versus predicted plots of the PCR models built with the prototype VIS spectrometer for total polyphenols index (A), tannins (B), anthocyanins (C) and color intensity (D). RMSEC = Root Mean Square Error in calibration. RMSECV = Root Mean Square Error in cross validation. R^2 =correlation coefficient in calibration. R^2 =correlation coefficient in cross validation.

Discussion

As described above, the focus of the current study centers on the advantages and potential applications of VIS spectroscopy, with particular attention to polyphenol and color analysis in wine during fermentation. Accurate predictive performance was achieved by the commercial benchtop VIS spectrophotometer model, confirming that VIS spectroscopy can be a proficient substitute to wet chemistry, substantially helping in reducing the usage of chemical reagents and greatly limiting the environmental impact of oenological analyses as well as the time required for performing them. Moreover, the fact that visible spectral data was used to predict UV absorbing compounds such as tannins and the majority of the phenolics of the TPI adds value to this VIS

spectroscopy application. VIS spectrophotometric methods offer a unique set of benefits and numerous techniques have been suggested for the measurement of phenolic compounds (Harbertson et al., 2003; Singleton et al., 1965; Somers et al., 1974). Nevertheless, despite the widespread adoption of spectrophotometric approaches, primarily because of their recognized advantages such as cost-effectiveness and simplified experimental procedures, they do exhibit notable limitations. For instance, an important drawback is their lack of specificity and reproducibility (Riberau-Gayon et al., 2006). This is because the reagents used in the assays are not exclusively selective for the intended target substances, and other substances may absorb at the same wavelengths of interest, leading to potential interference. The use of spectroscopy calibrations overcomes these limitations.

Even though the predicting aptitude of the spectral prototype device here tested was lower, a significant consideration that deserves to be highlighted is the strong difference in terms of cost between the two devices. The prototype VIS spectrometer made by Nature 4.0 has a significantly lower cost: the production cost which includes lamp, sensors, electrical and structural components is under 50 euros. Predictive models computed on data from the VIS prototype coupled to destructive analyses generally perform positively, even though always presenting lower correlations than those achieved employing commercial instrumentation. The best values were measured for the prediction of the total polyphenol index with comparable cross validation errors of 12.85 and 14.07 index values for the commercial and prototype instrument, respectively. With respect to the models built for the prediction of total anthocyanins the observed difference on the computed cross validation error was 108.62 and 117.45 mg/L of anthocyanins for the commercial and prototype spectrometer, respectively. These differences in prediction errors associated to the performed predicting models show the potential of the prototype compared to the commercial device.

The results showed that the predictive efficacy of PCR models generated using spectra derived from the VIS prototype device slightly underperformed those produced with the commercial instrument. The lower performance of the prototype is distinctly apparent from the percentage error in prediction (Table 1), where the observed error rates consistently exhibit double the values observed with the commercial spectrophotometer. This unequivocally underscores the prototype's limitations and emphasizes the imperative of further refinement and optimization in future iterations. The differences between the predictive accuracy are likely attributed to the disparity in the number of wavelength data points employed in instrumental detection: 8 for the prototype and 104 for the commercial apparatus. Moreover, the lack of an absorption wavelength at 280 nm

1958 makes the prediction of tannin content and the total polyphenol index particularly complex, as 280
1959 nm corresponds to the peak absorption wavelength associated to this specific phenolic category.
1960 Furthermore, it is imperative to incorporate wavelengths around 320 nm into the
1961 spectrophotometer to enhance its analytical performance. This wavelength range encompasses
1962 hydroxycinnamic acids (comprising free molecules such as caffeic, p-coumaric, ferulic, and
1963 sinapic acids) and their tartaric esters, typified by caftaric acid. Additionally, it includes benzoic
1964 acids characterized by their predominant form, gallic acid (Costa-Pérez et al., 2023). Furthermore,
1965 to ascertain the presence of specific stilbenes, the inclusion of a detection wavelength at 307 nm
1966 would be advantageous for monitoring the presence of resveratrol and its conjugated forms.
1967 Analogous to tannins, numerous polyphenolic families exhibit absorbance peaks at 280 nm and
1968 365 nm (e.g., quercetin), thereby contributing to a diminished predictive performance, even within
1969 the overall polyphenol index. Tannins constitute a prominent class of phenolic compounds,
1970 exerting substantial influence on the overall polyphenolic composition of red wine (Gutierrez-
1971 Escobar et al., 2021; Puyo et al., 2023; Claus et al., 2020; Giacosa et al., 2019). Wine's color
1972 components are critical parameters contributing significantly to its sensory attributes, including
1973 color perception and astringency, as well as playing a crucial role in determining its antioxidant
1974 properties (Waterhouse et al., 2002; López-Vélez et al., 2003). The objective measurement of
1975 wine color components is an integral part of modern winemaking and the development of new
1976 methods for quantifying pigments has become crucial.

1977 As known, several studies have demonstrated the efficacy of visible spectroscopy for the prediction
1978 of phenolic content in wine (Aleixandre-Tudo et al., 2015; Boulet et al., 2016; Beaver et al., 2016;
1979 Aleixandre-Tudo et al., 2018): Innovative methods of detection have also been developed. For
1980 instance, Gu et al., (2023) developed an UV-VIS prototype coupled with machine learning
1981 computation to identify red wines coming from different geographical areas of production (Gu et
1982 al., 2023). Moreover, UV-Vis combined with chemometric tools was recently used to predict
1983 phenolic content of Pinot noir wines (Giglio et al., 2023). However, low cost, easy to use and
1984 portable spectrophotometers are quite rare. Consequently, once the clear gaps and technical
1985 limitations of the prototype here proposed have been addressed, especially regarding the addition
1986 of important wavelengths, the prospect of employing such an analytical tool within winemaking
1987 operations holds significant appeal.

1988 Conclusions

1989 PCR models obtained throughout the spectral detections performed using the commercial VIS
1990 spectrophotometer were found to be suitable for predicting total polyphenol index, anthocyanins

and tannins. This confirms that non-destructive VIS techniques can be used as valid alternative to wet chemistry for monitoring polyphenol extraction during vinification processes. On the other hand, regression models built with a VIS prototype showed only slightly less accurate models. This is likely explainable because of the different reading points in the two instruments (only 8 wavelengths in the prototype). As such, to increase the accuracy of the prototype other wavelengths need to be included and, among them, certainly those referred to 280, 320 and 365nm, which are the wavelengths of greatest interest for correlating polyphenol families. These additions could certainly bring the prototype accuracy at least to similar level as commercial instruments. Moreover, there is a need to find an effective and reproducible sampling protocol for fermenting musts that can facilitate the measurements directly in the cellar.

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References

1. Buljeta, I.; Pichler, A.; Šimunović, J.; Kopjar, M. Beneficial Effects of Red Wine Polyphenols on Human Health: Comprehensive Review. *Curr. Issues Mol. Biol.* 2023, 45, 782-798.
2. Giovinozzo, G., Grieco, F. Functional Properties of Grape and Wine Polyphenols. *Plant Foods Hum Nutr* 70, 454–462 (2015).
3. Jorge Garrido, Fernanda Borges, Wine and grape polyphenols — A chemical perspective, *Food Research International*, Volume 54, Issue 2, 2013, Pages 1844-1858, ISSN 0963-9969.
4. Rodrigo R, Miranda A, Vergara L (2001) Modulation of endogenous antioxidant system by wine polyphenols in human disease. *Clin Chim Acta* 412:410–412
5. Rustioni, L., Rossoni, M., Failla, O., & Scienza, A. (2013). Anthocyanin esterification in Sangiovese grapes. *Ital. J. Food Sci*, 25, 133-141.
6. Visioli, F.; Panaite, S.-A.; Tomé-Carneiro, J. Wine's phenolic compounds and health: A pythagorean view. *Molecules* 2020, 25, 4105.

7. Baiano, A. Recovery of biomolecules from food wastes—A review. *Molecules* 2014, 19.
8. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nat Protoc.* 2007;2(4):875–7.
9. Pękal Anna, Pyrzynska Krystyna, 2014, Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay *Food Analytical Methods*, 1776-1782-7-9
10. Somers, T.C. and Evans, E.E. (1977) Spectral evaluation of young red wines:anthocyanin equilibria, total phenolics, free molecular SO₂, and 'chemical age'. *Journal of the Science of Food and Agriculture* 28, 279– 287.
11. De Santis, Diana, Andrea Bellincontro, Roberto Forniti, and Rinaldo Botondi. 2021. "Time of Postharvest Ethylene Treatments Affects Phenols, Anthocyanins, and Volatile Compounds of Cesanese Red Wine Grape" *Foods* 10, no. 2: 322. <https://doi.org/10.3390/foods10020322>
12. Jia-Huan Qu, Dan Liu, Jun-Hu Cheng, Da-Wen Sun, Ji Ma, Hongbin Pu & Xin-An Zeng (2015) Applications of Near-infrared Spectroscopy in Food Safety Evaluation and Control: A Review of Recent Research Advances, *Critical Reviews in Food Science and Nutrition*, 55:13, 1939-1954
13. Bellincontro, A.; Nicoletti, I.; Valentini, M.; Tomas, A.; De Santis, D.; Corradini, D.; Mencarelli, F. Integration of nondestructive techniques with destructive analyses to study postharvest water stress of winegrapes. *Am. J. Enol. Vitic.* 2009, 60, 57–65.
14. Cozzolino D. 2012. Recent trends on the use of infrared spectroscopy to trace and authenticate natural and agricultural food products. *Appl Spectrosc Rev.* 47(7):518–530. doi:10.1080/05704928.2012.667858
15. Chapman, J., Gangadoo, S., Truong, V. K., & Cozzolino, D. (2019). Spectroscopic approaches for rapid beer and wine analysis. *Current Opinion in Food Science*, 28, 67-73. <https://doi.org/10.1016/j.cofs.2019.09.001>
16. Z. Genisheva, C. Quintelas, D.P. Mesquita, E.C. Ferreira, J.M. Oliveira, A.L. Amaral. New PLS analysis approach to wine volatile compounds characterization by near infrared spectroscopy (NIR), *Food Chem*, 246 (2018), pp. 172-178
17. J.L. Aleixandre-Tudo, H. Nieuwoudt, J.L. Aleixandre, W. du Toit. Chemometric compositional analysis of phenolic compounds in fermenting samples and wines using different infrared spectroscopy techniques. *Talanta*, 176 (2018), pp. 526-536
18. M. Martelo-Vidal, M. Vazquez. Determination of polyphenolic compounds of red wines by UV–VIS–NIR spectroscopy and chemometrics tools. *Food Chem*, 158 (2014), pp. 28-

19. M. Martelo-Vidal, M. Vazquez. Evaluation of ultraviolet, visible and near infrared spectroscopy for the analysis of wine compounds. *Czech J Food Sci*, 32 (2014), pp. 37-47
20. C. Grijalva-Verdugo, M. Hernández-Martínez, O.G. Meza-Márquez, T. Gallardo-Velázquez, G. Osorio-Revilla. FT-MIR spectroscopy and multivariate analysis for determination of bioactive compounds and antioxidant capacity in cabernet sauvignon wines. *CyTA J Food*, 16 (2017), pp. 561
21. É. Dufour, Principles of infrared spectroscopy, D.-W. Sun (Ed.), *Infrared spectroscopy for food quality analysis and control*, Elsevier/Academic Press, Amsterdam, London (2009), pp. 3-27
22. Pettinelli, Stefano, Luca Pardini, Giorgio De Angeli, Alessandro Bianchi, Basma Najar, Raffaele Cerreta, Andrea Bellincontro, Giuseppe Floridia, and Fabio Mencarelli. 2022. "Innovative “Soft” Maceration Techniques in Red Grape Fermentation" *Beverages* 8, no. 4: 62.
23. Cozzolino, D., et al., A feasibility study on the use of visible and short wavelengths in the near-infrared region for the non-destructive measurement of wine composition, *Anal Bioanal Chem* 2007, 387(6): 2289-2295.
24. Martelo-Vidal, M.J. and M. Vázquez, Application of artificial neural networks coupled to UV–VIS–NIR spectroscopy for the rapid quantification of wine compounds in aqueous mixtures. *Cyta-J Food* 2015, 13(1): 32-39
25. Liu, F., et al., Feasibility of the use of visible and near infrared spectroscopy to assess soluble solids content and pH of rice wines, *J Food Eng* 2007, 83(3): 430-435
26. Martelo-Vidal, M.J. and M. Vázquez, Classification of red wines from controlled designation of origin by ultraviolet-visible and near-infrared spectral analysis. *Cienc Tec Vitivinic* 2014, 29(1): 35-43. 83.
27. Acevedo, F.J., et al., Classification of wines produced in specific regions by UV–visible spectroscopy combined with support vector machines, *J Agric Food Chem* 2007, 55(17): 6842-6849.
28. Yu, J.; Wang, H.; Zhan, J.; Huang, W. Review of recent UV–Vis and infrared spectroscopy researches on wine detection and discrimination. *Appl. Spectrosc. Rev.* 2018, 53, 65–86.
29. Bellincontro A., A. Taticchi, M. Servili, S. Esposto, D. Farinelli, and F. Mencarelli, 2012, Feasible Application of a Portable NIR-AOTF Tool for On-Field Prediction of Phenolic Compounds during the Ripening of Olives for Oil Production, *Journal of Agricultural and Food Chemistry* 2012 60 (10), 2665-2673

30. Bellincontro, A., Mencarelli, F., Forniti, R. and Valentini, M. (2009). USE OF NIR-AOTF SPECTROSCOPY AND MRI FOR QUALITY DETECTION OF WHOLE HAZELNUTS. *Acta Hortic.* 845, 593-598
31. Bellincontro, A.; Cozzolino, D.; Mencarelli, F. (2011). Application of NIR-AOTF Spectroscopy to Monitor Aleatico Grape Dehydration for Passito Wine Production. *American Journal of Enology and Viticulture*, 62(2), 256–260
32. Ribereau-Gayon, J. Le dosage des composés phénoliques totaux dans les vins rouges (The determination of total phenolic compounds in red wines). *Chem. Anal.* 1970, 52,
33. B. Lorrain, I. Ky, L. Pechamat, P.L. Teissedre. Evolution of analysis of polyphenols from grapes, wines, and extracts. *Molecules*, 18 (1) (2013), pp. 1076-1100,
34. Ribéreau-Gayon P, Dubourdieu D, Done`che B, Lonvaud A (2006) Handbook of enology, vol 1: the microbiology of wine and vinifications. Wiley, Hoboken
35. Glories, Y. La couleur des vins rouges. Ire partie: Les équilibres des anthocyanes et des tanins. *OENO ONE* 2016, 18, 195.
36. Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich, M.J.; Smith, P.A. Quantification of Condensed Tannins by Precipitation with Methyl Cellulose: Development and Validation of an Optimised Tool for Grape and Wine Analysis. *Aust. J. Grape Wine Res.* 2006, 12, 39–49.
37. Mercurio, M. D., Dambergs, R. G., Herderich, M. J., & Smith, P. A. (2007). High throughput analysis of 658 red wine and grape phenolics - Adaptation and validation of methyl cellulose precipitable 659 tannin assay and modified somers color assay to a rapid 96 well plate format. *Journal of 660 Agricultural and Food Chemistry*, 55(12), 4651–4657
38. Taglieri, I., Mencarelli, F., Bellincontro, A., Modesti, M., Cerreta, R., Zinnai, A., Sanmartin, C., Cecchini, M., Agostini, M. and Gagliardi, M. (2023). Development of a micro-Vis-NIR and SAW nanobiosensor to measure polyphenols in must/wine on-time and online. *Acta Hortic.* 1370, 39-46.
39. Pascual, O.; González-Royo, E.; Gil, M.; Gómez-Alonso, S.; García-Romero, E.; Canals, J. M.; Hermosín-Gutiérrez, I.; Zamora, F., Influence of Grape Seeds and Stems on Wine Composition and Astringency. *Journal of Agricultural and Food Chemistry* 2016, 64 (34), 6555-6566.
40. J.F. Harbertson, E.A. Picciotto, D.O. Adams. Measurement of polymeric pigments in grape berry extract sand wines using a protein precipitation assay combined with bisulfite bleaching. *Am. J. Enol. Vitic.*, 54 (4) (2003), pp. 301-306.

41. V.L. Singleton, J.A. Rossi. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16 (3) (1965), pp. 144-158.
42. T.C. Somers, M.E. Evans. Wine quality: correlations with color density and anthocyanin equilibria in a group of young red wines. *J. Sci. Food Agric.*, 25 (11) (1974), pp. 1369-1379.
43. Costa-Pérez, A.; Medina, S.; Sánchez-Bravo, P.; Domínguez-Perles, R.; García-Viguera, C. The (Poly)phenolic Profile of Separate Winery By-Products Reveals Potential Antioxidant Synergies. *Molecules* 2023, 28, 2081.
44. Gutiérrez-Escobar, R., Aliaño-González, M. J., & Cantos-Villar, E. (2021). Wine Polyphenol Content and Its Influence on Wine Quality and Properties: A Review. *Molecules* (Basel, Switzerland), 26(3), 718.
45. Puyo M, Simonin S, Klein G, David-Vaizant V, Quijada-Morín N, Alexandre H, Tourdot-Maréchal R. Use of Oenological Tannins to Protect the Color of Rosé Wine in a Bioprotection Strategy with *Metschnikowia pulcherrima*. *Foods*. 2023; 12(4):735.
46. Claus H. How to Deal with Uninvited Guests in Wine: Copper and Copper-containing Oxidases. *Fermentation*. 2020; 6(1):38.
47. Giacosa, S., Segade, S. R., Cagnasso, E., Caudana, A., Rolle, L., & Gerbi, V. (2019). SO₂ in wines: Rational use and possible alternatives. In *Red wine technology* (pp. 309-321). Academic Press
48. Waterhouse, A.L., 2002. The phenolic wine antioxidants. In: Cadenas, E. & Packer, L. (eds). *Handbook of Antioxidants*. Marcel Dekker, New York. pp. 401 – 416.
49. López-Vélez, M., Martínez-Martínez, F. & Del Valle-Ribes, C., 2003. The study of phenolic compounds as natural antioxidants in wine. *Crit. Rev. Food Sci. Nutr.* 43, 233-244.
50. J.L. Aleixandre-Tudo, H. Nieuwoudt, J.L. Aleixandre, W.J. Du Toit Robust ultraviolet–visible (UV–Vis) partial least-squares (PLS) models for tannin quantification in red wine. *J. Agric. Food Chem.*, 63(2015).
51. J.C. Boulet, C. Trarieux, J.M. Souquet, M.A. Ducasse, S. Caillé, A. Samson, V. Cheynier Models based on ultraviolet spectroscopy, polyphenols, oligosaccharides and polysaccharides for prediction of wine astringency. *Food Chem.*, 190 (2016), pp. 357- 363, 10.1016/j.foodchem.2015.05.062.
52. C.W. Beaver, J.F. Harbertson Comparison of multivariate regression methods for the analysis of phenolics in wine made from two *Vitis vinifera* cultivars. *Am. J. Enol. Vitic.*, 67 (1) (2016), pp. 56-64.

53. J.L. Aleixandre-Tudo, H. Nieuwoudt, A. Olivieri, J.L. Aleixandre, W. du Toit. Phenolic profiling of grapes, fermenting samples and wines using UV-Visible spectroscopy with chemometrics. Food Control, 85 (2018), pp. 11-22.
54. Gu ui-Wen , Hui-Hui Zhou, Yi Lv, Qiao Wu, Yuan Pan, Zhi-Xin Peng, Xiao-Hua Zhang, Xiao-Li Yin. Geographical origin identification of Chinese red wines using ultraviolet-visible spectroscopy coupled with machine learning techniques, Journal of Food Composition and Analysis, Volume 119, 2023, 105265, ISSN 0889-1575,
55. Giglio, C., Yang, Y., & Kilmartin, P. (2023). Analysis of phenolics in New Zealand Pinot noir wines using UV-visible spectroscopy and chemometrics. Journal of Food Composition and Analysis, 117, 105106.

Supplementary materials

Table S1 Color intensity (CI), total polyphenol index (TPI), anthocyanins and tannins measured with analytical methods in Syrah musts at different degrees of ripeness (Sample 1 to 9) fermented without (series a) and with (series b) stems. Values are the mean of three replicate $\pm$ standard deviation.

		CI	TPI	Anthocyanins mg/L	Tannins mg/L	Polymeric pigments mg/L
Sampling day 1	syrah1a	1.4	10.31 $\pm$ 0.83	543.46 $\pm$ 18.31	439.3 $\pm$ 17.9	0.00
	syrah2a	1.26	15.33 $\pm$ 0.89	605.46 $\pm$ 23.33	390.4 $\pm$ 5.1	0.00
	syrah3a	1.27	14.25 $\pm$ 0.79	538.78 $\pm$ 5.51	309.2 $\pm$ 6	0.00
	syrah1b	1.39	26.85 $\pm$ 0.43	566.91 $\pm$ 3.71	1098.8 $\pm$ 36.5	0.00
	syrah2b	1.21	36.20 $\pm$ 1.29	464.41 $\pm$ 1.3	1177.6 $\pm$ 20.8	0.00
	syrah3b	1.38	27.22 $\pm$ 1.07	621.29 $\pm$ 18.38	766.4 $\pm$ 17.5	0.00
Sampling day 2	syrah1a	1.4	19.85 $\pm$ 0.05	702.84 $\pm$ 15.16	637.4 $\pm$ 52.1	0.00
	syrah2a	1.41	24.48 $\pm$ 0.02	722.75 $\pm$ 27.47	630.1 $\pm$ 14.3	0.00
	syrah3a	1.43	25.72 $\pm$ 0.02	669.24 $\pm$ 20.11	810.8 $\pm$ 18	0.00
	syrah1b	1.39	51.39 $\pm$ 0.01	763.96 $\pm$ 8.29	2187.8 $\pm$ 108	0.00
	syrah2b	1.96	54.63 $\pm$ 0.04	891.76 $\pm$ 3.65	2228.3 $\pm$ 273.2	0.00
	syrah3b	1.54	44.16 $\pm$ 0.04	791.22 $\pm$ 18.99	1498.0 $\pm$ 112.3	0.00
Sampling day 3	syrah1a	1.58	19.33 $\pm$ 1.6	738.59 $\pm$ 11.38	468.2 $\pm$ 57.6	0.00
	syrah2a	1.61	24.40 $\pm$ 1.33	766.28 $\pm$ 7.36	667.1 $\pm$ 41	0.00
	syrah3a	1.61	26.14 $\pm$ 1.14	803.69 $\pm$ 45.91	627.5 $\pm$ 50.8	0.00
	syrah1b	1.62	60.86 $\pm$ 1.03	799.36 $\pm$ 1.55	2310.9 $\pm$ 170	0.00
	syrah2b	2	63.53 $\pm$ 3.61	1007.91 $\pm$ 22.89	2441.5 $\pm$ 196.9	9.15 $\pm$ 0.49
	syrah3b	1.74	50.84 $\pm$ 0.16	829.85 $\pm$ 9.16	1508.3 $\pm$ 89.9	3.85 $\pm$ 2.32
Sampling day 4	syrah1a	1.72	22.92 $\pm$ 0.13	688.49 $\pm$ 9.22	817.7 $\pm$ 33.4	0.45 $\pm$ 0.62
	syrah2a	1.32	28.69 $\pm$ 1.17	776.74 $\pm$ 28.09	870.7 $\pm$ 44.4	5.05 $\pm$ 3.21
	syrah3a	1.54	29.42 $\pm$ 0.99	763.13 $\pm$ 33.6	756.0 $\pm$ 58	6.15 $\pm$ 0.37

	syrah1b	1.67	56.38 ± 2.36	841.93 ± 2.35	2269.9 ± 236	3.85 ± 1.55
	syrah2b	2.05	79.66 ± 5.61	939.75 ± 0.37	2441.5 ± 81.4	23.05 ± 1.24
	syrah3b	1.77	55.53 ± 5.36	924.13 ± 3.03	2012.5 ± 117.8	18.75 ± 7.36
Sampling day 5	syrah1a	1.82	25.89 ± 0.21	840.66 ± 6.5	891.7 ± 51.8	0.0000
	syrah2a	1.84	34.67 ± 0.81	882.22 ± 31.37	621.3 ± 5	23.15 ± 3.76
	syrah3a	1.82	34.14 ± 1.68	808.68 ± 12	415.4 ± 18.1	1.25 ± 1.79
	syrah1b	1.87	61.35 ± 0.2	896.13 ± 2.29	2158.2 ± 143	0.82 ± 1.01
	syrah2b	2.31	78.32 ± 1.82	1002.40 ± 3.22	2774.1 ± 146	24.75 ± 1.73
	syrah3b	2.18	66.77 ± 5.23	946.97 ± 24.69	2059.8 ± 152.8	19.25 ± 1.06
Sampling day 6	syrah1a	1.9	28.16 ± 1.98	809.59 ± 36.81	808.0 ± 114.4	64.25 ± 0.8
	syrah2a	1.9	38.39 ± 0.26	859.12 ± 14.17	1106.3 ± 43.6	79.75 ± 1.98
	syrah3a	1.99	45.85 ± 7.34	866.21 ± 43.37	1037.2 ± 79.2	86.25 ± 13.05
	syrah1b	2.14	60.39 ± 2.51	967.79 ± 35.21	2286.3 ± 134.5	76.30 ± 2.35
	syrah2b	2.74	84.99 ± 0.08	1229.94 ± 11.2	2872.7 ± 147.1	102.75 ± 7.42
	syrah3b	2.44	68.78 ± 1.41	1092.39 ± 22.83	2390.9 ± 174.5	100.95 ± 0.74
Sampling day 7	syrah1a	1.67	34.65 ± 2.48	824.38 ± 22.34	951.8 ± 57.2	42.25 ± 4.02
	syrah2a	1.81	37.53 ± 3.09	948.33 ± 10.15	993.1 ± 30.5	56.75 ± 2.54
	syrah3a	1.67	55.98 ± 2.30	858.16 ± 3.03	1174.8 ± 54.3	52.75 ± 1.42
	syrah1b	1.88	60.74 ± 0.02	1009.31 ± 72.39	2639.1 ± 60.5	56.75 ± 2.04
	syrah2b	2.27	84.14 ± 3.09	1090.78 ± 8.91	3007.4 ± 23.4	80.75 ± 2.29
	syrah3b	1.98	71.02 ± 2.98	1046.50 ± 4.33	2828.5 ± 7.4	74.90 ± 5.32
Sampling day 8	syrah1a	1.61	32.91 ± 1.78	892.89 ± 48.2	933.0 ± 9.4	83.75 ± 6.87
	syrah2a	2.09	41.04 ± 0.04	1068.46 ± 10.15	1095.8 ± 85.2	98.75 ± 0.19
	syrah3a	1.66	32.82 ± 0.62	1008.22 ± 3.4	1049.1 ± 23.5	98.95 ± 3.96
	syrah1b	1.74	69.33 ± 0.05	952.26 ± 31.18	2839.7 ± 11.4	13.35 ± 0.74
	syrah2b	2.2	90.86 ± 0.06	1153.78 ± 1.61	2977.7 ± 18.9	33.75 ± 2.78
	syrah3b	1.9	72.99 ± 0.05	979.96 ± 4.15	2549.0 ± 0.5	25.25 ± 0.06
Sampling day 9	syrah1a	1.41	56.44 ± 0.72	758.23 ± 46.71	1010.2 ± 38.2	46.59 ± 5.43
	syrah2a	1.62	67.24 ± 0.59	993.13 ± 1.98	1049.6 ± 24.1	17.76 ± 0.99
	syrah3a	1.44	30.84 ± 0.41	854.83 ± 21.1	1289.8 ± 13	8.79 ± 1.18
	syrah1b	1.4	93.85 ± 0.96	860.91 ± 3.22	2419.5 ± 61.5	10.50 ± 2.72
	syrah2b	1.15	116.10 ± 1.55	1202.82 ± 20.48	3233.1 ± 199.4	39.90 ± 1.73
	syrah3b	1.62	95.74 ± 1.37	944.96 ± 11.57	2530.4 ± 73.4	29.05 ± 5.44

Table S2 Color intensity (CI), total polyphenol index (TPI), anthocyanins and tannins measured with analytical methods in Bobal musts at different degrees of ripeness (Sample 1 to 9) fermented without (series a) and with (series b) stems. Values are the mean of three replicate ± standard deviation.

		CI	TPI	Anthocyanins mg/L	Tannins mg/L	Polymeric pigments mg/L
	bobal1a	1.17	14.46 ± 1.16	484.66 ± 17.08	447.05 ± 7.09	0.00
	bobal2a	0.97	5.93 ± 0.06	400.71 ± 9.71	347 ± 12.47	0.00

Sampling day 1	bobal3a	1	23.79 ± 1.44	554.97 ± 1.42	1000 ± 62.21	0.00
	bobal1b	0.86	19.15 ± 0.21	354.99 ± 7.8	907.99 ± 16.21	0.00
	bobal2b	1.23	27.9 ± 0.1	490.53 ± 2.6	886.5 ± 55.58	0.00
	bobal3b	0.95	30.77± 0.05	458.19 ± 3.16	1677.02 ± 14.68	0.00
Sampling day 2	bobal1a	1.27	24.39 ± 0.07	664.43 ± 15.03	810.14 ± 25.26	0.00
	bobal2a	1	11.24 ± 0.01	566.13 ± 19.8	543.14 ± 14.38	0.00
	bobal3a	1.05	38.41 ± 0.03	794.72 ± 19.49	1701.6 ± 27.18	0.00
	bobal1b	0.74	30.72 ± 0.01	491.05 ± 5.82	1248.82 ± 10.17	0.00
	bobal2b	1.27	29.03 ± 0-04	620.81 ± 36.31	1475.04 ± 99.26	0.00
	bobal3b	1.1	54.98 ± 0.02	661.68 ± 20.54	2699.5 ± 24.61	0.00
Sampling day 3	bobal1a	1.35	25.79 ± 2.03	759.24 ± 10.77	812.78 ± 12.55	0.00
	bobal2a	1.1	14.82 ± 3.25	606.46 ± 0.99	569.85 ± 34.67	0.00
	bobal3a	1.2	40.72 ± 1.51	771.93 ± 14.48	1743.69 ± 32.55	18.20 ± 0.49
	bobal1b	0.85	29.07 ± 0.23	513.84 ± 2.66	1395.92 ± 59.1	0.00
	bobal2b	1.41	30.83 ± 3.14	672.39 ± 7.86	1523.75 ± 108.99	0.00
	bobal3b	1.31	59.13 ± 2.49	742.92 ± 35.08	2816.2 ± 104.82	17.06 ± 2.23
Sampling day 4	bobal1a	1.54	26.65 ± 0.51	638.88 ± 15.16	915.04 ± 42.25	2.80 ± 1.24
	bobal2a	1.26	15.37 ± 1.13	584.15 ± 17.82	623.91 ± 21.71	0.65 ± 0.49
	bobal3a	1.44	41.01 ± 3.1	778.53 ± 24.69	1967.27 ± 37.99	21.15 ± 1.55
	bobal1b	1.02	34.02 ± 0.22	463.05 ± 4.08	1415.43 ± 18.14	3.75 ± 2.54
	bobal2b	1.49	32.34 ± 1.52	586.43 ± 1.48	1597.02 ± 129.91	1.75 ± 0.56
	bobal3b	1.31	62.27 ± 3.71	731.72 ± 14.42	2868.32 ± 45.62	25.75 ± 3.4
Sampling day 5	bobal1a	1.69	29.39 ± 1.67	862.09 ± 4.02	976.53 ± 76.64	7.25 ± 0.68
	bobal2a	1.25	18.53 ± 0.11	691.29 ± 6.37	657.41 ± 32.06	3.55 ± 1.05
	bobal3a	1.44	48.24 ± 3.22	840.04 ± 8.72	2002.09 ± 90.61	22.45 ± 8.04
	bobal1b	1.16	37.42 ± 1.57	556.85 ± 1.24	1413.22 ± 99.79	8.25 ± 4.52
	bobal2b	1.81	41.77 ± 1.69	800.14 ± 11.08	1604.52 ± 49.86	2.71± 1.11
	bobal3b	1.63	67.03 ± 3.43	840.39 ± 24.32	2899.06 ± 16.86	28.00 ± 1.73
Sampling day 6	bobal1a	1.94	30.77 ± 2.97	641.29 ± 22.29	1092.01 ± 67.92	11.25 ± 0.31
	bobal2a	1.63	22.12 ± 1.25	687.66 ± 10.02	826.89 ± 54.88	6.25 ± 0.25
	bobal3a	1.48	48.11 ± 4.47	921.33± 20.48	2146.23 ± 19.28	25.55 ± 1.24
	bobal1b	1.23	41.1 ± 1.53	524.78 ± 7.49	1686.72 ± 35.03	0.0000
	bobal2b	1.89	46.58 ± 1.4	726.82 ± 3.77	1615.98 ± 18.14	0.0000
	bobal3b	1.89	68.6 ± 1.9	834.18 ± 9.22	2986.01 ± 159.25	21.75 ± 2.29
Sampling day 7	bobal1a	1.91	35.95 ± 1.2	836.46 ± 36.94	1262.92 ± 19.71	19.25 ± 0.8
	bobal2a	1.38	26.65 ± 0.75	672.7 ± 5.32	957.47 ± 45.08	25.15 ± 1.36
	bobal3a	1.82	56.74 ± 2.66	947.19 ± 13.86	2564.41 ± 20.06	56.03± 0.49
	bobal1b	1.27	59.19 ± 3.18	533.84 ± 5.94	1821.49 ± 75.46	39.22 ± 2.97
	bobal2b	2.6	59.64 ± 0.85	754.69 ± 2.72	1884.74 ± 52.34	40.61 ± 1.73
	bobal3b	2.08	70.42 ± 1.66	872.51 ± 7.98	3583.47 ± 18.06	62.75 ± 0.62
Sampling day 8	bobal1a	1.58	37.63 ± 1.24	819.7 ± 7.42	1475.7 ± 68.44	80.25 ± 0.56
	bobal2a	1.39	42.86 ± 0.01	665.64 ± 30.94	1450.03 ± 41.42	83.75 ± 2.41
	bobal3a	1.91	54.45 ± 0.03	1032.46 ± 26.42	2535.1 ± 96.5	89.25 ± 0.8
	bobal1b	0.99	70.45 ± 0.52	646.06 ± 26.05	1745.45 ± 49.66	66.25 ± 6.5
	bobal2b	1.57	86.92 ± 3.33	816.99 ± 38.48	2140.57 ± 22.43	71.95 ± 6.81
	bobal3b	1.5	77.94 ± 8.5	1043.53 ± 7.18	3438.9 ± 24.14	74.25 ± 2.41

Sampling day 9	bobal1a	1.47	62.97 ± 0.36	861.57 ± 5.63	1257.41 ± 11.81	15.265 ± 4.27
	bobal2a	1.01	47.12 ± 1.11	649.64 ± 22.95	727.82 ± 33.45	4.81 ± 4.21
	bobal3a	1.26	80.3 ± 3.03	897.44 ± 60.33	2458.51 ± 61.82	28 ± 4.83
	bobal1b	0.79	66.01 ± 1.41	588.35 ± 35.54	1618.18 ± 98.16	13.16 ± 5.09
	bobal2b	1.31	71.13 ± 1.58	751.84 ± 2.29	2180.83 ± 15.17	6.47 ± 0.62
	bobal3b	1.33	96.66 ± 1.79	835.76 ± 27.66	2459.5 ± 86.98	36.25 ± 2.66

Table S3 Color intensity (CI), total polyphenol index (TPI), anthocyanins and tannins measured with analytical methods in Cabernet Sauvignon musts at different degrees of ripeness (Sample 1 to 9) fermented without (series a) and with (series b) stems. Values are the mean of three replicate ± standard deviation.

		CI	TPI	Anthocyanins mg/L	Tannins mg/L	Polymeric pigments mg/L
Sampling day 1	cabernet 1a	0.76	7.01 ± 0.46	418.12 ± 38.92	230.1 ± 13.7	0
	cabernet 2a	1.1	29.61 ± 0.89	546.04 ± 2.04	558.2 ± 18.1	0
	cabernet1b	0.81	14.05 ± 0.20	461.69 ± 3.28	573 ± 21.7	0
	cabernet 2b	1.56	32.92 ± 2.11	718.07 ± 3.03	1191.5 ± 23	0
Sampling day 2	cabernet 1a	0.99	17.77 ± 0.06	753.38 ± 24.01	569.5 ± 10.5	0
	cabernet 2a	1.33	26.6 ± 0.06	748.56 ± 4.21	734.4 ± 16.1	0
	cabernet1b	0.85	30.57 ± 0.01	696.06 ± 23.88	1277.1 ± 41.7	0
	cabernet 2b	1.99	53.89 ± 0.02	920.24 ± 3.09	1609 ± 23.8	0
Sampling day 3	cabernet 1a	1.07	21.77 ± 0.46	880.51 ± 2.72	576.3 ± 12.9	1.18 ± 1.67
	cabernet 2a	1.41	27.18 ± 0.30	786.98 ± 21.53	764.2 ± 49	4.25 ± 3.2
	cabernet1b	1.04	30.19 ± 0.07	686.57 ± 7.86	1535.9 ± 80	16.75 ± 12.2
	cabernet 2b	2.26	49.91 ± 4.05	937.04 ± 1.3	1742.5 ± 43.3	15.04 ± 2.41
Sampling day 4	cabernet 1a	1.21	26.6 ± 0.38	778.97 ± 12.81	830.9 ± 15.1	9.8 ± 2.23
	cabernet 2a	1.55	29.49 ± 1.60	732.94 ± 3.16	871.3 ± 45.4	7.575 ± 1.67
	cabernet1b	1.13	37.65 ± 2.26	703.55 ± 12.68	1427.7 ± 35.6	12.25 ± 1.86
	cabernet 2b	2.19	57.61 ± 4.78	964.86 ± 15.96	1905.8 ± 54.3	27.75 ± 0.12
Sampling day 5	cabernet 1a	1.33	31.12 ± 2.80	831.21 ± 27.04	903.7 ± 11.9	59.75 ± 5.543
	cabernet 2a	1.8	35.09 ± 2.58	845.6 ± 19.18	927.9 ± 20.9	12.15 ± 2.47

	cabernet1b	1.14	39.58 ± 2.11	737.84 ± 47.32	1614.1 ± 60.4	12.55 ± 0.56
	cabernet 2b	2.45	65.02 ± 3.48	997.11 ± 41.95	2036.5 ± 19.8	31.75 ± 0.43
Sampling day 6	cabernet 1a	1.61	35.59 ± 0.55	853.96 ± 0.31	1050.1 ± 73.5	91.075 ± 4.15
	cabernet 2a	1.85	38 ± 1.36	858.29 ± 8.17	1236.3 ± 52.7	98.25 ± 3.28
	cabernet1b	1.37	44.32 ± 1.02	730.14 ± 5.51	1738.7 ± 42.8	98.15 ± 1.18
	cabernet 2b	2.64	67.9 ± 0.69	1017.93 ± 40.65	2570.1 ± 93.3	116.05 ± 0.37
Sampling day 7	cabernet 1a	1.25	40.9 ± 0.77	802.11 ± 41.21	1157.1 ± 31.1	54.25 ± 3.65
	cabernet 2a	2.04	41.64 ± 1.94	976.11 ± 1.55	1289.1 ± 75.6	56.75 ± 4.15
	cabernet1b	1.31	45.65 ± 0.62	890.62 ± 3.16	1813.3 ± 9.6	57.75 ± 1.92
	cabernet 2b	2.48	69 ± 0.43	1157.49 ± 61.93	2690.2 ± 30.4	85.55 ± 5.01
Sampling day 8	cabernet 1a	1.18	60.58 ± 1.34	756.76 ± 17.45	1286.9 ± 44.4	48.25 ± 3.09
	cabernet 2a	2.75	49.15 ± 3.36	845.64 ± 14.79	1385.1 ± 18.4	13.25 ± 1.48
	cabernet1b	1.09	50.1 ± 0.02	688.23 ± 31.49	2113.8 ± 55.4	13.55 ± 0.99
	cabernet 2b	2.31	77.82 ± 0.06	1298.58 ± 21.78	2786.9 ± 64.9	8.4 ± 2.23
Sampling day 9	cabernet 1a	0.85	61.4 ± 5.53	681.67 ± 15.65	1228.7 ± 67.7	18.06 ± 0.43
	cabernet 2a	1.35	63.26 ± 1.32	797.67 ± 0.56	1295.8 ± 16.7	28.17 ± 0.62
	cabernet1b	0.87	73.48 ± 2.24	622.04 ± 29.57	1991.4 ± 80	21.43 ± 13.61
	cabernet 2b	2.01	105.18 ± 3.07	1175.65 ± 3.46	2370.2 ± 66.8	50.90 ± 1.98

3. Chapter III - Feasibility assessment of a low-cost NIR spectroscopy-based prototype for monitoring polyphenol extraction in fermenting musts.

This chapter is based on the article entitled "*Feasibility assessment of a low-cost NIR spectroscopy-based prototype for monitoring polyphenol extraction in fermenting musts*", accepted for publication by the *Journal of the Science of Food and Agriculture*. This work describes the design, building-up, and validation of an additional spectrophotometric prototype, based on Near-Infrared (NIR) technology, always aimed at the non-destructive monitoring of polyphenols in fermenting musts.

This study represents the natural follow-up of the work reported in Chapter II, where polyphenol monitoring was entrusted to a prototype VIS spectroscopy-based. The employ of NIR technology allows for the exploration of a different spectral range, providing a complementary low-cost approach for polyphenol characterization.

Complementary, it offers improved predictive performances with respect these chemical categories, if compared to the prototype working on visible spectroscopy.

As compared to the published version, the text has been modified to ensure stylistic consistency with all the thesis and to include additional methodological details essential for the overall research framework.

Abstract

Background

The winemaking industry increasingly relies on non-destructive techniques to support processing activities, particularly in response to climate change-induced imbalances between technological and phenolic grape maturity. Spectroscopy-based instruments have become widely available for monitoring key parameters such as total polyphenols, anthocyanins, and tannins during maceration and fermentation. Near-infrared (NIR) spectroscopy presents a promising solution for rapid and efficient analysis, potentially enhancing process control and decision-making in winemaking.

Discussion

This study evaluates the feasibility of a low-cost NIR spectroscopy-based prototype for monitoring phenolic extraction in small-scale fermentations. The device's performance was assessed by correlating spectral data with conventional reference measurements, including total polyphenol index, tannin concentration, colour intensity, anthocyanins, and polymeric pigments. Predictive models were developed using partial least squares regression and compared with those obtained from a commercial FT-NIR spectrophotometer to assess the prototype's accuracy and reliability.

Conclusions

The results indicate that the low-cost NIR prototype shows potential for monitoring phenolic extraction during fermentation, providing real-time and non-destructive measurements. While its predictive performance was slightly lower than that of the commercial FT-NIR system, the prototype demonstrated promising accuracy, suggesting that with further optimization, it could serve as a cost-effective alternative for wineries seeking rapid and affordable phenolic analysis.

Keywords: Polyphenols; near-infrared spectroscopy (NIRs); tannins; anthocyanins, low-cost prototype.

Introduction

Winemaking is a multifaceted bioprocess influenced by different factors, such as environmental conditions during production (e.g., temperature, pressure, and atmospheric composition) and specific practices implemented in the cellar (Cavaglia et al., 2020). These variables significantly affect the extraction of phenolic compounds during maceration, the progression of alcoholic fermentation, and the final composition of the wine. As technological advancements continue, wineries are actively exploring innovative methods to enhance their operational protocols while maintaining high-quality standards. This pursuit is especially relevant in light of challenges posed by climate change, labor shortages, and rising production costs. Concurrently, consumer expectations are becoming increasingly demanding, and the competitive nature of the market underscores the need for tools that optimize winemaking processes. In this context, wineries benefit from fast and cost-effective methods that provide timely information to ensure precise control at every stage of production (Littarru et al., 2024). The application of spectroscopy technologies in the wine industry has now reached a position of significant importance. Their aptitude to be integrated into systems, supported by predictive statistical models that provide

multiparametric information on grapes, must or wine, makes them highly valuable (Dos santos costa et al., 2019; Croce et al., 2020; Anjos et al., 2021). Traditional laboratory analyses, often categorized as wet chemistry or destructive measurements, are time-consuming, labor-intensive, require highly specialized personnel, and expensive due to the reagents used. They also have a high environmental impact due to the need to dispose of these chemical reagents after processing (Arvanitoyannis et al., 2006). Although these techniques provide accurate information about wine composition, often do not correspond to the needs of modern wine production in a global market demands. As such, ultraviolet (UV) (Weingerl et al., 2009), visible (VIS) (Giglio et al., 2023; Gu et al., 2023; Shahrajabian et al., 2024), and infrared (IR) (Bai et al., 2023; da Costa Fulgêncio et al., 2023; Dong et al., 2023; Ta et al., 2023) are the most commonly used spectroscopy techniques addressed to measure various enological parameters nowadays. These techniques function by interacting with the natural organic molecules in the wine. By leveraging chemometric techniques, non-destructive data from these approaches can be combined with destructive analysis results to develop predictive models that correlate sensor signals with molecule concentrations. These spectroscopy techniques, with their corresponding wavelength ranges, are usually exploited for detecting a large number of wine parameters, such as total soluble sugar content Cornehl et al., 2023; Lu et al., 2023), total acidity and individual acids (Cornehl et al., 2023; Singla et al., 2024), alcohol content (Feifel et al., 2024; He et al., 2024), nitrogen levels in musts (Miao et al., 2023), free and total sulfur dioxide concentrations (Ozdemir et al., 2023), volatile acidity (Regmi et al., 2012), total polyphenol (Cozzolino et al., 2004; Baca-Bocanegra et al., 2011), and anthocyanins content (Ferrearì et al., 2011). Recently, in addition to established companies that produce expensive and functional commercial spectrophotometers, many small startups are moving for developing more affordable instruments, being the characteristics of the low-cost prototype, that can be used in production processes. Some authors are already working with prototype spectrophotometers for measuring quality parameters in grapes (Pampuri et al., 2022), for the quantification of procyanidins (tannins) (Iaccheri et al., 2021), and for measuring polyphenols in wines (Taglieri et al., 2022). Very recently, our previous published research demonstrated the potential of a low-cost and portable VIS spectrometer prototype to monitor polyphenol extraction during winemaking (Alfieri et al., 2023). The prototype proved affordable and user-friendly and had minimal operating costs compared to a traditional benchtop VIS spectrometer. However, some limitations were identified in the previous VIS prototype, which highlighted the need for enhanced refinement and optimization. For instance, the predictive accuracy of the VIS prototype was hindered by the limited number of wavelength data points—only eight compared to 104 in the commercial spectrometer. This discrepancy significantly impacted the reliability of the

predictions. To overcome these limitations, the main goal of the present study was to develop and evaluate a new low-cost NIR spectrophotometer created in partnership with Nature 4.0 (Viterbo, Italy). The NIR spectrophotometer leverages the consistent bonding patterns of phenolic compounds to detect and quantify their presence effectively. Key absorption bands associated with polyphenol identification include the stretching of C-H bonds at the third (928 and 940 nm), second (1148 nm), and first overtones (1620 and 1652 nm). The region between 1100 and 1300 nm corresponds to combination bands involving symmetric and antisymmetric O-H stretching, as well as the second overtone of aromatic C-H vibrations and the third overtone of C-H vibrations. Additionally, the range around 1600 nm is linked to condensed tannins (Littarru et al., 2024). These distinct spectral features, attributed to the chemical structure of phenolics, allow NIR technology to discriminate between varying concentrations of critical phenolic molecules. By integrating chemometric analysis, the proposed NIR prototype offers a rapid, cost-effective solution for monitoring phenolic compounds during the vinification process.

Materials and methods

Sampling and experimental design

The grapes analysed in this study were sourced from the 'Coloraos' experimental vineyard located in the municipality of Requena during the 2022 harvest. Three grape varieties, *Vitis vinifera* L. cv Syrah, Bobal, and Cabernet Sauvignon, were collected at different ripening stages from September 15 to September 29. Three sampling points were designated for Syrah and Bobal, while Cabernet Sauvignon was sampled at two points (Figure 1) (Alfieri et al., 2023). The fermentation of the grapes was carried out independently for each batch. Some batches were crushed and destemmed, "a" series, while others were crushed and fermented with their stems, the "b" series, resulting in different polyphenol concentrations. Altogether, six fermentation processes were conducted for Syrah and Bobal and four for Cabernet Sauvignon.

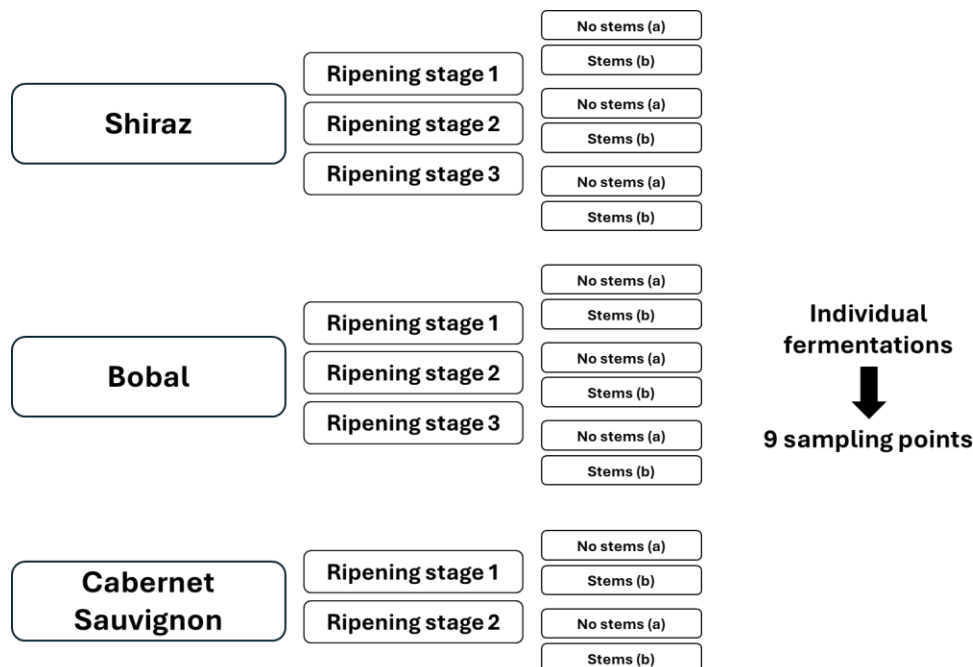


Figure 1. Experimental design.

Winemaking

Approximately 5 kg of grape per sample were crushed (and destemmed for the “a” series) in 10-liter glass jars. Each sample was inoculated with 30 mg/L of commercial yeast (Zymaflore RX60 red wine yeast, Renteria, Spain), and 0.03 g/kg of sulfur dioxide was added as an 8% potassium metabisulfite solution. To induce variability in the phenolic extraction, the destemmed grapes were fermented on their skins under controlled conditions at 18 ± 1.5 °C and 80 % relative humidity. For the batches with stems included, fermentation was performed at room temperature. Samples of must/wine were collected daily over 9 days, centrifuged and filtered through a 0.45 µm filter.

Analytical analyses

Fermenting must samples were analyzed to measure several phenolic parameters. The Total Polyphenol Index (TPI) was determined by diluting the wine 50-fold with distilled water and measuring the absorbance at 280 nm using 10 mm quartz cuvettes (Ribéreau-Gayon et al., 2006). Distilled water served as the blank. Total and bleachable anthocyanins were quantified using the bisulfite bleaching method, allowing for monitoring of total anthocyanins and polymer pigments (Glories et al., 2016). Colour Intensity (CI) was calculated by measuring the absorbance of the wine samples at three wavelengths (420 nm, 520 nm, and 620 nm) and adding these values to obtain the CI index (Sarneckis et al., 2006). The methylcellulose precipitation (MCP) method was used to assess total tannins (Mercurio et al., 2007). Except for colour intensity, all measurements

were performed in triplicate. These analyses were conducted using a commercial UV-VIS spectrophotometer (Jasco 730 UV-visible) covering a range from 200 to 705 nm, with 2 nm increments and a 1 mm quartz cuvette. These measurements provided data to evaluate correlations between NIR spectra and analytical values for prediction purposes.

Spectral acquisitions via the prototype device

The low-cost NIR prototype used in this study was developed by Nature 4.0 (Viterbo, Italy). The spectrometer covers almost the entire NIR region (1350nm-2150nm). The device has a halogen lamp and three MEMS-FPI spectral sensors from Hamamatsu Photonics (Hamamatsu City, Japan) (Figure 2).

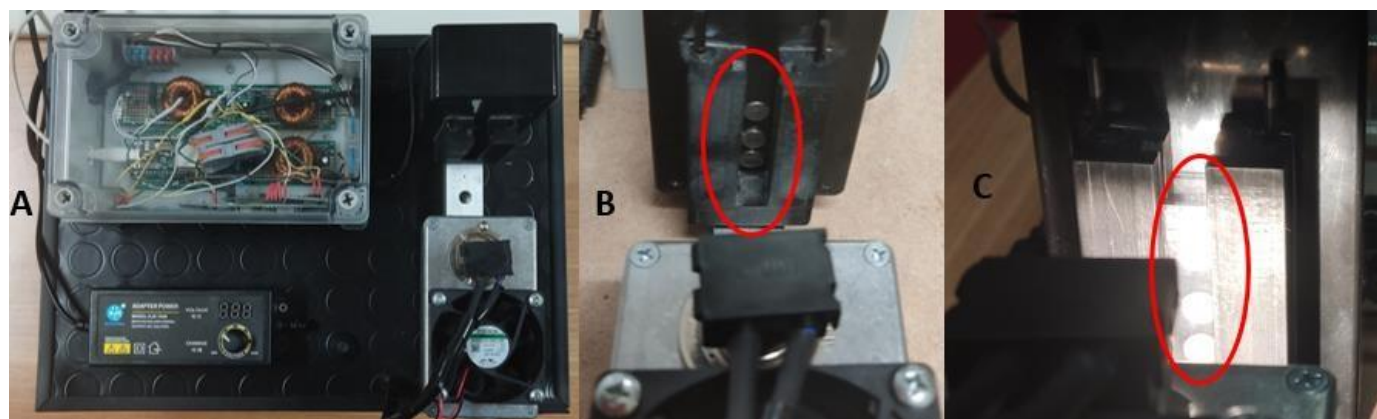


Figure 2. Low-cost NIR spectrophotometer components: (A) Low-cost NIR spectrophotometer; (B) 3 sensors Hamamatsu; (C) Low-cost lamp of the NIR spectrophotometer with 1 mm cuvette housing.

The MEMS-FPI is an ultra-compact sensor that houses a MEMS-FPI (Fabry-Perot Interferometer) tunable filter that can vary its transmission wavelength depending on the applied voltage and an InGaAs PIN photodiode in a single package. It has a simple structure in which the MEMS-FPI tunable filter and photosensor are arranged on the same axis in the direction of the incident light. MEMS-FPI tunable filter has an upper mirror and a lower mirror opposite each other with an air gap between them. The three sensors have activities between 1350 -1650 nm, 1550-1850 nm and 1750-2150 nm, and have a 6 nm resolution. The prototype is designed with the 'Arduino software interface and is connected to a laptop. Must/wine sample readings were instrumentally performed using a 1mm quartz cuvette and transmittance (T) as a detection method. The prototype takes ten readings for each sample and returns an average spectrum. Raw spectra collected in transmittance were transformed into absorbance ($\log 1/T$) and later used for chemometric analysis.

Spectral acquisitions via commercial NIR device

To compare the performance of the prototype NIR spectrophotometer, spectral acquisitions were also taken by using an FT-IR (Fourier Transformed Infrared) spectroscopy (Cary 630 spectrometer, Agilent Technologies Inc., Santa Clara, CA, USA) instrument covering the range from 1350 to 2150 nm with a 4 cm⁻¹ resolution. Ten readings were acquired for each sample (including replicates), and the computed average spectrum, in transmittance, represented the final reference, which was transformed into absorbance (log 1/T) and used for subsequent computations and modelling.

Data analysis

The absorbance values obtained with commercial and prototype NIR spectrometers were used to build linear regressive models using the partial least square (PLS) regression algorithm. During model optimization, the NIR spectral information was used as independent variables (X-block) while the reference data (i.e. colour intensity, total polyphenol index, anthocyanins (mg/L), tannins (mg/L) and polymer pigments (mg/L)) was used as the dependent variable (Y-block). The datasets were first auto-scaled, and Venetian blinds (blind thickness = 1) were used as a cross-validation method. After reviewing the scree plot, the ideal number of latent variables (LVs) was selected to identify the best relationship between the observed and latent variables, corresponding to the point of error minimization in calibration (RMSEC) and prediction (RMSECV). The correlation coefficients (R^2), in calibration (R^2_c) and prediction or cross-validation (R^2_{cv}), and the ratio performance to deviation (RPD) were also calculated and considered to assess model performance. Multivariate computations were performed in Matlab R2013a (MathWorks®, Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA).

Results

The phenolic parameters analytically measured (colour intensity, total polyphenol index, anthocyanins, and tannins) are shown in Tables S1-S3 of Chapter II. Very briefly, colour density exhibits a consistent pattern across the variety, with Shiraz showing the highest values, followed by Cabernet and Bobal. This parameter generally increases throughout the fermentation process until a slight decrease observed towards the end of the process (Aleixandre-Tudo et al., 2018a). In line with other studies, the presence of stems along vinification process favored higher colour density values (Pascual et al., 2016; Suriano et al., 2015).

The total polyphenol index showed the highest values in Cabernet Sauvignon, followed closely by Shiraz and Bobal. This index shows a substantial increase over the fermentation period and is markedly higher in samples fermented with stems. Overall, observed anthocyanin and tannin concentrations are most prominent in Shiraz, followed by Cabernet and Bobal.

Similarly to the total polyphenol index, tannins notably increased during fermentation especially in fermentation with stems (Suriano et al., 2015). Polymeric pigments emerged after few days from the beginning of the fermentation, typically after day 3 or 4, and were more abundant in Shiraz and Cabernet than Bobal. The grape maturity stage also influences the phenolic composition, with riper grapes (W3) generally yielding wines with higher concentrations of phenolic compounds than less mature grapes (W1) (Pérez-Magariño et al., 2006; Canals et al., 2005).

These data were used to build predictive models aimed at verifying and comparing the aptitude of both NIR spectrophotometers (prototype and commercial) to predict the different phenolic parameters by non-destructive approach. For the building-up of the predictive model referred to polymeric pigments, data from the first four days of fermentation were excluded, as the concentrations of these compounds were still zero, indicating they had yet to dissolve into the fermenting musts.

Discussion

The spectral acquisitions performed using both the commercial NIR device and the NIR prototype were processed with first-order polynomial fitting and 25-point smoothing, as shown in Figures 3 and 4, respectively. The plotted spectra already reveal significant differences between the two acquisition. The spectra from the commercial device appear clearer and more refined, while those from the prototype exhibit fewer peaks. Commercial NIR devices often utilize advanced or dispersive elements, which contribute to higher spectral resolution and clarity (Barton et al., 1996).

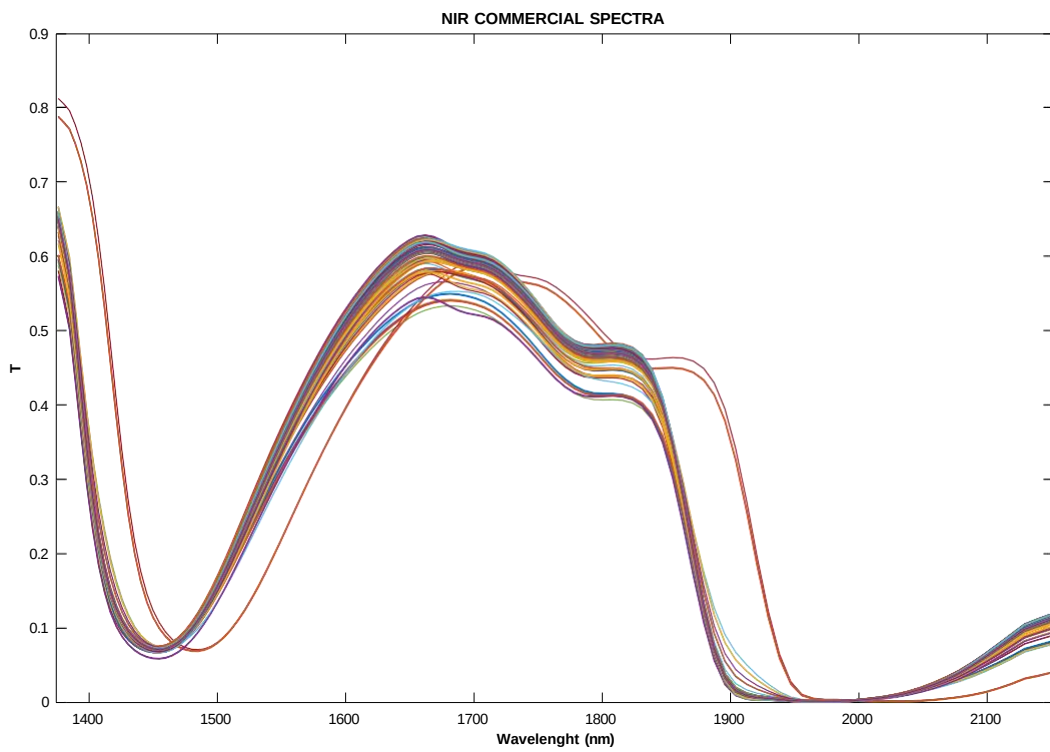


Figure 3. Spectral acquisitions performed with the NIR commercial, processed with first-order polynomial fitting and 25-point smoothing.

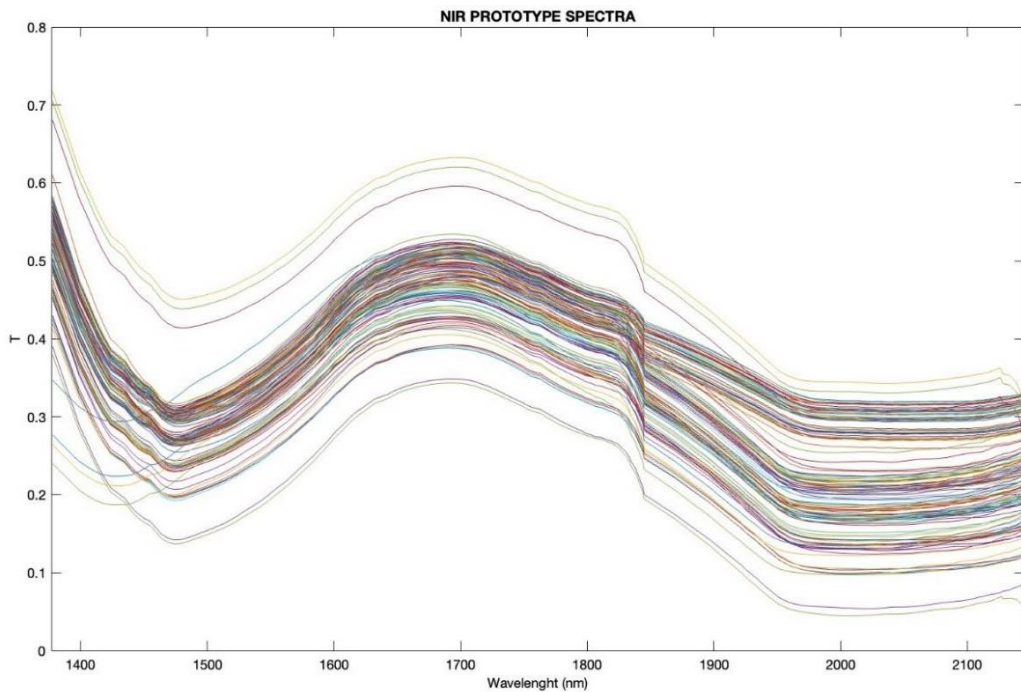


Figure 4. Spectral acquisitions performed with the NIR prototype, processed with first-order polynomial fitting and 25-point smoothing.

In contrast, prototypes, while innovative, typically rely on simpler configurations which can affect spectral clarity, as these setups have less sophisticated light dispersion and detection mechanisms. However, the prototypes must prioritize miniaturization and cost reduction.

To compare the ability, reliability and accuracy of the two NIR spectrophotometer in predicting the content of the different phenolic fractions, different PLS models were built and specific statistical indexes have been considered. For instance, R^2_{cal} , which measures how well the predicted data fit the calibration line across all samples, reflect the robustness of the model. On the other hand, R^2_{cv} evaluates the model's ability to predict new, randomly removed data by assessing how well these data fit the calibration line. A high R^2_{cal} and low R^2_{cv} may indicate that the model is overly dependent on the specific samples used for calibration and may need to perform better with external or new samples (Miramont et al., 2020). Another useful index is the ratio between the reference data's standard deviation and the model's cross-validation error (SD/RMSECV). According to the literature, an RPD (Residual Predictive Deviation) value below 1.4 indicates that the model is unreliable for prediction and should only be used for rough screening. When the RPD is between 1.4 and 2, the model starts to be reliable for predictive purposes. An RPD over 2 indicates that the model is acceptable for prediction purposes, while an RPD that exceeds the value of 3 indicates that the model has excellent prediction capabilities (Cozzolino et al., 2011a; Ferrer-Gallego et al., 2011; Martelo-Vidal et al., 2014).

Overall, the optimized PLS models for the investigated parameters showed quite positive statistics indexes for both commercial and prototype devices, indicating the suitability of NIR spectroscopy to quantify phenolic fractions in wines.

As far as the commercial device is concerned, the R^2 were sufficiently positive, with values ranging from 0.78 to 0.91 (Figure 5 and Table 1), even though numerous latent variables were necessary to minimizing the residual error.

Table 1 Statistical indexes of the PLS models built with the commercial NIR spectrometer for polymer pigments, tannins, anthocyanins, color intensity and total polyphenols index. RMSEC = Root Mean Square Error in calibration. RMSECV = Root Mean Square Error in cross validation. R^2 =correlation coefficient in calibration. R^2 = correlation coefficient in cross validation. RPD = Relative Percentage Difference.

<i>Commercial NIR</i>	<i>Sample n.</i>	<i>R^2_{Cal}</i>	<i>RMSEC</i>	<i>R^2_{Cv}</i>	<i>RMSECV</i>	<i>LV</i>	<i>RPD</i>
<i>Polymeric pigments</i>	144	0.91	9.48	0.89	10.40	17	3.03

Tannins	144	0.88	277.30	0.84	313.30	21	2.59
Anthocyanins	144	0.89	32.25	0.88	33.80	19	5.49
Colour intensity	144	0.80	0.19	0.78	0.20	20	2.32
Total polyphenols index	144	0.78	10.42	0.75	11.00	18	2.08

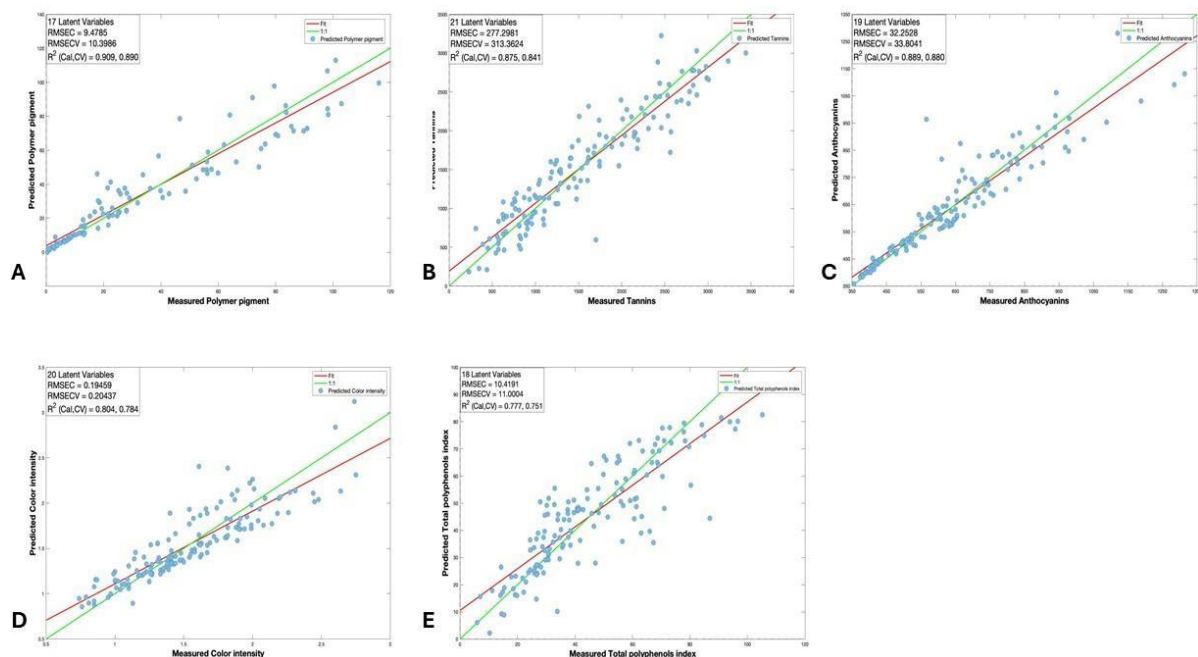


Figure 5. Observed versus predicted plots of the PLS models built with the commercial NIR spectrometer for polymer pigments (A), tannins (B), anthocyanins (C), color intensity (D) and total polyphenols index (E). RMSEC = Root Mean Square Error in calibration. RMSECV = Root Mean Square Error in cross validation. R^2 =correlation coefficient in calibration. R^2 = correlation coefficient in cross validation. RPD = Relative Percentage Difference.

Specifically, for polymeric pigments, R^2 of 0.909 and 0.890 were obtained in calibration and cross-validation respectively by using 17 latent variables (LVs) (Figure 5. a). Significant prediction performance was also observed for tannins (R^2 of 0.875 in calibration and 0.841 in cross-validation, 21 LVs), anthocyanins, (R^2 of 0.889 in calibration and 0.880 in cross-validation with 19 LVs) and color intensity (R^2 of 0.804 and 0.784 for calibration and cross-validation with 20 LVs) (Figure 5. b, c and d). Although the TPI model exhibited the lowest performance, it still delivered an acceptable prediction, with R^2 values of 0.777 in calibration and 0.751 in cross-validation, using 18 LVs (Figure 5. e). The RPD statistic for the selected models confirms the prediction aptitude of the optimized models. Hence, the lowest RPD was observed for the TPI and CI indexes, 2.08 and 2.32, respectively. These values are just below the threshold of good predictive performance, considered equal to 2.5. The other models presented RPD values of 2.59 for tannins , 3.03 for polymeric pigments, and 5.49 in the case of anthocyanins (mg/L) (Table 1).

For NIR prototype regression models the same PLS modelling criteria used for the commercial spectrometer were employed thus allowing a performance comparison between the prototype and

the commercial device. As expected, the models performance obtained with the NIR prototype were overall lower than those derived from the commercial device (Figure 6 and Table 2).

Table 2. Statistical indexes of the PLS models built with the prototype NIR spectrometer for polymer pigments, tannins, anthocyanins, color intensity and total polyphenols index. RMSEC = Root Mean Square Error in calibration. RMSECV = Root Mean Square Error in cross validation. R2=correlation coefficient in calibration. R2= correlation coefficient in cross validation. RPD = Relative Percentage Difference.

<i>prototype NIR</i>	<i>Sample n.</i>	<i>R² Cal</i>	<i>RMSEC</i>	<i>R² Cv</i>	<i>RMSECV</i>	<i>LV</i>	<i>RPD</i>
<i>Polymeric pigments</i>	144	0.87	11.26	0.84	12.46	27	2.53
<i>Tannins</i>	144	0.81	345.59	0.75	393.74	21	2.06
<i>Anthocyanins</i>	144	0.78	47.50	0.73	51.20	26	3.63
<i>Colour intensity</i>	144	0.35	0.35	0.27	0.37	29	1.24
<i>Total polyphenols index</i>	144	0.72	11.57	0.67	12.80	25	1.79

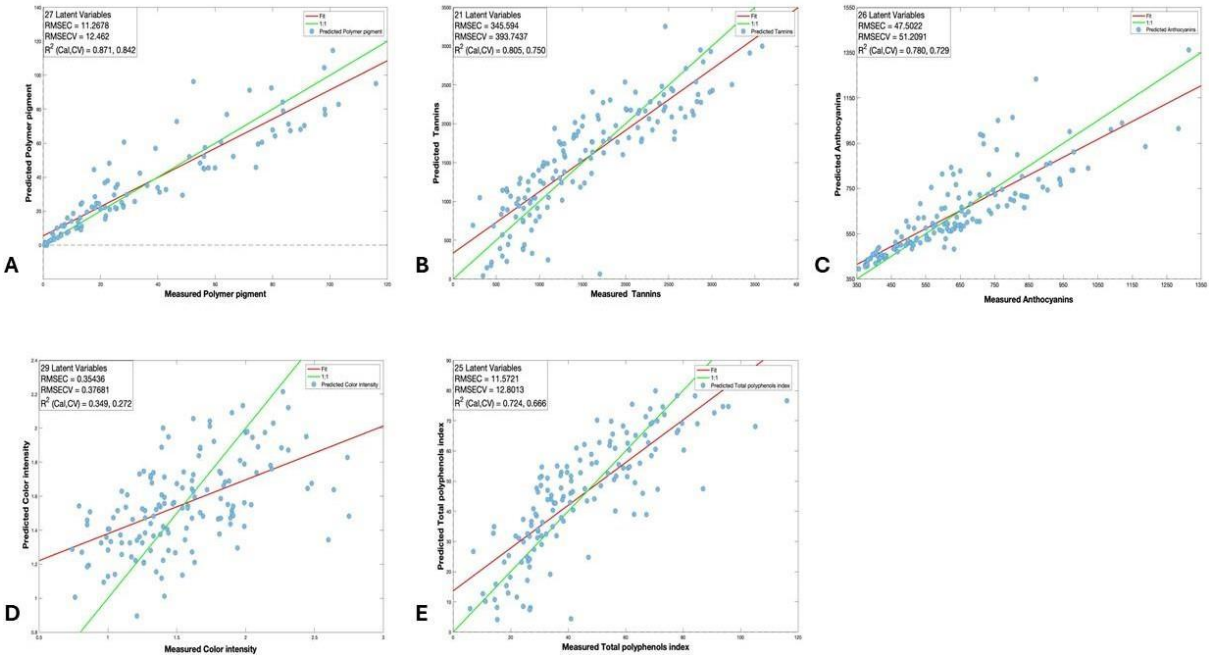


Figure 6. Observed versus predicted plots of the PLS models built with the prototype NIR spectrometer for polymer pigments (A), tannins (B), anthocyanins (C), color intensity (D) and total polyphenols index (E). RMSEC = Root Mean Square Error in calibration. RMSECV = Root Mean Square Error in cross validation. R2=correlation coefficient in calibration. R2= correlation coefficient in cross validation. RPD = Relative Percentage Difference.

The model for polymeric pigments prediction achieved R² values of 0.871 in calibration and 0.842 in cross-validation, using 27 LVs (Figure 6. A). For tannins, slightly lower R² values were obtained using 21 LVs (0.805 in calibration and 0.750 in cross-validation) (Figure 6. B). The model for anthocyanins prediction showed an R²c of 0.780, and R²cv 0.729, using 26 LVs (Figure 6. C) while for the TPI an R² equal to 0.724 in calibration and 0.666 in cross-validation, using 25 LVs were obtained (Figure 6. E). Differentially from what obtained for the commercial device, the lowest performance was observed for the colour intensity prediction, with notably lower R² values of 0.349 in calibration and 0.272 in cross-validation, using 29 LVs (Figure 6.D). The underperformance obtained with the prototype is also reflected in the RPD values (Table 2). RPD

above the quantification threshold were only obtained for anthocyanins (3.63) and polymeric pigments (2.53). The other models showed an RPD ranging between 2.06 and 1.24. One of the key steps in PLS modeling is determining the optimal number of latent variables (LVs) or dimensionalities. This decision is critical as it directly impacts the balance between model complexity and predictive accuracy. Selecting too many LVs can result in overfitting, where the model becomes overly tailored to the training data, compromising its ability to generalize to unseen data. In this study, the quantification of phenolic fraction using NIR spectroscopy dealt with complex datasets characterized by a large number of predictors and observations (Hawkins et al., 2004). The intricate spectral patterns associated with phenolic compounds, along with overlapping signals and subtle variations, necessitated models with a higher LVs (Cuadrado et al., 2005). Notably, the use of a higher number of LVs is not unprecedented. Studies addressing similar challenges in the field of wine analysis and phenolic quantification have also utilized models with a substantial number of LVs to achieve robust predictions (Miramont et al., 2020). Thus, while the use of 20 LVs may seem extensive, it is justified by the complexity of the data.

Overall the data obtained from the chemometric modelling highlight the ability of NIR spectroscopy to predict various phenol fractions even though some important limitation are evident. The RPD values obtained in the predictive models of NIR prototype acquisition were mostly over 1.4, except for the colour intensity, which presented an RPD of 1.24. This model also has a marked difference between the R^2_{cv} and R^2_{cal} , indicating its reliance on the specific data used for calibration and, consequently, its reduced predictive aptitude with unknown data. In contrast, the commercial instrument achieved an RPD of 2.32 and a much higher R^2_{cv} (0.78). However, the prediction of color intensity with NIR wavelength can be tricky, as demonstrated in previous studies (Fernández-Navales et al., 2011; Aleixandre-Tudo et al., 2018b). Hence, color intensity is generally analyzed by specific wavelengths of the visible spectrum (420, 520, and 620 nm), which are missing in the NIR spectra.

A higher RPD value (1.79) was obtained for the prediction of TPI using the prototype. The model presented an RMSEC of 11.57 and an RMSECV of 12.80, suggesting a reasonable absolute mean predicting error. In comparison, the commercial NIR device showed an RPD of 2.08 and lower RMSEC (10.42) and RMSECV (11.00) values indicating slightly higher accurate predictive capabilities. Variations between different NIR spectrometers can, of course, affect the accuracy of predictions. For instance, FT-NIR spectroscopy has been shown to accurately predict the TPI in red wine during fermentation and aging, however working with different configuration and with a much larger sample size (569 vs 144) (Fragoso et al., 2011). For NIR-based models, an adequate

sample size ensures that the model can generalize well to unseen data, minimizing overfitting and increasing prediction reliability. Small sample sizes may lead to models that perform well on the training data but fail to predict accurately on new, unseen samples. However, in our previously study, the VIS prototype reached much lower R^2 values for the prediction of TPI (R^2_{cal} of 0.603 and R^2_{cv} equal to 0.592, with an RPD of 1.64) with a similar sample size (Alfieri et al., 2023). Thus the result can still be considered acceptable.

Other studies investigated the use of FT-MIR for the prediction of TPI and reported higher predictive performance (R^2_{cv} higher than 0.95 and RPD exceeding 4.0). However, MIR technology utilized broader wavelength bands such as spectral regions between 979 to 2989 cm^{-1} , which lack in the NIR region (Fragoso et al., 2011). Spectral signatures in MIR range result from the fundamental stretching, bending, and rotating vibrations of the organic molecules, whereas NIR spectra are due to complex overtones and high-frequency combinations at shorter wavelengths. As a result, MIR spectral peaks are often sharper and better resolved compared to those in the NIR domain (Cozzolino et al., 2006; Cozzolino et al., 2011b; Gishen et al., 2005). Nevertheless, higher overtones referred to O-H, N-H, C-H, and S-H bands observed in the MIR region are also present in the NIR spectrum, even though they are much weaker than the fundamental frequencies in MIR. Additionally, combination bands, such as CO stretch and NH band in proteins, result in a crowded NIR spectrum with strongly overlapping bands (Cozzolino et al., 2010; Gishen et al., 2010). Thus, due to these complex interactions of absorption mechanisms and overlapping bands in the NIR region, establishing a direct correlation between the concentration of phenolic compounds and the size of these bands remains a challenge.

The model developed for total anthocyanins with spectral data obtained with the NIR prototype showed an RMSEC of 47.5 and an RMSECV of 51.20. These values are 32.1% and 34% higher than those obtained in the model developed for the commercial device. These errors are however acceptable considering the high anthocyanin content measured. In addition, the RPD indicates excellent performance, reaching a value of 3.63 for the prototype and 5.49 for the commercial device. A previous study by Martelo-Vidal et al. (2014) encountered significant challenges in utilizing UV/VIS/NIR spectra (190–2500 nm) to develop PLS models for monitoring anthocyanin content in red wines, achieving only an R^2_{cal} of 0.55 and an RPD of 1.29 (Martelo-Vidal et al., 2014). However, other authors achieved better performance, although using commercial instruments. and, again, larger samples size ($n=569$) (Aleixandre-Tudo et al., 2018b; Lambrecht et al., 2022).

Significant statistical results were also obtained in the PLS models developed for tannin content prediction. The RPD of the model of the NIR prototype was 2.06, whereas an RPD of 2.59 was obtained for the commercial FT-NIR. These results indicated the ability of both systems to predict tannin content during the fermentation process of red wines. The prototype system model showed an RMSEC of 345.59, while the RMSECV was 393.74; these values were reduced by 19.7% and 20.4%, respectively, in the model developed with the commercial NIR device. The model is based on a wide range of tannin concentrations. However, assuming that the model is applied during the final stages of maceration—such as the sample collected on the seventh day of fermentation in the Bobal variety vinified with stems—the prediction error would be notably reduced. Previous studies have also explored the non-destructive prediction of tannin content in wines using spectroscopic devices (Beaver et al., 2020; Fernandez et al., 2007).

A recent study reported a hyperspectral system to monitor tannin content using direct measurement of the wine grapes. This study reported similar results to those reported in our study. The hyperspectral system obtained an R^2_{cal} of 0.78 and an R^2_{cv} of 0.77, with calibration errors of 15.07% and prediction. However, most authors seem to use instruments operating in the UV-VIS range, as the maximum absorption values for various catechin families and procyanidins are around 280 nm (Aleixandre-Tudo et al., 2018a; Liu et al., 2024).

Interestingly, the most significant results were observed in the models that attempted to predict polymeric pigment content. The commercial NIR instrument showed an R^2_{cal} of 0.91, while the prototype instrument yielded an R^2_{cal} equal to 0.87. In prediction, these values slightly decreased. These slight decreases suggest a minimal model dependence on the specific samples used for calibration, and a significant predictive aptitude. The RPD values measured for both models are also acceptable (RPD of 2.53 for the prototype and RPD of 3.03 for the commercial device). The RMSEC value for the NIR prototype system was equal to 11.27, while the RMSECV increased to 12.46. In the case of the model obtained with the commercial instrument, the values were 9.48 for the RMSEC and 10.40 for the RMSECV. A recent study attempted to quantify polymeric pigment content in red wines using spectral data from commercial UV-VIS and FT-NIR. A comparison between the two techniques and a combination of them was reported. The R^2 range obtained was between 0.95 (R^2_{cal}) and 0.83 (R^2_{cv}), while the obtained RPD index was equal to 2.53 using 20 latent variables (LVs).

Lastly, the contribution of each variable (wavelength) is quantified using the VIP scores, and their significance is evaluated against an efficacy threshold of 1. In the predictive models developed with the commercial NIR instrument, the wavelengths that contribute most significantly to the

explained variability are associated (Figure S1) to spectral regions between 1350 nm-1450 nm, 1700 nm-1750 nm, 1880 nm-1950 nm, and 2080 nm-2150 nm

Similarly, figure S2 report the VIP scores for the prediction of the most relevant phenolic parameters developed using the prototype system. The VIPs strongly align with those obtained with the commercial instrument but with fewer wavelengths observed above the threshold of 1. The only variation is due to a slight forward shift of the spectral portions with greater significance, likely resulting from a slight delay in the acquisition system. The most important variable for the prediction of tannins with the prototype (Figure S7b) were found to be 1700-1750 nm and 1900-1950 nm. The relevant ranges for anthocyanins (Figure S7c) were 1350-1400 nm, 1700-1720 nm, and 2100-2150 nm. For polymeric pigments (Figure S7a), the range between 1900-2100 nm. Lastly, the range between 1350-1420 nm and 1700-1750 nm was significant for TPI prediction (Figure S7 d). Other authors have reported similar wavelengths as being of great interest for predicting total polyphenols and anthocyanins (Chen et al., 2015; Sen et al., 2016). In contrast, several authors confirm the strong influence of the spectral portion between 1060 nm and 1557 nm for the quantification of tannins (Jensen et al., 2008), with a prominent signal near 1300 nm attributed to the C-O stretching vibration associated with the pyran-derived ring structure of flavonoids derived from tannins (Edelmann et al., 2002). Thus demonstrating that phenolic compounds exhibit consistent bonding pattern. Specifically, C-H bonds show stretching at the third (928 and 940 nm), second (1148 nm), and first overtones (1620 and 1652 nm). The wavelength range between 1100 and 1300 nm corresponds to a combination band involving symmetric and antisymmetric O-H stretching vibrations, as well as a combination band linked to the second overtone of C-H aromatic vibrations and the third overtone of C-H vibrations. These characteristics can be attributed to the chemical structure of phenolic compounds.

Several studies have indicated that NIR spectroscopy is poised to become a routine method in the modern grape and wine industry (Shahrajabian et al., 2024; Bai et al., 2023). However, various challenges are impeding its widespread adoption, preventing winemakers from fully benefiting from its potential advantages. The primary barrier is the lack of understanding of the technology, which is often compounded by a shortage of trained personnel. Furthermore, the expertise needed to interpret the data generated by these devices is frequently absent.

Conclusions

The regression models obtained using a commercial FT-NIR spectrometer have demonstrated a solid ability to predict the total polyphenol index, tannins, anthocyanins, and polymeric pigments in fermenting samples. The results indicate that this technology can effectively reduce the costs associated with traditional wet chemistry methods while offering a lower environmental impact. In contrast, the low-cost prototype NIR system showed slightly lower model performance. However, some results remain promising, specifically for the prediction of tannin, anthocyanin and polymeric pigment. Overall, even though the results are promising, overoptimistic outcomes are frequently reported, and the prediction accuracy of models should be approached with caution, as in the case of early stage prototypes. This is even more important when working with a limited number of samples, or rely on specific experimental winemaking conditions. Thus additional tests are necessary to enhance the interpretation of the results and increase the prediction reliability of the prototype. Options to improve the prototype's sensitivity include amplifying the electrical signal measured by the sensor, stabilizing the light source, and calibrating the instrument against a commercial product. Incorporating a segment of the visible spectrum into the prototype NIR instrument could significantly enhance its performance, especially for measuring color intensity and the total polyphenol index. This enhancement would increase the practicality of using the prototype directly in winemaking applications and represent a valuable direction for future research.

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References

1. Cavaglia, J., Schorn-García, D., Giussani, B., Ferré, J., Busto, O., Aceña, L., ... & Brizzolara, S. (2020). ATR-MIR spectroscopy and multivariate analysis in alcoholic fermentation monitoring and lactic acid bacteria spoilage detection. *Food Control*, 109, 1–7. <https://doi.org/10.1016/j.foodcont.2019.106877>
2. Littarru, E., Modesti, M., Alfieri, G., Pettinelli, S., Floridia, G., Bellincontro, A., ... & Brizzolara, S. (2024). Optimizing the winemaking process: NIR spectroscopy and e-nose

- analysis for the online monitoring of fermentation. *Journal of the Science of Food and Agriculture*. <https://doi.org/10.1002/jsfa.12009>
3. dos Santos Costa, D., Mesa, N. F. O., Freire, M. S., Ramos, R. P., & Mederos, B. J. T. (2019). Development of predictive models for quality and maturation stage attributes of wine grapes using vis-NIR reflectance spectroscopy. *Postharvest Biology and Technology*, 150, 166–178. <https://doi.org/10.1016/j.postharvbio.2019.01.018>
4. Croce, R., Malegori, C., Oliveri, P., Medici, I., Cavaglioni, A., & Rossi, C. (2020). Prediction of quality parameters in straw wine by means of FT-IR spectroscopy combined with multivariate data processing. *Food Chemistry*, 305, 125512. <https://doi.org/10.1016/j.foodchem.2019.125512>
5. Anjos, O., Caldeira, I., Fernandes, T. A., Pedro, S. I., Vitória, C., Oliveira-Alves, S., ... & Canas, S. (2021). PLS-R calibration models for wine spirit volatile phenols prediction by near-infrared spectroscopy. *Sensors*, 22(1), 286. <https://doi.org/10.3390/s22010286>
6. Arvanitoyannis, I. S., Ladas, D., & Mavromatis, A. (2006). Wine waste treatment methodology. *International Journal of Food Science & Technology*, 41(10), 1117–1151. <https://doi.org/10.1111/j.1365-2621.2006.01235.x>
7. Weingerl, V., Strlič, M., & Kočar, D. (2009). Comparison of methods for determination of polyphenols in wine by HPLC-UV/VIS, LC/MS/MS, and spectrophotometry. *Acta Chimica Slovenica*, 56(3), 798–802.
8. Giglio, C., Yang, Y., & Kilmartin, P. (2023). Analysis of phenolics in New Zealand Pinot noir wines using UV-visible spectroscopy and chemometrics. *Journal of Food Composition and Analysis*, 117, 105106. <https://doi.org/10.1016/j.jfca.2023.105106>
9. Gu, H. W., Zhou, H. H., Lv, Y., Wu, Q., Pan, Y., Peng, Z. X., ... & Yin, X. L. (2023). Geographical origin identification of Chinese red wines using ultraviolet-visible spectroscopy coupled with machine learning techniques. *Journal of Food Composition and Analysis*, 119, 105265. <https://doi.org/10.1016/j.jfca.2023.105265>
10. Shahrajabian, M. H., & Sun, W. (2024). Assessment of wine quality, traceability, and detection of harmful substances in alcohol: Composition analysis. *Letters in Drug Design & Discovery*, 21(8), 1377–1399. <https://doi.org/10.2174/1570180819666230217152831>
11. Bai, X., Xu, Y., Chen, X., Dai, B., Tao, Y., & Xiong, X. (2023). Analysis of near-infrared spectral properties and quantitative detection of rose oxide in wine. *Agronomy*, 13(4), 1123. <https://doi.org/10.3390/agronomy13041123>
12. da Costa Fulgêncio, A. C., Resende, G. A. P., Teixeira, M. C. F., Botelho, B. G., & Sena, M. M. (2023). Combining portable NIR spectroscopy and multivariate calibration for the

- determination of ethanol in fermented alcoholic beverages by a multi-product model. *Talanta Open*, 7, 100180. <https://doi.org/10.1016/j.talo.2023.100180>
13. Dong, R., Sun, J., Cui, P., Shi, J., Li, Y., & Ma, X. (2023). Quantitative and classification analysis of red wine by infrared spectra and gas chromatography-mass spectrometry data coupling with a new variable selection method. *Journal of Food Composition and Analysis*, 120, 105325. <https://doi.org/10.1016/j.jfca.2023.105325>
14. Ta, N., Wei, H., Hu, Z., Cao, X., & Xiao, M. (2023). Wine component tracing method based on near infrared spectrum fusion machine learning. *Frontiers in Sustainable Food Systems*, 7, 1197508. <https://doi.org/10.3389/fsufs.2023.1197508>
15. Cornehl, L., Krause, J., Zheng, X., Gauweiler, P., Schwander, F., Töpfer, R., ... & Kicherer, A. (2023). Determination of sugars and acids in grape must using miniaturised near-infrared spectroscopy. *Sensors*, 23(11), 5287. <https://doi.org/10.3390/s23115287>
16. Lu, B., Tian, F., Chen, C., Wu, W., Tian, X., Chen, C., & Lv, X. (2023). Identification of Chinese red wine origins based on Raman spectroscopy and deep learning. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 291, 122355. <https://doi.org/10.1016/j.saa.2022.122355>
17. Singla, M., Gill, K. S., Chauhan, R., Pokhariya, H. S., & Chanti, Y. (2024, February). Analysing red wine quality data exploratorily using support vector machines and other machine learning methods. In *2024 2nd International Conference on Computer, Communication and Control (IC4)* (pp. 1–6). IEEE. <https://doi.org/10.1109/IC4.2024.00010>
18. Feifel, S., Weilack, I., Markusevics, E., Zimmermann, D., Wegmann-Herr, P., Weber, F., ... & Durner, D. (2024). Influence of potential alcohol in grapes on phenolic and sensory characteristics of red wine. *Journal of Agricultural and Food Chemistry*. <https://doi.org/10.1021/acs.jafc.4c01162>
19. He, S., & Sun, A. (2024). Non-contact measurement of alcohol content based on near infrared spectroscopy. *International Journal of Computer Science and Information Technology*, 2(1), 189–197. <https://doi.org/10.11648/j.ijcsit.2024.02.01.19>
20. Miao, X., Miao, Y., Liu, Y., Tao, S., Zheng, H., Wang, J., ... & Tang, Q. (2023). Measurement of nitrogen content in rice plant using near-infrared spectroscopy combined with different PLS algorithms. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 284, 121733. <https://doi.org/10.1016/j.saa.2023.121733>
21. Ozdemir, E., Alcaay, Y., Yavuz, O., Yildirim, M. S., Aribuga, H., Ertugral, U., ... & Yilmaz, I. (2023). Colorimetric and near-infrared spectrophotometric monitoring of bisulfite using

- glyoxal modified chromenylium-cyanine chemosensor: Smartphone and paper strip applications for on-site food and beverages control. *Talanta*, 261, 124660. <https://doi.org/10.1016/j.talanta.2023.124660>
22. Regmi, U., Palma, M., & Barroso, C. G. (2012). Direct determination of organic acids in wine and wine-derived products by Fourier transform infrared (FT-IR) spectroscopy and chemometric techniques. *Analytica Chimica Acta*, 732, 137–144. <https://doi.org/10.1016/j.aca.2012.03.039>
23. Cozzolino, D., Kwiatkowski, M. J., Parker, M., Cynkar, W. U., Damberg, R. G., Gishen, M., & Herderich, M. J. (2004). Prediction of phenolic compounds in red wine fermentations by visible and near-infrared spectroscopy. *Analytica Chimica Acta*, 513(1), 73–80. <https://doi.org/10.1016/j.aca.2004.05.017>
24. Baca-Bocanegra, B., Nogales-Bueno, J., Hernández-Hierro, J. M., & Heredia, F. J. (2018). Evaluation of extractable polyphenols released to wine from cooperage byproduct by near-infrared hyperspectral imaging. *Food Chemistry*, 244, 206–212. <https://doi.org/10.1016/j.foodchem.2017.10.019>
25. Ferrari, E., Foca, G., Vignali, M., Tassi, L., & Ulrici, A. (2011). Adulteration of the anthocyanin content of red wines: Perspectives for authentication by Fourier Transform-Near Infrared and ¹H NMR spectroscopies. *Analytica Chimica Acta*, 701(2), 139–151. <https://doi.org/10.1016/j.aca.2011.05.009>
26. Pampuri, A., Tugnolo, A., Giovenzana, V., Casson, A., Pozzoli, C., Brancadoro, L., & Beghi, R. (2022). Application of a cost-effective visible/near-infrared optical prototype for measuring qualitative parameters of Chardonnay grapes. *Applied Sciences*, 12(10), 4853. <https://doi.org/10.3390/app12104853>
27. Iaccheri, E., Ricci, A., Parpinello, G. P., Versari, A., & Ragni, L. (2021, November). Portable photometer for procyanidins quantitation in red wine. In 2021 IEEE International Workshop on Metrology for Agriculture and Forestry (MetroAgriFor) (pp. 253–257). IEEE. <https://doi.org/10.1109/MetroAgriFor52389.2021.9628707>
28. Taglieri, I., Mencarelli, F., Bellincontro, A., Modesti, M., Cerreta, R., Zinnai, A., & Gagliardi, M. (2022, August). Development of a micro-Vis-NIR and SAW nano biosensor to measure polyphenols in must/wine on time and online. In XXXI International Horticultural Congress (IHC2022): International Symposium on the Vitivinicultural Sector (pp. 39–46).
29. Alfieri, G., Modesti, M., Bellincontro, A., Renzi, F., & Aleixandre-Tudo, J. L. (2024). Feasibility assessment of a low-cost visible spectroscopy-based prototype for monitoring

- polyphenol extraction in fermenting musts. *Journal of the Science of Food and Agriculture*.
<https://doi.org/10.1002/jsfa.12234>
30. Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2006). *Handbook of enology*, Vol. 1: The microbiology of wine and vinifications. Wiley.
31. Glories, Y. (2016). La couleur des vins rouges. 1re partie: Les équilibres des anthocyanes et des tanins. *OENO One*, 18, 195. <https://doi.org/10.20870/oeno-one.2016.18.4.151>
32. Sarneckis, C. J., Damberg, R. G., Jones, P., Mercurio, M., Herderich, M. J., & Smith, P. A. (2006). Quantification of condensed tannins by precipitation with methylcellulose: Development and validation of an optimized tool for grape and wine analysis. *Australian Journal of Grape and Wine Research*, 12, 39–49. <https://doi.org/10.1111/j.1755-0238.2006.tb00046.x>
33. Mercurio, M. D., Damberg, R. G., Herderich, M. J., & Smith, P. A. (2007). High-throughput analysis of red wine and grape phenolics: Adaptation and validation of methylcellulose precipitable tannin assay and modified Somers color assay to a rapid 96-well plate format. *Journal of Agricultural and Food Chemistry*, 55(12), 4651–4657. <https://doi.org/10.1021/jf070140j>
34. Alexandre-Tudo, J. L., & du Toit, W. (2018a). The role of UV-visible spectroscopy for phenolic compounds quantification in winemaking. *Frontiers and New Trends in the Science of Fermented Food and Beverages*, 200–204.
35. Pascual, O., González-Royo, E., Gil, M., Gómez-Alonso, S., García-Romero, E., Canals, J. M., & Zamora, F. (2016). Influence of grape seeds and stems on wine composition and astringency. *Journal of Agricultural and Food Chemistry*, 64(34), 6555–6566. <https://doi.org/10.1021/acs.jafc.6b02286>
36. Suriano, S., Alba, V., Tarricone, L., & Di Gennaro, D. (2015). Maceration with stem contact fermentation: Effect on proanthocyanidins compounds and color in Primitivo red wines. *Food Chemistry*, 177, 382–389. <https://doi.org/10.1016/j.foodchem.2015.01.048>
37. Pérez-Magariño, S., & González-San José, M. L. (2006). Polyphenols and color variability of red wines made from grapes harvested at different ripeness grades. *Food Chemistry*, 96(2), 197–208. <https://doi.org/10.1016/j.foodchem.2005.02.025>
38. Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry*, 53(10), 4019–4025. <https://doi.org/10.1021/jf047801k>

39. Barton, F. E., Himmelsbach, D. S., & Archibald, D. D. (1996). Two-dimensional vibration spectroscopy. V: Correlation of mid-and near-infrared of hard red winter and spring wheats. *Journal of Near Infrared Spectroscopy*, 4(1), 139–152. <https://doi.org/10.1255/jnirs.78>
40. Miramont, P. L., et al. (2020). Development of UV-vis and FTIR partial least squares models: Comparison and combination of two spectroscopy techniques with chemometrics for polyphenols quantification in red wine. *OENO One*, 54(4), 779–792. <https://doi.org/10.20870/oeno-one.2020.54.4.3497>
41. Cozzolino, D., Cynkar, W. U., Shah, N., & Smith, P. (2011a). Multivariate data analysis applied to spectroscopy: Potential application to juice and fruit quality. *Food Research International*, 44(7), 1888–1896. <https://doi.org/10.1016/j.foodres.2010.10.013>
42. Ferrer-Gallego, R., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2011). Determination of phenolic compounds of grape skins during ripening by NIR spectroscopy. *LWT-Food Science and Technology*, 44(4), 847–853. <https://doi.org/10.1016/j.lwt.2010.12.013>
43. Martelo-Vidal, M. J., & Vázquez, M. (2014). Determination of polyphenolic compounds of red wines by UV-VIS-NIR spectroscopy and chemometrics tools. *Food Chemistry*, 158, 28–34. <https://doi.org/10.1016/j.foodchem.2014.02.099>
44. Aleixandre-Tudo, J. L., Nieuwoudt, H., Olivieri, A., Aleixandre, J. L., & du Toit, W. (2018c). Phenolic profiling of grapes, fermenting samples, and wines using UV-visible spectroscopy with chemometrics. *Food Control*, 85, 11–22. <https://doi.org/10.1016/j.foodcont.2017.09.003>
45. Hawkins, D. M. (2004). The problem of overfitting. *Journal of Chemical Information and Computer Sciences*, 44(1), 1–12. <https://doi.org/10.1021/ci0342472>
46. Cuadrado, M. U., De Castro, M. L., Juan, P. P., & Gómez-Nieto, M. A. (2005). Comparison and joint use of near-infrared spectroscopy and Fourier transform mid-infrared spectroscopy for the determination of wine parameters. *Talanta*, 66(1), 218–224. <https://doi.org/10.1016/j.talanta.2004.11.013>
47. Fernández-Novales, J., Sánchez, M. T., López, M. I., García-Mesa, J. A., & Ramírez, P. (2011). Feasibility of using a miniature fibre optic UV-VIS-NIR spectrometer to assess total polyphenol index, color intensity, and volumic mass in red wine fermentations. *Journal of Food Process Engineering*, 34(4), 1028–1045. <https://doi.org/10.1111/j.1745-4530.2009.00521.x>

48. Aleixandre-Tudo, J. L., Nieuwoudt, H., Aleixandre, J. L., & du Toit, W. (2018b). Chemometric compositional analysis of phenolic compounds in fermenting samples and wines using different infrared spectroscopy techniques. *Talanta*, 176, 526–536. <https://doi.org/10.1016/j.talanta.2017.08.079>
49. Fragoso, S., Aceña, L., Guasch, J., Mestres, M., & Busto, O. (2011). Quantification of phenolic compounds during red winemaking using FT-MIR spectroscopy and PLS-regression. *Journal of Agricultural and Food Chemistry*, 59(20), 10795–10802. <https://doi.org/10.1021/jf201718u>
50. Cozzolino, D., Cynkar, W., Janik, L., Damberg, R. G., & Gishen, M. (2006). Analysis of grape and wine by near-infrared spectroscopy—A review. *Journal of Near Infrared Spectroscopy*, 14, 279–289. <https://doi.org/10.1255/jnirs.706>
51. Cozzolino, D., Shah, N., Cynkar, W. U., & Smith, P. (2011b). Technical solutions for analysis of grape juice, must, and wine: The role of infrared spectroscopy and chemometrics. *Analytical and Bioanalytical Chemistry*, 401(5), 1479–1488. <https://doi.org/10.1007/s00216-011-5127-3>
52. Gishen, M., Damberg, R. G., & Cozzolino, D. (2005). Grape and wine analysis in the Australian wine industry—Enhancing the power of spectroscopy with chemometrics. *Australian Journal of Grape and Wine Research*, 11(3), 296–305. <https://doi.org/10.1111/j.1755-0238.2005.tb00029.x>
53. Cozzolino, D., & Damberg, R. G. (2010). Instrumental analysis of grape, must, and wine. In A. G. Reynolds (Ed.), *Managing Wine Quality: Volume One, Viticulture and Wine Quality* (pp. 415–441). Woodhead Publishing Ltd.
54. Gishen, M., Cozzolino, D., & Damberg, R. G. (2010). The analysis of grapes, wine, and other alcoholic beverages by infrared spectroscopy. In *Handbook of Vibrational Spectroscopy* (pp. 415–441).
55. Lambrecht, K., Nieuwoudt, H., du Toit, W., & Aleixandre-Tudo, J. L. (2022). Moving towards in-line monitoring of phenolic extraction during red wine fermentations using infrared spectroscopy technology: Influence of sample preparation and instrumentation. *Journal of Food Composition and Analysis*, 110, 104542. <https://doi.org/10.1016/j.jfca.2022.104542>
56. Beaver, C., Collins, T. S., & Harbertson, J. (2020). Model optimisation for the prediction of red wine phenolic compounds using ultraviolet–visible spectra. *Molecules*, 25(7), 1576. <https://doi.org/10.3390/molecules25071576>

- 2947 57. Fernández, K., & Agosin, E. (2007). Quantitative analysis of red wine tannins using
2948 Fourier-transform mid-infrared spectrometry. *Journal of Agricultural and Food Chemistry*,
2949 55(18), 7294–7300. <https://doi.org/10.1021/jf071463q>
- 2950 58. Liu, C., Gao, S., Ma, J., Lu, Y., Prejanòd, M., & Li, Y. (2024). Real-time monitoring of
2951 chromatic and phenolic dynamics of vinification employing UV-Vis spectroscopy, Python,
2952 and chemometrics. *Journal of Food Composition and Analysis*, 106359.
2953 <https://doi.org/10.1016/j.jfca.2023.106359>
- 2954 59. Chen, S., Zhang, F., Ning, J., Liu, X., Zhang, Z., & Yang, S. (2015). Predicting the
2955 anthocyanin content of wine grapes by NIR hyperspectral imaging. *Food Chemistry*, 172,
2956 788–793. <https://doi.org/10.1016/j.foodchem.2014.09.141>
- 2957 60. Sen, I., Ozturk, B., Tokatli, F., & Ozen, B. (2016). Combination of visible and mid-infrared
2958 spectra for the prediction of chemical parameters of wines. *Talanta*, 161, 130–137.
2959 <https://doi.org/10.1016/j.talanta.2016.08.017>
- 2960 61. Jensen, J. S., Egebo, M., & Meyer, A. S. (2008). Identification of spectral regions for the
2961 quantification of red wine tannins with Fourier-transform mid-infrared spectroscopy.
2962 *Journal of Agricultural and Food Chemistry*, 56(10), 3493–3499.
2963 <https://doi.org/10.1021/jf703573f>
- 2964 62. Edelmann, A., & Lendl, B. (2002). Toward the optical tongue: Flow-through sensing of
2965 tannin-protein interactions based on FTIR spectroscopy. *Journal of the American Chemical*
2966 *Society*, 124(49), 14741–14747. <https://doi.org/10.1021/ja026309v>

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Supplementary materials

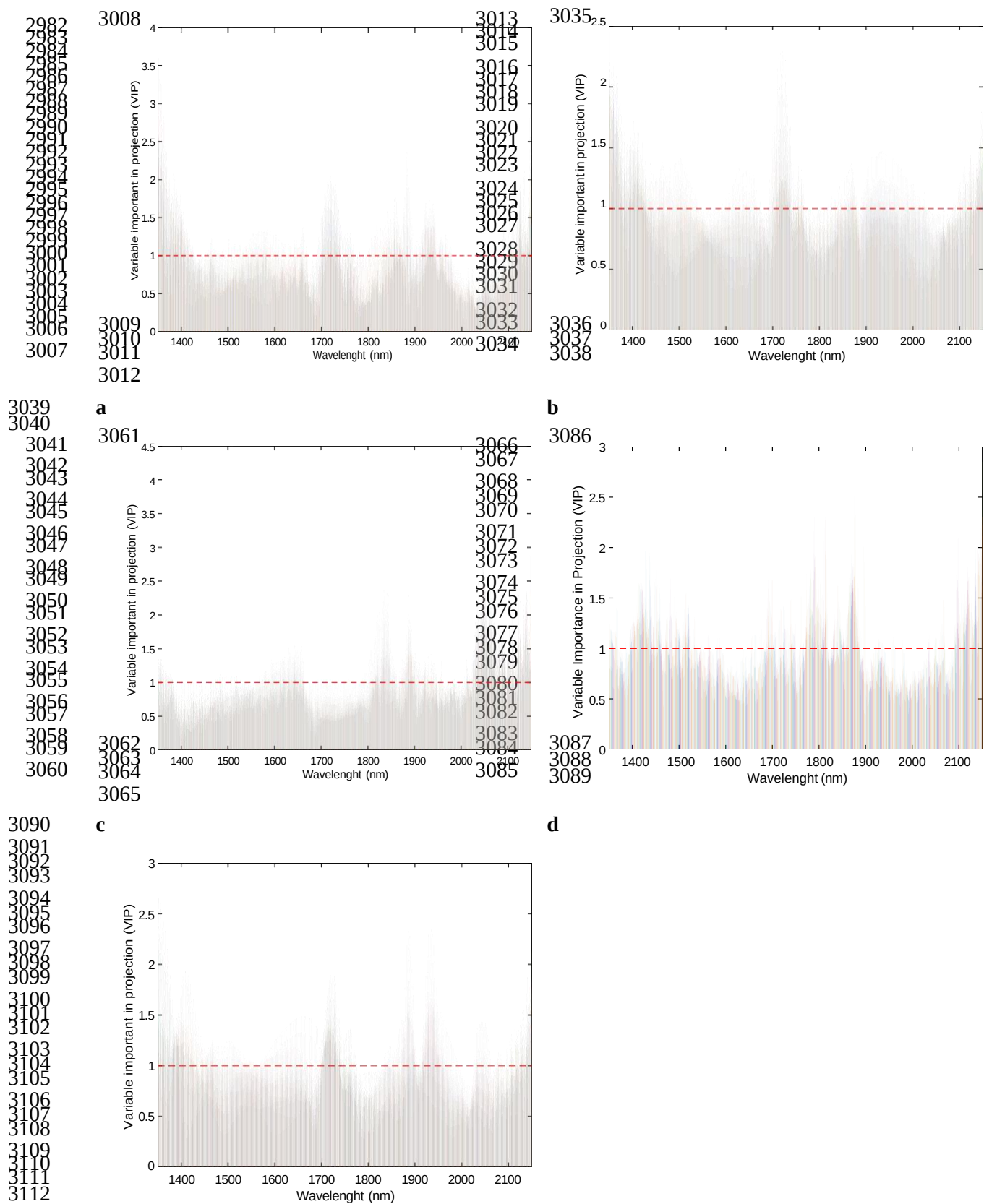
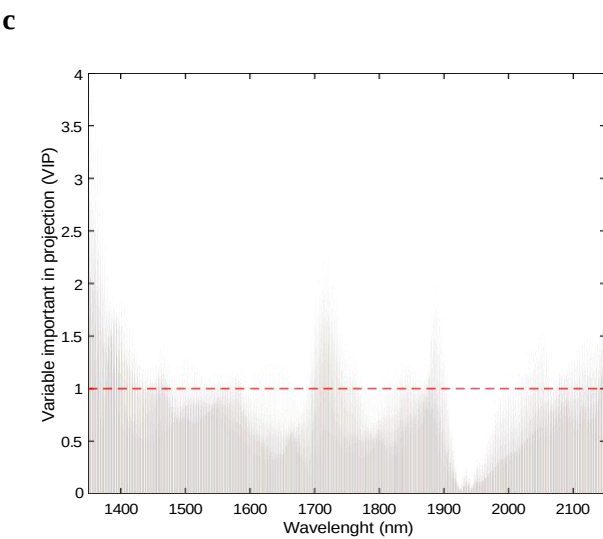
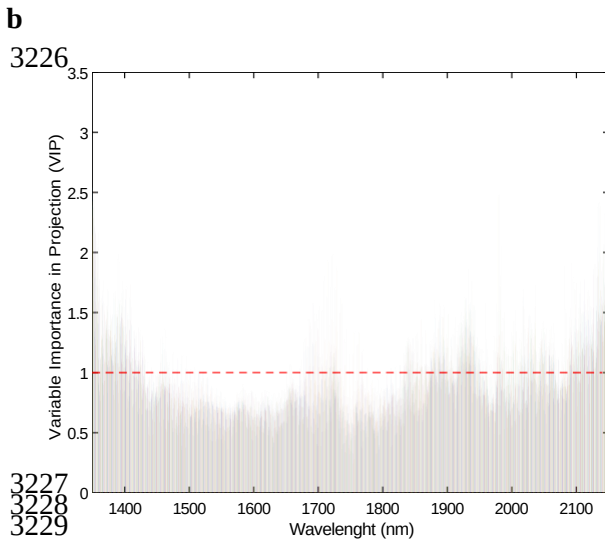
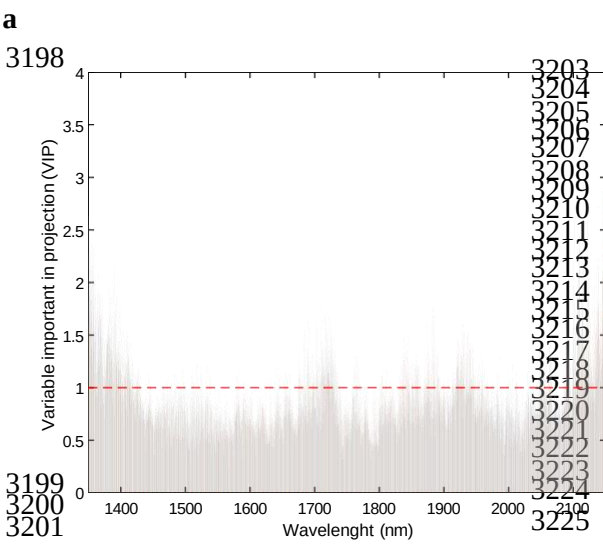
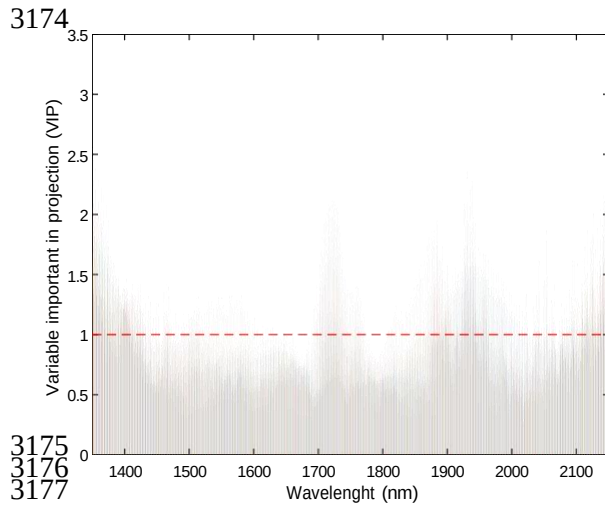
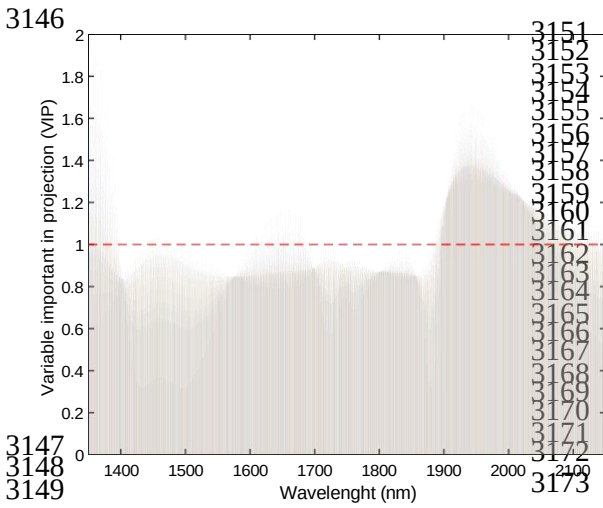


Figure S1. Variable Importance in Projection (VIP) scores quantifying the contribution of each variable (wavelength) to the recognition performance of different qualitative parameters are presented. These scores were obtained using Partial Least Squares (PLS) regression applied to commercial NIR sample acquisitions. The VIP analysis is conducted for different qualitative parameters, including polymer pigments (A), tannins (B), anthocyanins (C), color intensity (D),

and total polyphenols index (E). Each VIP score is evaluated against an efficacy threshold of 1 to determine its significance in the model.



e

Figure S2. Variable Importance in Projection (VIP) scores quantifying the contribution of each variable (wavelength) to the recognition performance of different qualitative parameters are presented. These scores were obtained using Partial Least Squares (PLS) regression applied to prototype NIR sample acquisitions. The VIP analysis is conducted for different qualitative parameters, including polymer pigments (A), tannins (B), anthocyanins (C), color intensity (D), and total polyphenols index (E). Each VIP score is evaluated against an efficacy threshold of 1 to determine its significance in the model.

4. Chapter IV - Exploring Gelatin-A and Murine Salivary Protein 5 as Probes for Wine Polyphenols analysis by Quartz Crystal Microbalance with Dissipation Monitoring Experiments

This chapter is based on the manuscript entitled "*Exploring Gelatin-A and Murine Salivary Protein 5 as Probes for Wine Polyphenols Analysis by Quartz Crystal Microbalance with Dissipation Monitoring Experiments*", submitted after revision to *Advanced Sensor Research* and first reviewed in November 2024. The article explores the use of a Lab-on-Chip prototype based on Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D), functionalized for the detection of polyphenols in red wine, employing both Type A Gelatin (Gel-A) and Murine Proline-Rich Protein 5 (MP5) as sensitive materials.

Compared to the version of the manuscript right now under editorial process after first review, some introductory paragraphs have been modified to better connect this study with the previous works described.

The approach followed in this study is complementary to those presented in Chapter II and Chapter III, as it investigates a novel methodology for analyzing polyphenols in wines and musts. This technology demonstrates distinct sensitivity characteristics toward specific phenolic families, enabling a more selective discrimination compared to the technologies explored previously in the thesis, and significantly shortening the measurement time for individual phenolic families.

Abstract

Background

Polyphenols constitute a substantial group of compounds that play a pivotal role in the winemaking process by influencing wine quality, color, astringency, bitterness, and chemical stability. Traditionally, the polyphenolic content of wine is assessed through conventional analytical techniques, which are costly and time-consuming. Consequently, the development of novel strategies which overcome these limitations would be highly desirable.

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) is a gravimetric sensor, which is now broadly acknowledged as a fast, reliable, and label-free tool. QCM-D has consistently shown remarkable performance in both biomedical and agri-food fields.

Results

In this study, QCM-D is employed to investigate Gelatin Type A (Gel-A), derived from porcine skin, and Mouse Proline-Rich Protein 5 (MP5) in the analysis of polyphenols in red wines. The results demonstrate the ability of Gel-A and MP5 to detect polyphenols within the complex matrix of red wines. Moreover, using MP5, a linear response of the dissipation signal is observed with both the total polyphenol and hydroxybenzoic acid concentrations. Conversely, the frequency response of Gel-A is correlated with the entire polyphenolic profile of wines, indicating that it is influenced by the combined effects of different polyphenolic subfamilies within the wine matrix.

Discussion

The use of QCM-D for polyphenol analysis in red wine demonstrated high sensitivity and specificity in detecting and quantifying polyphenols through two different sensing surfaces: Gel-A and MP5. MP5 exhibited a linear response to total polyphenol concentration and hydroxybenzoic acids, suggesting potential application for rapid quantitative analysis. On the other

hand, Gel-A proved to be more sensitive to the overall polyphenolic profile, suggesting a more complex and nonlinear interaction with various polyphenolic components present in the wine matrix. The ability to distinguish and correlate specific polyphenols with QCM-D signals highlights the potential of this technique as an alternative tool to conventional analytical methods. Furthermore, the identification of key polyphenols such as Malvidin 3-O-glucoside, Quercetin 3-O-glucoside, Procyanidin B3, t-Resveratrol, Gallic Acid, and Caffeic Acid in determining Gel-A behavior provides further insights for optimizing QCM-D applications in wine characterization.

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Introduction

Gravimetric sensors based on acoustic waves are now widely recognized as a robust approach for detecting extremely small masses, while also enabling the assessment of other physical parameters such as film thickness, viscosity, and elasticity (Voinova et al., 2009). These devices have already demonstrated excellent performance in various research field such as the detection of clinically relevant proteins (Agostini et al., 2021), viruses (Agostini et al., 2021), and bacteria (Gagliardi et al., 2023; Kimet al., 2012).

Among gravimetric sensors, the *Quartz Crystal Microbalance with Dissipation Monitoring* (QCM-D) is widely acknowledged as the primary piezoelectric detection method. In this

apparatus, the transducer element is typically based on an AT-cut quartz crystal sandwiched between two circular electrodes (Fogel et al., 2016). When an alternating current (AC) voltage is applied across the electrodes, the crystal undergoes an oscillatory deformation in a thickness shear mode. Maximum displacement amplitudes occur at a specific acoustic frequency (and associated overtones), called resonance frequency (Fogel et al., 2016). When mass adsorption or desorption events occurs, the resonance frequency changes. Therefore, monitoring the variations of the resonance frequency enables for the collection of information on surface-level phenomena (Fogel et al., 2016). The coupling of the aforementioned properties with surface functionalization of the quartz crystal surface enables the use of the QCM-D as a sensoristic technology.

Polyphenols are secondary metabolites synthesized by all higher plants, playing a crucial role in responding to both biotic factors, such as plant pathogens and herbivore aggressions, and abiotic stressors, including UV-rays (Daglia et al., 2012).

Polyphenols, present in the solid parts of grapes (such as skin, pulp and seeds), play also a crucial role in winemaking as they significantly influence the quality of wine. While pulp is the primary source of polyphenols for white and rosé wines, red wines derive them from all parts of the grape (including also skin and seeds) (Gutiérrez-Escobar et al., 2021). Polyphenols also affect the organoleptic properties of wine, with specific subclasses contributing to distinct features. For instance, anthocyanins, along with hydroxybenzoic and hydroxycinnamic acids, are responsible for the color of wine. Additionally, condensed tannins contribute to the astringency and bitterness of wine (Niculescu et al., 2018).

Currently, the gold standard techniques to evaluate the polyphenolic content of wine include spectrophotometric and chromatographic methods. However, these methods are time-consuming and expensive (Lorrain et al., 2013), leading the wine industry to explore alternative approaches to overcome these limitations.

Several examples of electrochemical (Gomes et al., 2003; Capannesi et al., 2000; Gomes et al., 2004; Di Fusco et al., 2010; Cetò et al., 2012; Gamella et al., 2006), optical (Jia et al., 2021; Abdullah et al., 2007) and acoustic biosensors for polyphenols are reported in literature. Considerable evidence supports the effectiveness of acoustic sensors in monitoring polyphenols in oenology. Among the acoustic biosensors, the QCM-D was reported as an efficient device for wine analysis for different purposes. In the realm of wine safety, the QCM-D enabled the detection of mycotoxins in wine, such as Ochratoxin A, achieving a Limit of Detection (LOD) of 0.16 ng mL⁻¹ (Karczmarczyk et al., 2017). Moreover, the QCM-D was used to identify *Brettanomyces*

bruxellensis genome in red wines. *B. bruxellensis* produces health-harming chemical compound and compromises the quality of wine (Poggesi et al., 2021).

Furthermore, the QCM-D was applied to better clarify the astringency mechanism in different beverages, such as wine, beer, and tea, demonstrating that the interaction between polyphenols, particularly tannins, and salivary proteins is involved in this sensory perception (Yan et al., 2009; Kaneda et al., 2004; Wang et al., 2021). Other studies (Zhao et al., 2023) provided novel insights about the role of both tartaric acid and pH in the astringency mechanism by QCM-D experiments. The study revealed that within the usual pH ranges (2.3 ~ 4.3) and tartaric acid concentrations (0.0 ~ 7.0 g L⁻¹) found in wine, the astringency effect is more pronounced at low pH confirming this condition mainly impact the astringency (Zhao et al., 2023).

Additionally, QCM-D was used to investigate the interaction between specific polyphenols, including (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), (-)-epigallocatechin gallate (EGCg) and theaflavin-3,3'-digallate, with alpha casein, bovine serum albumin (BSA) and human serum albumin (HAS) (Weerawatanakorn et al., 2014; Ali et al., 2019; Minoda et al., 2010; Kamihira et al., 2008).

Besides, QCM-D provided important insight into the interaction between polyphenols and lipid bilayers. The presence of galloyl units in polyphenols appear to enhance this interaction, as demonstrated by the higher the affinity of (-)-epicatechin gallate and (-)-epigallocatechin gallate to 1,2-dimyristoyl-sn-glycero-3-phospho-choline compared to (-)-epicatechin and (-)-epigallocatechin (Cosme et al., 2007).

This work explored Gelatin type A from porcine skin (Gel-A) and of a peptide fragment of Mouse Proline-Rich Protein 5 (MP5) for detecting polyphenols in authentic red wines. Gel-A is commonly used as a wine-finishing agent (Gagliardi et al., 2022) and has been consistently recognized as a reliable probe for polyphenol analysis (Gagliardi et al., 2022; Gagliardi et al., 2024). The selected MP5 fragment represents a single proline-rich repeat of the entire protein, known for its interaction with polyphenols containing galloyl units (Baxter et al., 1997). As previously demonstrated (Murray et al., 1994), this interaction is primarily driven by two forces: the hydrophobic interaction between the pyrrolidine ring of proline and the galloyl ring of polyphenols, and hydrogen bonding, which enhances the stabilization of the complex.

We previously tested these probes in the analysis of different tannins families in aqueous solutions and in artificial wines, demonstrating their effectiveness (Gagliardi et al., 2022). The novelty of this study was the use of Gel-A and MP5 for polyphenol detection in complete red wines using

3431 two optimized functionalization protocols that we recently developed (Wang et al., 2021). This
3432 posed a significant challenge given the highly complex matrix of red wines, predominantly
3433 composed of water, ethanol, alongside glycerol, polysaccharides, organic acids, polyphenols such
3434 as anthocyanin and tannins, minerals, volatile compounds (Nemzer et al., 2022).

Material and Methods

Reagents

Water, hydrogen peroxide solution (30% w/w), ammonium hydroxide solution (28-30% v/v), sodium dodecyl sulfate (20 % in water), phosphate buffer saline (140 mM NaCl, 10 mM phosphate buffer, and 3 mM KCl, pH 7.4 at 25 °C), sodium carbonate (purity degree $\geq 99.5\%$) and sodium bicarbonate (purity degree $\geq 99.5\%$) were obtained from Sigma Aldrich (St. Louis, MO, USA) and ethanol absolute anhydrous and isopropanol were purchased from Carlo Erba Reagents (Milan, Italy).

The functionalization based on the protein involved the use of the following reagents: 12-mercaptododecanoic acid (12-MDA, purity degree 96%, M_w 232.4 Da, Sigma Aldrich, St. Louis, MO, USA) was used for the formation of the adlayer; 1,4-Dithiothreitol (DTT, M_w 154.2 Da, Sigma Aldrich) for the reduction of 12-MDA molecules; N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI) and N-hydroxysuccinimide (NHS) for the activation of 12-MDA carboxylic groups (Sigma Aldrich, purity degree $\geq 98\%$); Gelatin type A from porcine skin (Gel-A, High bloom, M_w 75.0 kDa, Sigma Aldrich); ethylene diamine (EDA, Sigma Aldrich) was employed for the blocking of residual active ester after probe immobilization.

The functionalization based on the peptide required: a fragment of Mouse Proline-Rich Protein 5 (MP5, GPQQRPPQPGNQQGPPPQGPPQC, M_w 2350 Da) with an additional cysteine at the C-terminal end (synthesized by Biomatik Kitchener, Ontario, Canada, purity degree 95%); 1,4-Dithiothreitol (DTT, M_w 154.2 Da, Sigma Aldrich) for the reduction of MP5 molecules.

The wine samples analyzed included: Sensi Lungarno v.83, Sensi Gov-Fatt v144, Sensi Bio-Veg v63 and Sensi Gamay v221 provided by Sensi Vini (Lamporecchio, Italy), as shown in **Table 1**.

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)

Experiments

The QCM-D (E4 model, Q-Sense AB, Sweden) experiments were carried out using AT-cut quartz crystals, with a fundamental resonance frequency of 5 MHz (diameter = 14 mm, Biolin Scientific, Västra Frölunda, Sweden). The quartz crystals used in QCM-D experiments underwent a six-step cleaning process: i. rinsing with 2% sodium dodecyl sulfate, ii. treatment with oxygen plasma (Femto Diener, Diener electronic GmbH & Co., Ebhausen, Germany) for 2 minutes at a power of 100 W, iii. chemical treatment with a 5:1:1 solution of water, ammonia, and hydrogen peroxide for 15 minutes, iv. rinsing with water, v. rinsing with isopropanol, vi. treatment with oxygen plasma under the same conditions (2 minutes, 100 W).

The measurements were accomplished in temperature-controlled condition (25°C). QCM-D measurements involved monitoring changes in both resonance frequency (Δf) and energy dissipation (ΔD), focusing on odd overtone from the 1st to the 13th, by exciting the fundamental resonance frequency of the quartz crystal. Among the 7 overtones, the 3rd was selected as it showed more reliable performance throughout the acquisitions.

Sensor functionalization

The functionalization of the sensor surface was achieved by exploiting the thiol-gold chemistry. Since Gel-A lack in free thiol groups, the functionalization was accomplished in a three-steps process. The surface was previously modified using a linker, 12-MDA, with its thiol groups used for the immobilization. Subsequently, the carboxylic groups of 12-MDA were activated using EDCI/NHS chemistry and then the protein was immobilized. The linker solution was prepared by combining 12-MDA and DTT (0.1x mol/mol in respect to free -SH groups) in a 50% v/v water/ethanol mixture (12-MDA concentration 2 mg mL⁻¹). Before the injection of the linker solution the QCM-D apparatus was conditioned in a 50% v/v water/ethanol mixture, then the linker solution was injected and incubated for 30 minutes. Then, the sensor was rinsed with a 50% water/ethanol solution (5 minutes) and with water (5 minutes). This produced a self-assembled monolayer (SAM) of 12-MDA with carboxylic groups available for the subsequent protein immobilization. Afterward, a water solution of EDCI/NHS (10 mM each one) was injected and incubated for 15 minutes to activate the carboxylic functionalities. The sensor was rinsed with water for 5 minutes.

At this stage, the protein conjugation was performed at pH 10 using a carbonate/bicarbonate buffer. The protein immobilization was performed at pH 10, close to the isoelectric point of Gel-A. This

ensured that the Gel-A molecules predominantly exhibited a neutral net charge, minimizing repulsion among them. The apparatus was initially conditioned with the buffer for 5 minutes, and then the protein solution was injected (1 mg mL^{-1}) for 30 minutes. The protein solution was rinsed with the same buffer for 5 minutes and with water for 5 minutes.

Finally, residual NHS-esters were deactivated injecting a 0.1 M EDA solution in water for 5 minutes, followed by water rinsing (5 minutes). A representation of the functionalization steps for the protein is outlined in **Figure 1a**, with a corresponding typical trace of $\Delta f_3/3$ and ΔD_3 over time in **Figure 1b**.

In the case of MP5, the functionalization of the sensor surface was obtained in a single step, due to the cysteine at the C-terminal end of the peptide, as shown in **Figure 1c**. The apparatus was initially conditioned in PBS 1x buffer for 5 minutes, then MP5 solution in PBS 1x (1 mg mL^{-1}) supplemented with DTT ($3.5 \times \text{mol/mol}$ in respect to free -SH) was injected and incubated for 30 minutes. Finally, the sensor was rinsed with PBS 1x (5 minutes) and with water (5 minutes). In **Figure 1d** a typical trace of $\Delta f_3/3$ and ΔD_3 trace over time was shown.

Wine samples detection experiments

Total polyphenols concentration of the wine samples was measured via Folin-Ciocalteu method. The wine samples analyzed (**Table 1**) were stored at 4°C before use. Once the functionalization was completed, the signal was stabilized in a 14% water/ethanol blend (v/v) (5 minutes). This percentage was chosen to reflect the average alcohol content of the analyzed wines, which was between 12.6 and 14.7 (water/ethanol v/v). Then, the wine samples were injected and incubated for 15 minutes. Finally, the sensor was rinsed with 14% water-ethanol blend (v/v) (5 minutes) and with water (5 minutes). All the steps in sample detection stage are shown in **Figure 1b,d** (respectively for Gel-A and MP5).

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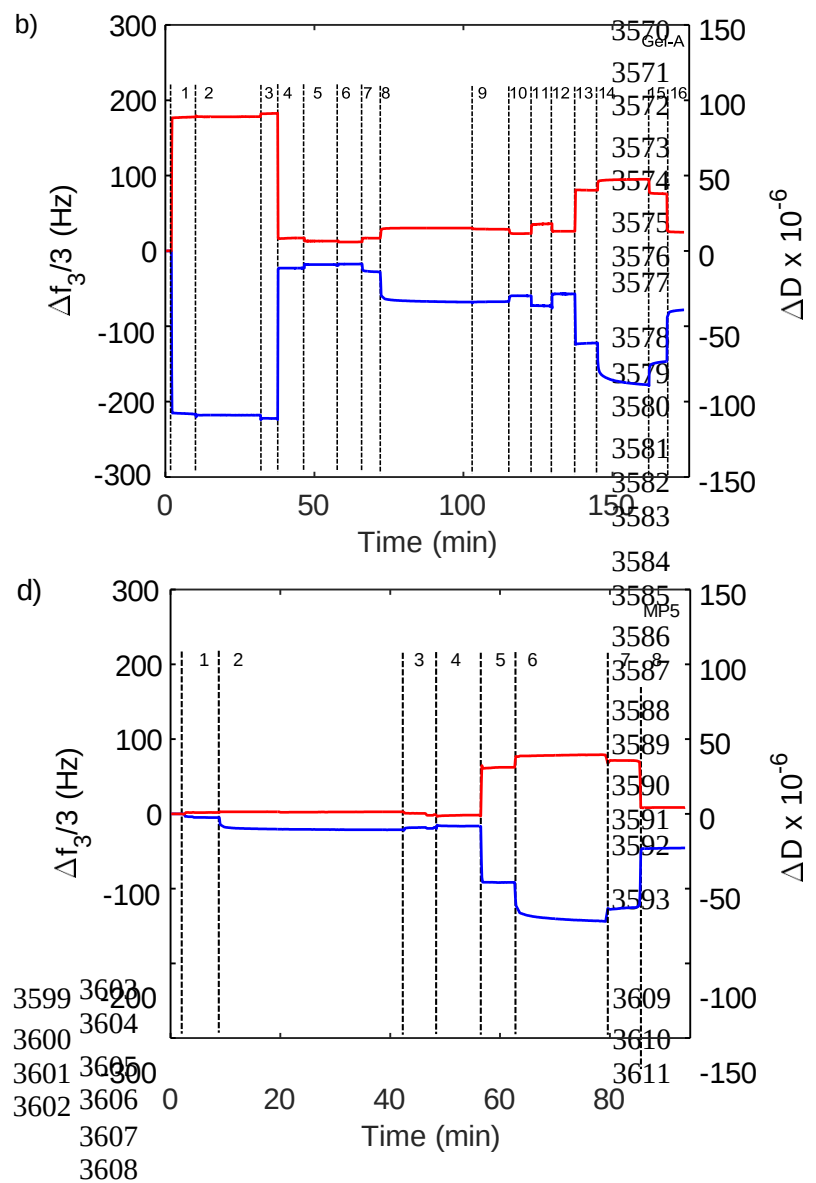
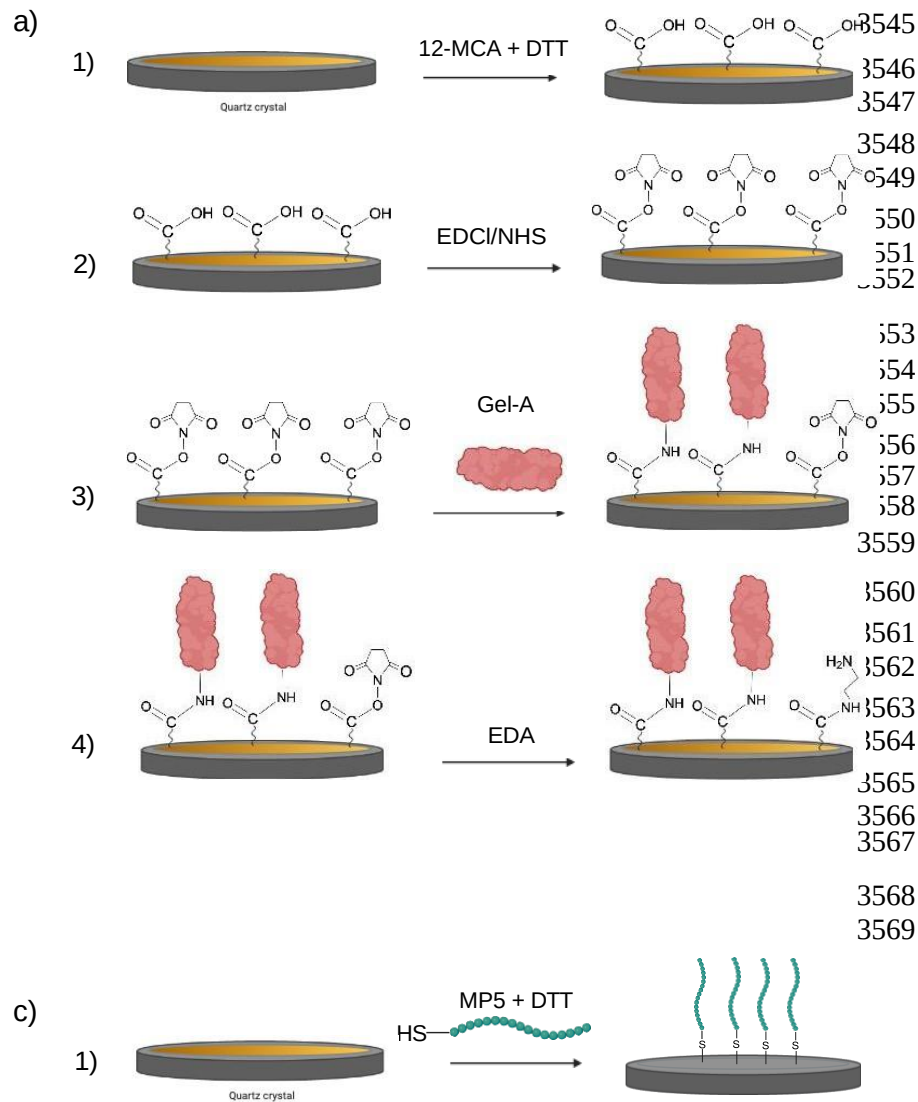


Figure 1. Schematic of the quartz crystal functionalization (not in scale) a) in 1. the formation of the 12-MDA adlayer in presence of DTT as a reducing agent; in 2. the activation of carboxylic acid groups of the adlayer via EDCI/NHS chemistry; in 3. the protein immobilization was achieved through the reaction between the activated adlayer and an aminic group of the selected protein (Gel-A); in 4. deactivation of the residual unreacted NHS-esters with EDA. b) Typical $\Delta f_3/3$ (blue line) and ΔD_3 (red line) traces over time in the case of Gel-A based functionalization (events: 1 pre-rinsing with water/ethanol, 2 injection of the adlayer solution, 3 rinsing with water/ethanol, 4 rinsing with water, 5 injection of the activation solution, 6 rinsing with water, 7 pre-rinsing with buffer carbonate/bicarbonate, 8 injection of the protein solution, 9 rinsing with buffer carbonate/bicarbonate, 10 rinsing with water, 11 injection of the blocking solution, 12 rinsing with water, 13 pre-rinsing with water/ethanol, 14 injection of the wine sample, 15 rinsing with water/ethanol, 16 rinsing with water). c) the peptide immobilization in presence of DTT as a reducing agent. d) Typical $\Delta f_3/3$ (blue line) and ΔD_3 (red line) traces over time in the case of MP5-based functionalization (events: 1 pre-rinsing with PBS 1x, 2 injection of peptide solution, 3 rinsing with PBS 1x, 4 rinsing with water, 5 pre-rinsing with water/ethanol, 6 injection of the wine sample, 7 rinsing with water/ethanol, 8 rinsing with water).

Sample Name	Nomenclature	Dilution	Polyphenol Concentration (g L ⁻¹)
Sensi Lungarno V83	L0	1:0	2.453 ± 0.047
	L1	1:1	1.241 ± 0.041
	L2	1:2	0.637 ± 0.019
Sensi Gov-Fatt V144	F0	0	1.999 ± 0.021
	F1	1:1	0.999 ± 0.032
	F2	1:2	0.509 ± 0.005
Sensi Bio-Veg V63	B0	0	1.444 ± 0.029
	B1	1:1	0.788 ± 0.037
	B2	1:2	0.437 ± 0.006
Sensi Gamay V221	G0	0	3.587 ± 0.003
	G1	1:1	1.825 ± 0.030
	G2	1:2	0.927 ± 0.004

Table 1. Wine samples analyzed by QCM-D experiments: sample name, nomenclature, dilution and polyphenol concentration in g L⁻¹ of Gallic Acid Equivalent, measured via the Folin–Ciocalteu method; each value represents mean ± standard deviation (n = 3).

High Performance Liquid Chromatography

A liquid chromatography apparatus, Dionex (Dionex Corporation Sunnyvale, Sunnyvale, CA, USA), controlled by Chromeleon software (version 6.50) and equipped with P680 quaternary pump, manual injector (Rheodyne) with 20 μ L loop, TCC-100 thermostatic oven, and PDA 100 detector (Photodiode Array Detector) was used. The separation was carried out with a Dionex Acclaim® 120 C18, 5 μ m, 4.6 $\times$ 250 mm column thermostated at 30 $^{\circ}$ C. The mobile phase consists of a ternary gradient consisting of: i. solvent A = 50 mM ammonium dihydrogen phosphate adjusted to pH 2.6 with orthophosphoric acid; ii. solvent B = 20% solvent A and 80% acetonitrile; iii. solvent C = 0.2 M orthophosphoric acid adjusted to pH 1.5 with NaOH according to the method developed by Ritchey and Waterhouse (Ritchey et al., 1999). For qualitative and quantitative analysis of the individual polyphenols, 33 analytical standards were selected and used to build different calibration curves. Phenolic compounds were identified on the basis of their retention time and the characteristics of their Uv-Vis spectra at wavelengths of 280 nm, 307nm, 316nm, 365nm e 520nm. The measurements were performed in triplicate.

Data analysis

Throughout the QCM-D experiments, $\Delta f_n/n$ and ΔD_n were continuously monitored. Each experimental condition was subjected to a minimum of three independent measurements. Despite QCM-D capability to record multiple harmonics of frequency and dissipation, our analysis was mainly focused on the third harmonic (Δf_3 , ΔD_3) due to its enhanced stability. The utilization of a single harmonic did not affect the final interpretation of the data.

The QCM-D raw data were analyzed using a MATLAB in house-script (MATLAB version R2022b, Mathworks, Natick, MA, USA). The reported $\Delta f_3/3$ and ΔD_3 values were derived by computing the difference between the initial baseline in water and the readings obtained after the water rinse.

The number of molecules per cm^2 was determined as follows. The areal mass Δm (ng cm^{-2}), that represents the mass adhered per unit area of the sensor, was derived using Sauerbrey equation (Saftics et al., 2021):

$$\Delta m = - C \cdot \Delta f_n / n \quad (\text{Eq. 1})$$

The subscript n indicates the overtone number. The constant C is called mass sensitivity constant and it is equal to 17.7 ng cm⁻² Hz⁻¹ for a 5 MHz quartz crystal. Δf_n refers to the frequency shift of a specific overtone.

Then the molecular density (molecules cm⁻²) was calculated as follows:

$$\text{molecular density} = \frac{\Delta m}{M_w} \cdot NA \text{ (Eq. 2)}$$

In Eq. 2 M_w (g mol⁻¹) represents the molecular weight of the immobilized molecule, and NA is the Avogadro number (6.022×10²³ mol⁻¹). For the calculation of the molecular density after the adlayer formation, an average molecular weight of 225.35 Da was used, because we accounted for the presence of DTT 0.1x alongside 12-MDA in the linker solution.

For the calculation of the molecular density after MP5 immobilization, an average molecular weight of 637.32 Da was used, considering the presence of DTT 3.5x in the peptide immobilization solution.

The Δf₃/3, ΔD₃ values obtained after the functionalization process were displayed in box plots, considering the values falling outside 1.5 times the interquartile range (IQR) from the box as outliers. The calculated molecular density was displayed in bar plots where the height of the bar represented the median, while the error bars correspond the IQR.

The Δf₃, ΔD₃ values gathered after sample detection were displayed in bar plot, where the height of the bar represented the mean of at least three replicates and the corresponding error bars representing the standard error.

The statistical analysis was performed with MATLAB software (v R2023b, Mathworks, Natick, MA, USA). The normality of the frequency and dissipation shift obtained with Gel-A and MP5 after the functionalization were checked via Shapiro-Wilk test. Then the frequency and dissipations shifts were compared using the Wilcoxon Rank Sum Test. The significance threshold was set to 0.05.

Multilinear regression model

To assess whether the behavior of the functionalizations, Gel-A and MP5, was influenced by the combined effect of different polyphenolic families, a multilinear regression model was built. This analysis was conducted using MATLAB software (v R2022b, Mathworks, Natick, MA, USA). In this model, the set of predictors X contained the polyphenolic compounds detected by HPLC, which represented the independent variables, while the frequency shifts (Δf₃/3) obtained with Gel-A or MP5

were chosen as dependent variable (response variable). The MATLAB function fitlm was used to build the multilinear regression model and the goodness of the fit was assessed by calculating the R-square. The use of the fitlm allowed for the creation of the model and visualization of the coefficients vector β associated with each predictor (β_j represented the coefficient estimate for the predictor x_j). Predictors with a coefficient estimate equal to zero were removed from the model.

Subsequently, the fit was graphically depicted using plotAdded function, which allow the representation of the multilinear model as a whole (except a constant term) in two-dimensional graph.

In these graphs, the y-axis of the graph displayed the $\Delta f_3/3$ values obtained with the wine samples, while the x-axis values were derived as follows. Let X denote the set of predictors and β the vector containing all the coefficients calculated using fitlm function. Next, we define u as a unit direction vector obtained as follows: $u = \beta/s$, where $s = \text{norm}(\beta)$, and consequently, $X\beta = (Xu)s$. The function plotAdded function treat Xu as a single predictor and constructs an added variable of $\Delta f_3/3$ respect to Xu, where s represents the coefficient of the fitted line (The MathWorks inc. 2023)

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3714 Results

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3716 Functionalization

3717 Regarding Gel-A, the median value of $\Delta f_3/3$ obtained after the adlayer formation (**Table 2**) and probe
3718 (**Figure 2, Table 2**) immobilization were negative. The median value yielded with MP5 were also
3719 negative.

3720 Absolute $\Delta f_3/3$ values were higher for Gel-A compared to MP5. The statistical analysis indicated a
3721 very highly significant difference between the two distributions ($p\text{-value} < 0.0001$).

3722 The median value of ΔD_3 was higher with Gel-A (5.9×10^{-6} , IQR= 1.7×10^{-6}) compared to MP5 (0.7
3723 $\times 10^{-6}$, IQR= 1.7×10^{-6}). The statistical analysis revealed a very highly significant difference between
3724 the two distributions ($p\text{-value} < 0.0001$).

3725 As described in the **Data Analysis** section, the molecular density (molecules cm^{-2}) was calculated.
3726 Focusing on Gel-A the median values of the molecular density after adlayer formation and probe
3727 immobilization were 1.1×10^{15} and 5.1×10^{12} molecules cm^{-2} , respectively. In the case of MP5, the

3728 median of the molecular density was 6.9×10^{13} molecules cm^{-2} . A statistically significant difference
3729 was also observed between the molecular density distributions of Gel-A and MP5.

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Probe	Step	$\Delta f_3/3$ (Hz)		$\Delta D_3 \times 10^{-6}$		Molecular density (molecules cm^{-2})	
		Median	IQR	Median	IQR	Median	IQR
Gel-A	Adlayer	-23.2	14.4	7.1	6.3	1.1×10^{15}	7.0×10^{14}
	Probe	-36.2	12.0	5.9	1.7	5.1×10^{12}	1.7×10^{12}
MP5	Probe	-12.3	7.9	0.7	1.7	6.9×10^{13}	4.4×10^{13}

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Table 2. Functionalization results: $\Delta f_3/3$ (Hz), $\Delta D_3 \times 10^{-6}$, Molecular density (molecules cm^{-2}) obtained with Gel-A and MP5.

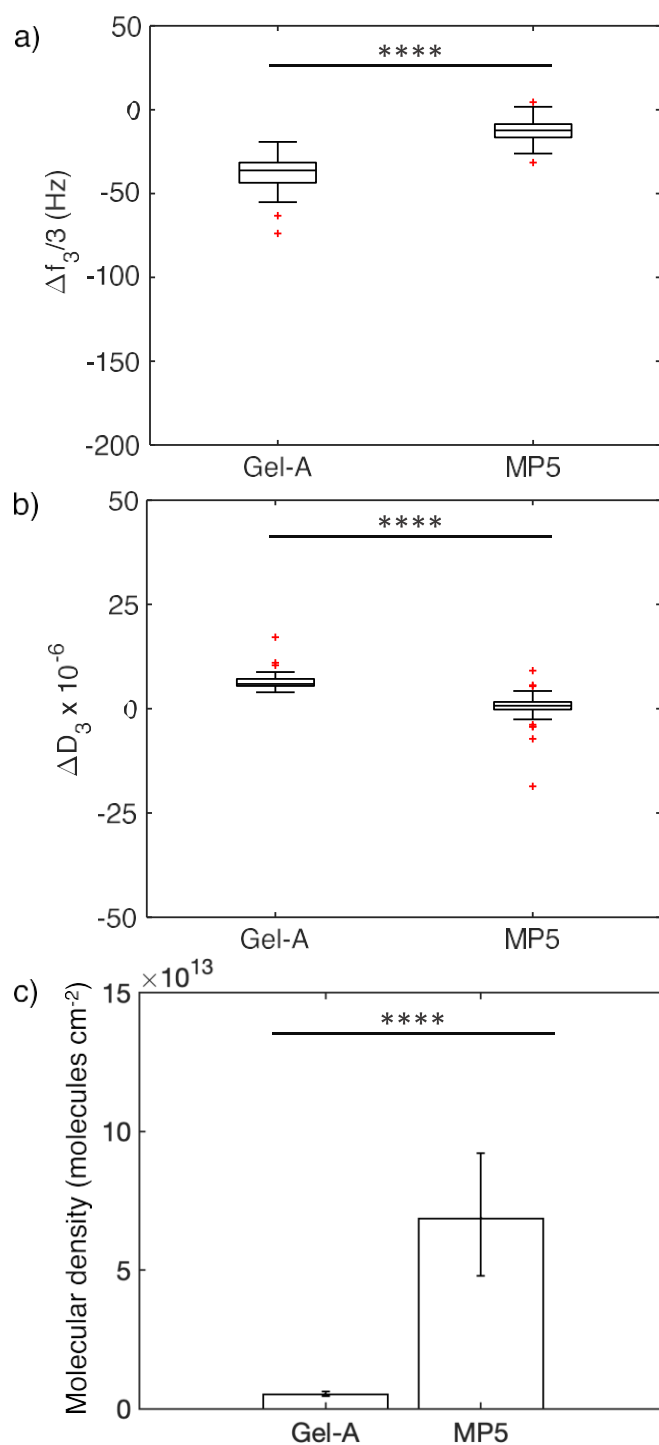


Figure 2. Characterization of the functionalizing layer: a) $\Delta f_3/3$, b) ΔD_3 and c) Molecular density (Molecules cm^{-2}) values after functionalization. The number of experiments for statistical analysis was 46 for Gel-A and 48 for MP5.

3767 HPLC analysis

3768 The results of the HPLC analysis are presented in **Tables S1-S2-S3-S4** in supplementary material.
3769 The major monomeric polyphenol detected in all wines was the gallic acid (the average values ranged
3770 from 53.88 mg L⁻¹ in B0 to 71.92 mg L⁻¹ in F0). This aligned with other HPLC studies (Tarola et al.,
3771 2007), which identified gallic acid as one of the predominant polyphenols in wine.

3772 According to the HPLC analysis the sample G0 exhibited the highest level of total anthocyanins,
3773 stilbenes, hydroxybenzoic acids and hydroxycinnamic acids, while total quercetin flavonols were
3774 fairly consistent among various wines.

3775 B0 showed a reduced content compared to the others in nearly all polyphenolic subfamilies except
3776 for the total flavonols.

3777 B0, G0 and L0 were the richest in total flavonols. However, a distinction can be highlighted: while in
3778 B0 and in G0 a substantial portion of the total flavonols consists of procyanidins, in the case of L0
3779 the contribution derives almost entirely from catechins and epicatechins.

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3782 Wine sample detection

3783 The effectiveness of Gel-A and MP5 in the analysis of polyphenols was investigated using red wine
3784 samples (**Table 1**). The average $\Delta f_3/3$ and ΔD_3 values (mean $\pm$ SE) measured for the wine samples
3785 were plotted in **Figure 3**.

3786 When using Gel-A, both negative (**Figure 3 a,e**) and positive (**Figure 3 c,g**) values of $\Delta f_3/3$ were
3787 observed. The absolute value of $\Delta f_3/3$ decreased with more diluted samples of L, G and F wines. On
3788 the other hand, B1 displayed a larger $\Delta f_3/3$ compared to B0.

3789 In all instances, ΔD_3 was negative (**Figure 3 b, d, f, h**) and remained relatively consistent across
3790 diluted samples in the case of G and B wines, while the dissipation was lower for L1 and F1 compared
3791 to the other.

3792 For MP5, negative shifts of $\Delta f_3/3$ were observed for all samples (**Figure 3a, c, e, g**), due to
3793 polyphenols mass loading. A decrease in the absolute value of $\Delta f_3/3$ was noted with G0, G1, G2 and
3794 B0, B1 and B2 (**Figure 3c, g**). The measured $\Delta f_3/3$ was consistent across F0, F1 and F2 (**Figure 3e**),
3795 while L1 exhibited a higher value compared to L0 (**Figure 3a**). Additionally, ΔD_3 was positive for all
3796 samples (**Figure 3 b, d, f, h**).

In the case of MP5, the $\Delta f_3/3$ observed with the sample G0 (**Figure 3c**), and the ΔD_3 values obtained with L1, L2 and F0 (**Figure 3a-f** respectively), were not normally distributed. However, the standard error was plotted for consistency with the other samples in the graphs.

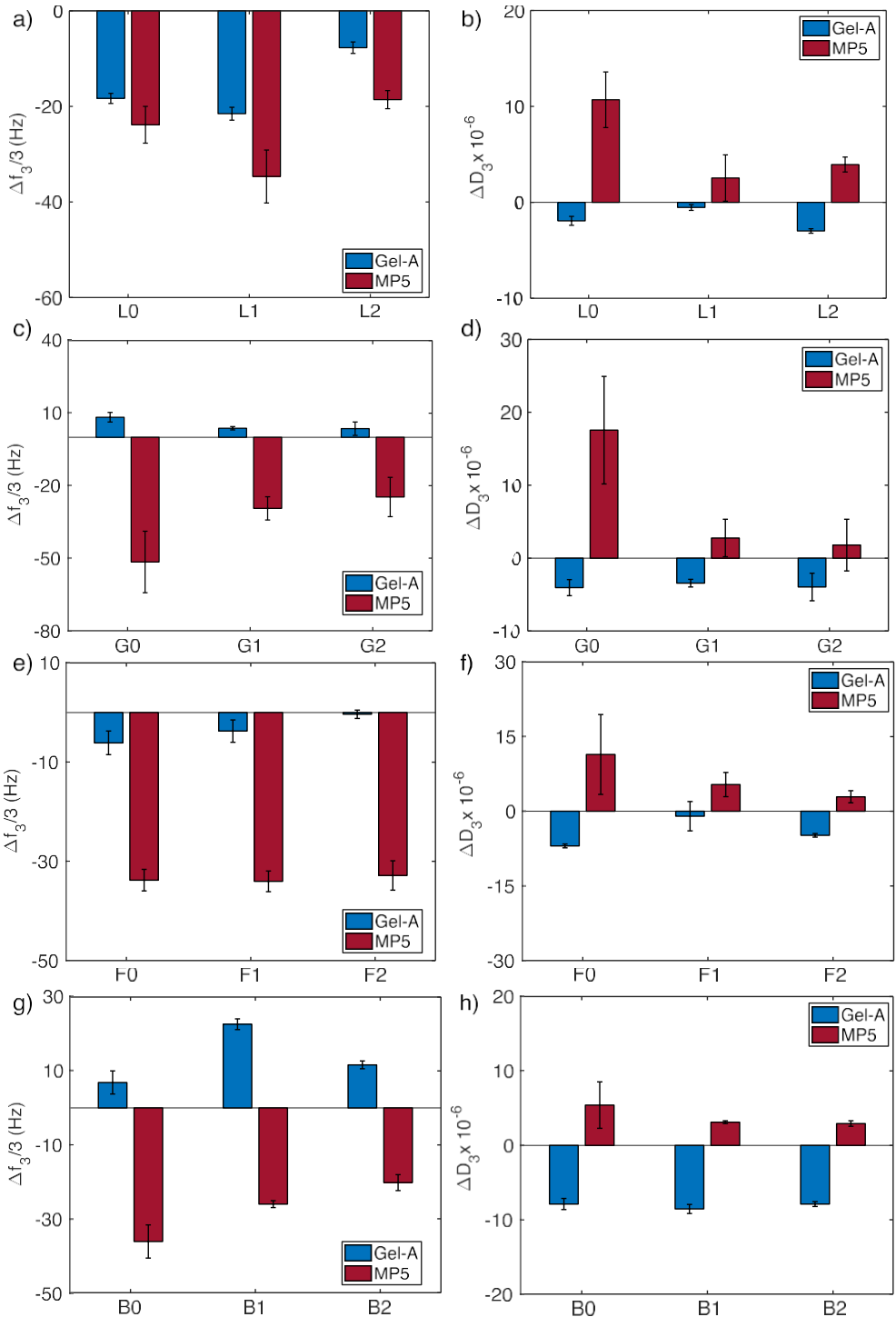


Figure 3. Detection of polyphenols in red wine samples. a), c), e), g) $\Delta f_3/3$ (mean $\pm$ SE) values with Gel-A and MP5 (blue and red respectively) and b), d), f), h) ΔD_3 (mean $\pm$ SE) values with Gel-A and MP5 (blue and red, respectively).

3813 The polyphenolic profiles of the red wines were analyzed using the HPLC technique as reported in
3814 **Supplementary Materials** section. Subsequently, these data were used to assess the correlation
3815 between $\Delta f_3/3$ and ΔD_3 signals and the concentration of different polyphenolic subfamilies. The
3816 relationship between $\Delta f_3/3$ and the concentration of total polyphenols was examined for both Gel-A
3817 and MP5, as depicted in **Figure 4**. Using Gel-A, no correlation with $\Delta f_3/3$ was observed (**Figure 4a**),
3818 while MP5 showed a decreasing trend with increasing polyphenol concentration (**Figure 4c**). In
3819 contrast, an increasing linear trend was observed in **Figure 4d** between ΔD_3 and the concentration of
3820 total polyphenols when MP5 was used. Furthermore, a linear trend between ΔD_3 and hydroxybenzoic
3821 acids concentration was seen using MP5.

3822 The HPLC data, along with QCM-D results, was used to build a multilinear regression model.
3823 Initially, all the polyphenols identified by the HPLC were incorporated in the model as predictors,
3824 while $\Delta f_3/3$ was treated as the response variable. Subsequently, the predictors associated to a
3825 coefficient estimate β equal to zero were removed from the model, resulting in 12 predictors
3826 remaining in the final version of the model. Finally, an added variable plot for both Gel-A and MP5
3827 was created to visualize the multilinear model (**Figure 5**). As regards the former, the significance of
3828 the model was appreciable ($p\text{-value} \ll 0.01$). The most significant predictors in the model were
3829 malvidin 3-*O*-glucoside, quercetin 3-*O*-glucoside, procyanidin B3, *t*-resveratrol, gallic acid and
3830 caffeic acid.

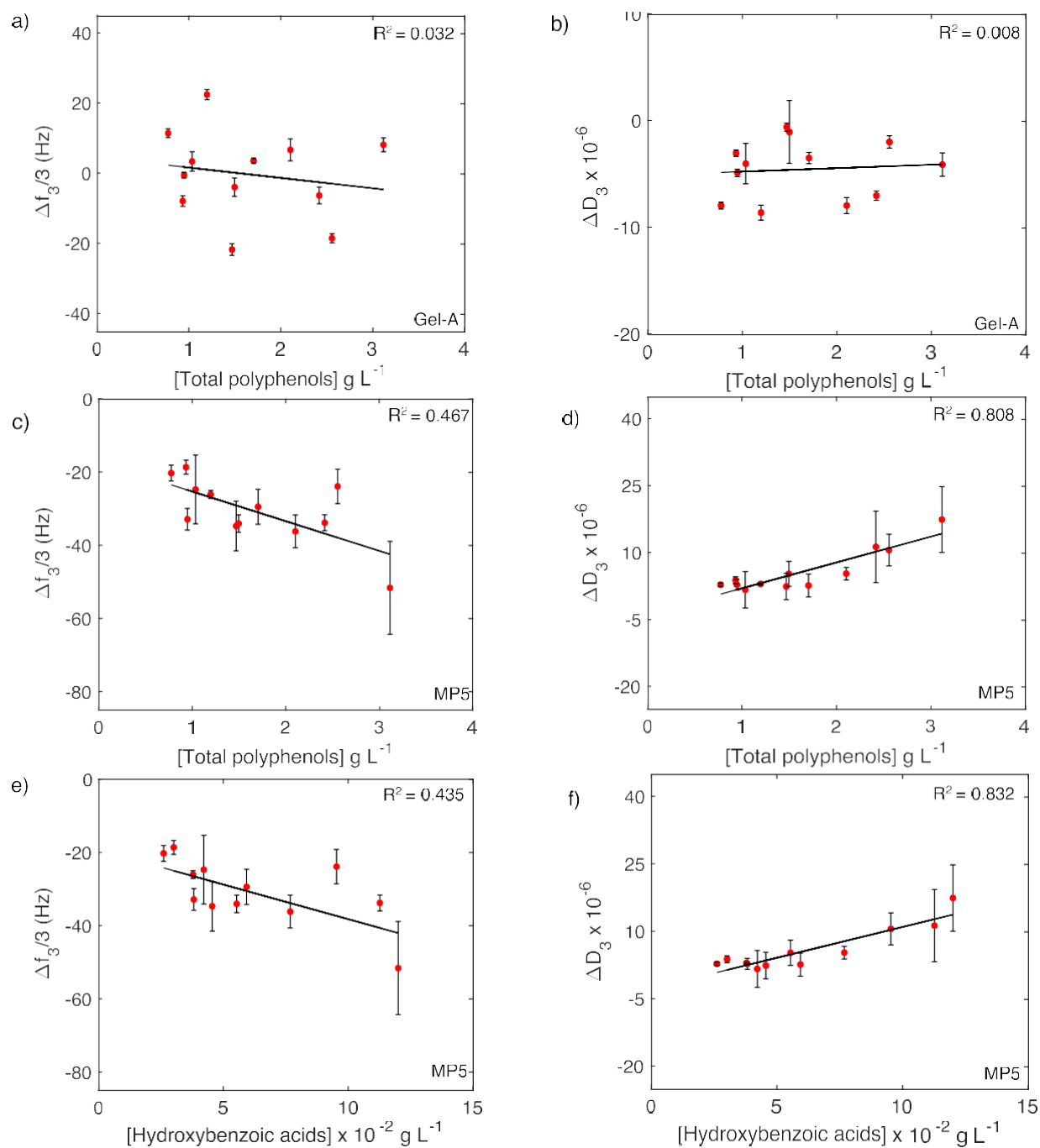


Figure 4. Regression of $\Delta f_3/3$ and ΔD_3 values over the concentration of total polyphenols or hydroxybenzoic acids. a), c) $\Delta f_3/3$ vs total polyphenols concentration with Gel-A and MP5 respectively. b), d) ΔD_3 vs total polyphenols concentration with Gel-A and MP5 respectively. e), f) $\Delta f_3/3$ and ΔD_3 vs hydroxybenzoic acids concentration with MP5.

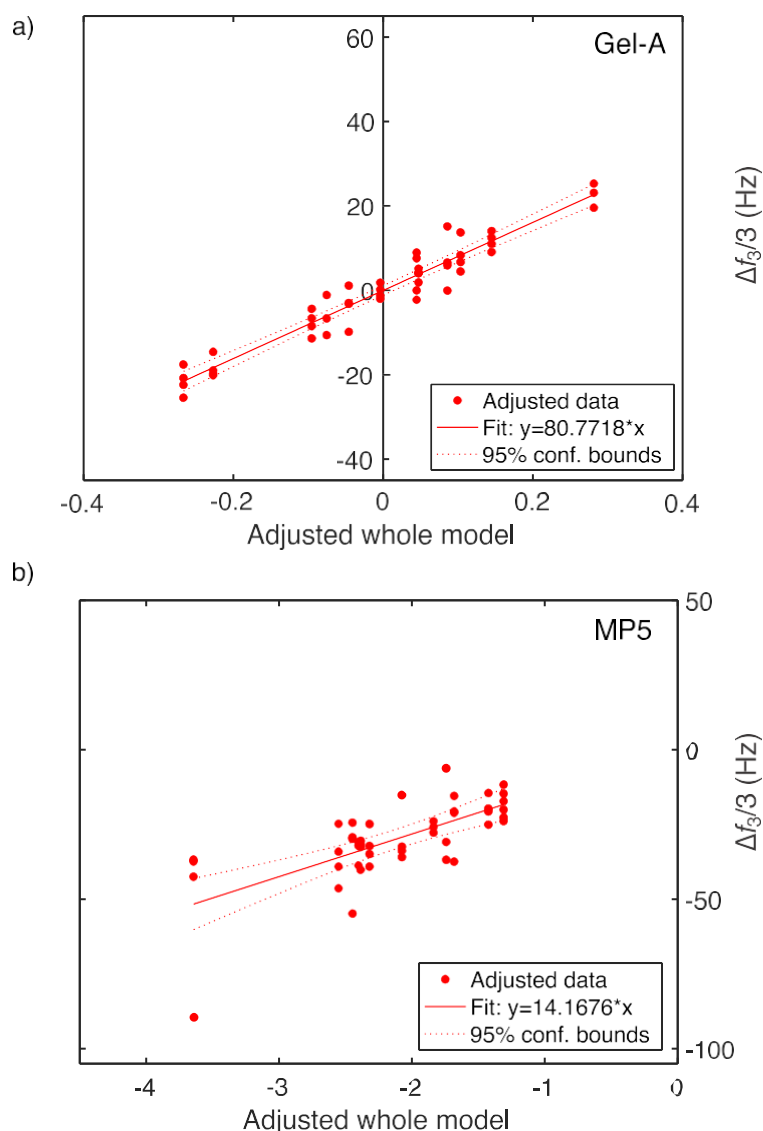


Figure 5. Added variable plot for the whole model, which allows to visualize the fit of 14 independent variables (different polyphenolic species in the wine matrix) against the dependent variable ($\Delta f_3/3$).

As regards the latter, even if the model was statistically significant ($p\text{-value} < 0.01$), the corresponding adjusted R squared was equal to $R^2_{adj} = 0.30$, indicating that the frequency shift was not well-explained by the multilinear regression model. MP5 allowed the interaction with phenolic groups, as demonstrated by the negative $\Delta f_3/3$ values, however, seemed less affected by the among various polyphenols compared to Gel-A.

Discussion

In this study the QCM-D was used to investigate the intriguing performance of Gel-A and MP5 in the analysis of polyphenols in red wines.

The functionalization of the QCM-D sensors was correctly accomplished with both Gel-A and MP5 as confirmed by the decrease of the resonance frequency. The $\Delta f_3/3$ values observed using Gel-A were consistent with those observed previously (Wang et al., 2021). We observed larger absolute values of $\Delta f_3/3$ with Gel-A compared to MP5. The molecular density (molecules cm^{-2}) was calculated for both Gel-A and MP5 as shown in **Data analysis** section using Sauerbrey model. The use of this model is recommended only for thin and rigid layers, otherwise the risk is an underestimation of the mass per unit area. Various studies debated its applicability under conditions different from the recommended and established three criteria: (i) minimal dependence of $\Delta f_n/n$ on n , (ii) ΔD_n values below 2×10^{-6} , and (iii) $\Delta D_n/(-\Delta f_n/n) \ll 4 \times 10^{-7}$ Hz. If any of these criteria are met, the model can be applied. In this study, the second criterion was used to evaluate the Sauerbrey equation's applicability (Saftics et al., 2021). While the dissipation was very low with MP5, indicating a rigid behavior, the dissipation values obtained with Gel-A were rather higher than 2×10^{-6} . However, contemplating this slight difference, the Sauerbrey model was applied.

The molecular coverage of MP5 was approximately one order of magnitude larger compared to that of Gel-A (the median molecular density was 6.9×10^{13} and 5.1×10^{12} molecules cm^{-2} for MP5 and Gel-A, respectively). The higher molecular density obtained with MP5 might be consistent with the significantly lower molecular weight of MP5 with respect to Gel-A.

In detection experiments with MP5, we observed negative frequency shift with all samples. This behavior was typically related to an increase in the mass adhering to the sensor surface, which we attributed in this scenario to the binding of polyphenols to MP5. Additionally, ΔD_3 was positive for all samples, suggesting that the pre-formed layer became more viscoelastic upon interaction with the sample. The dissipation showed a concentration-dependent effect with diluted samples resulting in lower ΔD_3 values.

Gel-A exhibited both negative and positive frequency shifts. This was consistent with our previous findings (Gagliardi et al 2022; Gagliardi et al., 2024), where positive $\Delta f_3/3$ values were attributed to the formation of cross-linked structures between Gel-A and polyphenols. These structures are determined by hydrogen bonds formation between hydroxylic groups of polyphenols and polar groups of Gel-A (Cao et al., 2007). In our interpretation, this occurred also in our system, as observed

3915 with G and B wines, where upon the formation of such structures, dehydration of the functionalizing
3916 layer occurred. Consequently, the mass per unit area removed surpassed that of the adhered
3917 polyphenols, resulting in a positive frequency shift. The dissipation resulted negative for all wines
3918 confirming the formation of a network between polyphenols and Gel-A.

3919 HPLC data were used to gain a deeper understanding of this unique behavior of Gel-A. As mentioned
3920 above, we supposed that Gel-A developed cross-linked structures with polyphenols, however we
3921 wondered why this effect was particularly pronounced with a subgroup of wine samples. A higher
3922 procyanidin content was noted in G and B compared to L and F samples. Procyanidins, also known
3923 as condensed tannins, are abundant in phenyl hydroxyl groups (Yang et al., 2021), which participate
3924 in formation of hydrogen bonds. In literature the role of procyanidins in the formation of cross-linked
3925 structures were well-established (Yang et al., 2021). Our hypothesis was that a higher content of
3926 procyanidins, which are known to interact with gelatin and act as natural cross-linkers, along with the
3927 abundance of hydroxyl groups, crucial for hydrogen bond formation, may explain the different
3928 behavior of G and B wines compared to F and L wines (He et al., 2011).

3929 Additionally, Gel-A, which is rich in proline, exhibits an open random coil structure (Frazier et al.,
3930 2010), which was reported to enhance interactions with high molecular weight procyanidins (Sarni-
3931 Manchado et al., 1999). Furthermore, other studies indicated that Gel-A exhibited an equilibrium
3932 binding constant two-order of magnitude higher for grape seeds proanthocyanidins compared to that
3933 of tea catechins (Frazier et al., 2010). In summary, the literature seemed to support our hypothesis,
3934 which, however, should be further investigated.

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3937 In a biosensor, a linear response of the signal within a specific concentration range of the analyte is
3938 desirable, as it facilitates a straightforward calibration of the sensors and maintains a constant
3939 sensitivity. In the developed biosensor, a linear correlation between the dissipation signal and the total
3940 polyphenol concentration was observed in the case of MP5. Additionally, a similar trend was noted
3941 between the dissipation shift and the concentration of hydroxybenzoic acids, aligning with the
3942 previously reported affinity of proline-rich proteins in saliva for hydrolysable tannins (Bacon et al.,
3943 2000).

3944 In the case of Gel-A, no linear trends were observed. One potential explanation might be that in this
3945 case the $\Delta f_3/3$ was influenced not only by polyphenol mass loading but also by water release resulting
3946 from the formation of cross-linked structures. According to this perspective, the presence of both
3947 phenomena might explain why the frequency data was not correlated with mass loading.

To assess if variations in the frequency signal were due to changes in the concentration of specific subfamilies rather than solely the total polyphenolic content, we constructed a multilinear model. In this case, our goal was not to construct a model to be used for calibration, which would allow to determine the properties of an unknown wine. Rather, our intention was to utilize this tool to gain a deeper understanding of the behavior exhibited by the tested probes. This model allowed to assess the effect on frequency shift due to variations in the concentration of individual subfamilies, highlighting, for example, if some of them had a greater influence. Using Gel-A, the model yielded intriguing results and suggested that the frequency shift was determined by the combined effect of specific polyphenolic subfamilies in the wine matrix. Our hypothesis was that Gel-A responded differently to changes in concentration of different polyphenolic subfamily, in relation to their distinct chemical structure. Additionally, procyanidin B3 was identified by the model as one of the most significant polyphenols, supporting the hypothesis that they may contribute to the behavior of Gel-A.

Conclusions

The tested probes exhibited noteworthy performance in the analysis of polyphenols in red wines. The primary advantage of our system lies in its high flexibility, which could allow its future adaptation to other gravimetric platforms suitable for on-field measurements. This could provide the desired responses in the winery directly, thus eliminating the need for transportation to specialized laboratories.

An additional development could be the adaptation of the system for the monitoring of wine characteristics over time, for example during fermentation, aging and blending.

All the presented findings underscore the potential of the system as a future strategy for assessing both the overall polyphenol content of a wine and the presence of specific polyphenolic subgroups.

The impact of this work could be extended in other areas of the agri-food field, such as the olive oil industry, where polyphenol content influences the flavor of the final product but also its nutritional aspect and storability (Angerosa et al., 2000).

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3979 References

- 3980 1. Voinova, M. V. (2009). On mass loading and dissipation measured with acoustic wave
3981 sensors: A review. *Journal of Sensors*, 2009, 1-13. <https://doi.org/10.1155/2009/943125>
- 3982 2. Agostini, M., Amato, F., Vieri, M. L., Greco, G., Tonazzini, I., Baroncelli, L., Caleo, M.,
3983 Vannini, E., Santi, M., Signore, G., & Cecchini, M. (2021). Glial-fibrillary-acidic-protein
3984 (GFAP) biomarker detection in serum-matrix: Functionalization strategies and detection by
3985 an ultra-high-frequency surface-acoustic-wave (UHF-SAW) lab-on-chip. *Biosensors and*
3986 *Bioelectronics*, 172, 112174 <https://doi.org/10.1016/j.bios.2020.112774>
- 3987 3. Agostini, M., Lunardelli, F., Gagliardi, M., Miranda, A., Lamanna, L., Luminare, A. G.,
3988 Gambineri, F., Lai, M., Pistello, M., & Cecchini, M. (2021). Surface-Acoustic-Wave (SAW)
3989 Induced Mixing Enhances the Detection of Viruses: Application to Measles Sensing in Whole
3990 Human Saliva with a SAW Lab-On-a-Chip. *Advanced Functional. Material*, 32(44), 2201958.
3991 <https://doi.org/10.1002/adfm.202201958>
- 3992 4. Gagliardi, M., Agostini, M., Lunardelli, F., Lamanna, L., Miranda, A., Bazzichi, A., Luminare,
3993 A. G., Cervelli, F., Gambineri, F., Totaro, M., Lai, M., Maisetta, G., Batoni, G., Pistello, M.,
3994 & Cecchini, M. (2023). Surface acoustic wave-based lab-on-a-chip for the fast detection of
3995 *Legionella pneumophila* in water. *Sensors and Actuators B: Chemical*, 379, 133299.
3996 <https://doi.org/10.1016/j.snb.2023.133299>
- 3997 5. Kim, Y. W., Sardari, S. E., Meyer, M. T., Iliadis, A. A., Wu, H. C., Bentley, W. E., & Ghodssi,
3998 R. (2012). An ALD aluminum oxide passivated Surface Acoustic Wave sensor for early
3999 biofilm detection. *Sensors and Actuators, B: Chemical*, 163(1), 136-145.
4000 <https://doi.org/10.1016/j.snb.2012.01.021>
- 4001 6. Fogel, R., Limson, J. & Seshia, A. (2016). Acoustic biosensors. *Essays in biochemistry*, 60(1),
4002 101–110. <https://doi.org/10.1042/EBC20150011>
- 4003 7. Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnolog*,
4004 23(2), 174-181. <https://doi.org/10.1016/j.copbio.2011.08.007>
- 4005 8. Gutiérrez-Escobar, R., Aliaño-González, M. J., & Cantos-Villar, E. (2021). Wine polyphenol
4006 content and its influence on wine quality and properties: A review. *Molecules* 26(3), 718.
4007 <https://doi.org/10.3390/molecules26030718>
- 4008 9. Niculescu, V.-C., Paun, N., & Ionete, R.-E. (2018). The Evolution of Polyphenols from Grapes
4009 to Wines. *Grapes and Wines-Advances in Production, Processing, Analysis and Valorization*,
4010 7, 119-140. <https://doi.org/10.5772/intechopen.72800>

10. Lorrain, B., Ky, I., Pechamat, L., & Teissedre, P. L. (2013). Evolution of analysis of polyphenols from grapes, wines, and extracts. *Molecules*, 18(1), 1076-1100.<https://doi.org/10.3390/molecules18011076>
11. Gomes, S. A. S. S., & Rebelo, M. J. F. (2003). A new laccase biosensor for polyphenols determination. *Sensors*, 3(6), 166-175. <https://doi.org/10.3390/s30600166>
12. Capannesi, C., Palchetti, I., Mascini, M., & Parenti, A. (2000). Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chemistry*, 71(4), 553-562. [https://doi.org/10.1016/S0308-8146\(00\)00211-9](https://doi.org/10.1016/S0308-8146(00)00211-9)
13. Gomes, S. A. S. S., Nogueira, J. M. F., & Rebelo, M. J. F. (2004). An amperometric biosensor for polyphenolic compounds in red wine. *Biosensors and Bioelectronics*, 20(6), 1211-1216. <https://doi.org/10.1016/j.bios.2004.05.013>
14. Di Fusco, M., Tortolini, C., Deriu, D., & Mazzei, F. (2010). Laccase-based biosensor for the determination of polyphenol index in wine. *Talanta*, 81(1-2), 235-240.<https://doi.org/10.1016/j.talanta.2009.11.063>
15. Cetó, X., Céspedes, F., & del Valle, M. (2012). BioElectronic Tongue for the quantification of total polyphenol content in wine. *Talanta*, 99, 544-551. <https://doi.org/10.1016/j.talanta.2012.06.031>
16. Gamella, M., Campuzano, S., Reviejo, A. J., & Pingarrón, J. M. (2006). Electrochemical estimation of the polyphenol index in wines using a laccase biosensor. *Journal of Agricultural and Food Chemistry*, 54(21), 7960-7967. <https://doi.org/10.1021/jf061451r>
17. Jia, M., Pan, Y., Zhou, J., & Zhang, M. (2021). Identification of Chinese teas by a colorimetric sensor array based on tea polyphenol induced indicator displacement assay. *Food Chemistry*, 335, 127566. <https://doi.org/10.1016/j.foodchem.2020.127566>
18. Abdullah, J., Ahmad, M., Heng, L. Y., Karupiah, N., & Sidek, H. J. (2007). An Optical Biosensor based on Immobilization of Laccase and MBTH in Stacked Films for the Detection of Catechol. *Sensors (Basel, Switzerland)*, 7(10), 2238-2250. <https://doi.org/10.3390/s7102238>
19. Karczmarczyk, A., Haupt, K., & Feller, K. H. (2017). Development of a QCM-D biosensor for Ochratoxin A detection in red wine. *Talanta*, 166, 193-197. <https://doi.org/10.1016/j.talanta.2017.01.054>
20. Poggesi, S., Zhou, L., Bariani, G. C., Mittapalli, R., Manzano, M., & Ionescu, R. E. (2021). Quartz crystal microbalance genosensing of *brettanomyces bruxellensis* yeast in wine using a rapid and efficient drop and collect protocol. *Crystals*, 11(5), 562. <https://doi.org/10.3390/cryst1105056>

21. Yan, Y., Hu, J., & Yao, P. (2009). Effects of Casein, ovalbumin, and dextran on the astringency of tea polyphenols determined by quartz crystal microbalance with dissipation. *Langmuir*, 25(1), 397-402. <https://doi.org/10.1021/la8030123>
22. Kaneda, H., Watari, J., Takashio, M., & Okahata, Y. (2004). Measuring Astringency of Beverages Using a Quartz-Crystal Microbalance. *Journal of the American Society of Brewing Chemists*, 61(3), 119-124. <https://doi.org/10.1094/asbcj-61-0119>
23. Wang, S., Olarte Mantilla, S. M., Smith, P. A., Stokes, J. R., & Smyth, H. E. (2021). Tribology and QCM-D approaches provide mechanistic insights into red wine mouthfeel, astringency sub-qualities and the role of saliva. *Food Hydrocolloids*, 120, 106918. <https://doi.org/10.1016/j.foodhyd.2021.106918>
24. Zhao, Q., Du, G., Wang, S., Zhao, P., Cao, X., Cheng, C., Liu, H., Xue, Y., & Wang, X. (2023). Investigating the role of tartaric acid in wine astringency. *Food Chemistry*, 403, 134385. <https://doi.org/10.1016/j.foodchem.2022.134385>
- Wang, X., Ho, C. T., & Huang, Q. (2007). Investigation of adsorption behavior of (-)-epigallocatechin gallate on bovine serum albumin surface using quartz crystal microbalance with dissipation monitoring. *Journal of Agricultural and Food Chemistry*, 55(13), 4987-4992. <https://doi.org/10.1021/jf070590l>
25. Weerawatanakorn, M., Huang, Q., & Ho, C. T. (2014). Monitoring the binding processes of (-)-epigallocatechin gallate and theaflavin-3,3'-digallate to alpha-casein surface using quartz crystal microbalance with dissipation. *International Food Research Journal*, 21(2), 493-499.
26. Ali, E. E., Elmakki, M. O., Gavette, M. L., Doyle, B. J., & Timpe, S. J. (2019). Protein Binding Characteristics of the Principal Green Tea Catechins: A QCM Study Comparing Crude Extract to Pure EGCG. *Biochemistry Research International*, 2019, 1-8. <https://doi.org/10.1155/2019/6154170>
27. Minoda, K., Ichikawa, T., Katsumata, T., Onobori, K. ichi, Mori, T., Suzuki, Y., Ishii, T., & Nakayama, T. (2010). Influence of the galloyl moiety in tea catechins on binding affinity for human serum albumin. *Journal of Nutritional Science and Vitaminology*, 56(5), 331-334. <https://doi.org/10.3177/jnsv.56.331>
28. Kamihira, M., Nakazawa, H., Kira, A., Mizutani, Y., Nakamura, M., & Nakayama, T. (2008). Interaction of tea catechins with lipid bilayers investigated by a quartz-crystal microbalance analysis. *Bioscience, Biotechnology and Biochemistry*, 72(5), 1372-1375. <https://doi.org/10.1271/bbb.70786>
29. Cosme, F., Ricardo-Da-Silva, J. M., & Laureano, O. (2007). Protein fining agents: Characterization and red wine fining assays. *Italian Journal of Food Science*, 19, 39-56.

30. Gagliardi, M., Tori, G., Agostini, M., Lunardelli, F., Mencarelli, F., Sanmartin, C., & Cecchini, M. (2022). Detection of Oenological Polyphenols via QCM-D Measurements. *Nanomaterials*, 12(1), 166. <https://doi.org/10.3390/nano12010166>
31. Gagliardi, M., Tori, G., Sanmartin, C., & Cecchini, M. (2024). The effect of probe density coverage on the detection of oenological tannins in quartz crystal microbalance with dissipation monitoring (QCM-D) experiments. *Journal of the science of food and agriculture*.<https://doi.org/10.1002/jsfa.13351>
32. Baxter, N. J., Lilley, T. H., Haslam, E., & Williamson, M. P. (1997). Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. *Biochemistry*, 36(18), 5566-5577. <https://doi.org/10.1021/bi9700328>
33. Murray, N. J., Williamson, M. P., Lilley, T. H., & Haslam, E. (1994). Study of the interaction between salivary proline-rich proteins and a polyphenol by ¹H-NMR spectroscopy. *European Journal of Biochemistry*, 219(3), 923-935. <https://doi.org/10.1111/j.1432-1033.1994.tb18574.x>
34. Nemzer, B., Kalita, D., Yashin, A. Y., & Yashin, Y. I. (2022). Chemical Composition and Polyphenolic Compounds of Red Wines: Their Antioxidant Activities and Effects on Human Health—A Review. *Beverages* 8(1), 1. <https://doi.org/10.3390/beverages8010001>
35. Saftics, A., Kurunczi, S., Peter, B., Szekacs, I., Ramsden, J. J., & Horvath, R. (2021). Data evaluation for surface-sensitive label-free methods to obtain real-time kinetic and structural information of thin films: A practical review with related software packages. *Advances in Colloid and Interface Science*, 294, 102431. <https://doi.org/10.1016/j.cis.2021.102431>
36. Cao, N., Fu, Y., & He, J. (2007). Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. *Food Hydrocolloids*, 21(4), 575-584.<https://doi.org/10.1016/j.foodhyd.2006.07.001>
37. Yang, H., Tuo, X., Wang, L., Tundis, R., Portillo, M. P., Simal-Gandara, J., Yu, Y., Zou, L., Xiao, J., & Deng, J. (2021). Bioactive procyanidins from dietary sources: The relationship between bioactivity and polymerization degree. *Trends in Food Science and Technology*, 111, 114-127. <https://doi.org/10.1016/j.tifs.2021.02.063>
38. He, L., Mu, C., Shi, J., Zhang, Q., Shi, B., & Lin, W. (2011). Modification of collagen with a natural cross-linker, procyanidin. *International Journal of Biological Macromolecules*, 48(2), 354-359. <https://doi.org/10.1016/j.ijbiomac.2010.12.012>
39. Frazier, R. A., Deaville, E. R., Green, R. J., Stringano, E., Willoughby, I., Plant, J., & Mueller-Harvey, I. (2010). Interactions of tea tannins and condensed tannins with proteins. *Journal of Pharmaceutical and Biomedical Analysis*, 51(2), 490-495. <https://doi.org/10.1016/j.jpba.2009.05.035>

40. Sarni-Manchado, P., Cheynier, V., & Moutounet, M. (1999). Interactions of grape seed tannins with salivary proteins. *Journal of Agricultural and Food Chemistry*, 47(1), 41-47.<https://doi.org/10.1021/jf9805146>
41. Bacon, J. R., & Rhodes, M. J. C. (2000). Binding affinity of hydrolyzable tannins to parotid saliva and to proline-rich proteins derived from it. *Journal of Agricultural and Food Chemistry*, 48(3), 838-843. <https://doi.org/10.1021/jf990820z>
42. Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2000). Virgin olive oil odour notes: Their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chemistry*, 68(3), 283-287. [https://doi.org/10.1016/S0308-8146\(99\)00189-2](https://doi.org/10.1016/S0308-8146(99)00189-2)
43. Ritchey, J. G., & Waterhouse, A. L. (1999). A standard red wine: Monomeric phenolic analysis of commercial Cabernet Sauvignon wines. *American Journal of Enology and Viticulture*, 50(1), 91-100. <https://doi.org/10.5344/ajev.1999.50.1.91>
44. The MathWorks Inc. (2023). Statistics and Machine Learning Toolbox: User's Guide (R2023b), Documentation, Natick, Massachusetts: The MathWorks Inc.<https://www.mathworks.com/help/stats/index.html>
45. Tarola, A. M., Milano, F., & Giannetti, V. (2007). Simultaneous determination of phenolic compounds in red wines by HPLC-UV. *Analytical Letters*, 40(12), 2433-2445. <https://doi.org/10.1080/00032710701577666>

Supplementary materials

Table S1. Polyphenolic profile of Sensi Lungarno v83 (L0, L1, L2 samples) wine obtained by HPLC analysis: monomer and concentration (mg L⁻¹) (mean ± SD). The displayed concentrations represent the mean of three replicates.

Monomer	Concentration mg L ⁻¹		
	Sensi Lungarno v83		
	L0	L1	L2
Cyanidin 3-O-glucoside	0.404	0.142	0.095
Delphinidin 3-O-glucoside	0.804	0.379	0.306
Peonidin 3-O-glucoside	0.415	0.152	0.118
Malvidin 3-O-glucoside	23.678	11.650	9.263
Petunidin 3-O-glucoside	0.942	0.465	0.344
Total anthocyanins	26.243	13.127	10.467
Myricetin	0.000	0.000	0.000
Quercetin 3-O-glucoside	4.152	3.945	1.208

Quercetin 3- <i>O</i> -rutinose	4.782	1.362	0.247
Quercetin	4.792	2.278	1.943
Kampferol	0.000	0.000	0.000
Quercetin 3- <i>O</i> -ramnose	6.085	3.183	2.134
Quercetin flavonols	17.132	7.825	5.269
Catechin	36.846	11.751	8.615
Procyanidin trimer C1	0.000	0.000	0.000
(-)-Epicatechin	32.067	12.666	7.400
Procyanidin B1	8.730	15.308	9.713
Procyanidin B2	0.000	0.000	0.000
Procyanidin B3	0.000	0.000	0.000
Epicatechin 3- <i>O</i> -gallate	0.000	0.000	0.000
Total flavonols	77.643	39.726	25.728
<i>t</i> -rasveratrol	0.826	0.299	0.146
Piceatannol	0.000	0.000	0.000
(+)- Epsilon Viniferin	1.006	0.505	0.302
Resveratrol	0.278	0.099	0.063
Total stilbene	2.109	0.903	0.511
Vanillic acid	0.000	0.000	0.000
Protocatechuic acid	3.801	1.293	0.809
Syringic acid	0.000	0.000	0.000
Gallic acid ethyl ester (Ethyl gallate)	30.585	14.829	10.137
4-Hydroxybenzoic acid	0.000	0.000	0.000
Gentisic acid	0.000	0.000	0.000
Gallic acid	60.920	29.452	19.220
Total hydroxybenzoic acids	95.306	45.575	30.165
Caffeoyl tartaric acid	0.825	0.000	0.000
<i>p</i> -Coumaric acid	0.000	0.000	0.000
Ferulic acid	0.185	0.000	0.000
Caffeic acid	9.212	4.461	2.922
Total hydroxycinnamic acids	10.222	4.461	2.922

Table S2. Polyphenolic profile of Sensi Gamay v221 (G0, G1 and G2) wine obtained by HPLC analysis: monomer and concentration (mg L⁻¹) (mean ± SD). The displayed concentrations represent the mean of three replicates.

Monomer	Concentration mg L ⁻¹
	Sensi Gamay v221

	G0	G1	G2
Cyanidin 3- <i>O</i> -glucoside	20.769	9.350	5.573
Delphinidin 3- <i>O</i> -glucoside	24.254	12.241	6.462
Peonidin 3- <i>O</i> -glucoside	2.527	1.570	0.891
Malvidin 3- <i>O</i> -glucoside	53.089	26.202	15.291
Petunidin 3- <i>O</i> -glucoside	13.122	6.226	3.662
Total anthocyanins	113.762	55.589	13.127
Myricetin	1.459	0.787	0.316
Quercetin 3- <i>O</i> -glucoside	7.630	7.037	2.771
Quercetin 3- <i>O</i> -rutinoside	0.000	0.000	0.000
Quercetin	5.815	5.324	1.632
Kampferol	0.000	0.000	0.000
Quercitin 3- <i>O</i> -ramnoside	4.032	1.948	1.158
Quercetin flavonols	15.898	11.180	4.915
Catechin	14.637	8.261	4.797
Procyanidin trimer C1	0.000	0.000	0.000
(-)-Epicatechin	0.000	0.000	0.000
Procyanidin B1	0.000	0.000	0.000
Procyanidin B2	0.000	0.000	0.000
Procyanidin B3	55.535	22.214	10.113
Epicatechin 3- <i>O</i> -gallate	0.000	0.000	0.000
Total flavonols	70.171	30.475	14.910
<i>t</i> -rasveratrol	31.384	15.134	10.277
Piceatannol	0.000	0.000	0.000
(+)- Epsilon Viniferin	2.770	1.335	0.812
Resveratrol	0.000	0.000	0.000
Total stilbene	35.154	16.469	11.089
Vanillic acid	0.000	0.000	0.000
Protocatechuic acid	5.006	2.831	1.853
Syringic acid	0.000	0.000	0.000
Gallic acid ethyl ester (Ethyl gallate)	53.929	26.920	21.090
4-Hydroxybenzoic acid	0.000	0.000	0.000
<i>Gentisic acid</i>	0.000	0.000	0.000
Gallic acid	61.062	29.590	19.250
Total hydroxybenzoic acids	119.998	59.341	42.193
Caffeoyl tartaric acid	1.745	0.835	0.551
p-Coumaric acid	0.000	0.000	0.000
Ferulic acid	0.185	0.000	0.000

Caffeic acid	27.996	16.370	9.854
Total hydroxycinnamic acids	29.740	17.204	10.405

4150 **Table S3.** Polyphenolic profile of Sensi Gov-Fatt v144 (F0, F1 and F2) wine obtained by HPLC
 4151 analysis: monomer and concentration (mg L⁻¹) (mean ± SD). The displayed concentrations represent
 4152 the mean of three replicates.
 4153

Monomer	Concentration mg L ⁻¹		
	Sensi Gov-Fatt v144		
	F0	F1	F2
Cyanidin 3- <i>O</i> -glucoside	0.628	0.200	0.138
Delphinidin 3- <i>O</i> -glucoside	1.187	0.469	0.330
Peonidin 3- <i>O</i> -glucoside	0.871	0.375	0.264
Malvidin 3- <i>O</i> -glucoside	14.702	6.387	4.483
Petunidin 3- <i>O</i> -glucoside	1.583	0.610	0.448
Total anthocyanins	18.971	13.127	10.467
Myricetin	0.787	0.316	0.260
Quercetin 3- <i>O</i> -glucoside	0.686	0.686	0.686
Quercetin 3- <i>O</i> -rutinoside	4.782	1.362	0.247
Quercetin	4.792	2.278	1.943
Kampferol	0.000	0.000	0.000
Quercitin 3- <i>O</i> -ramnoside	2.023	0.959	0.628
Quercetin flavonols	13.070	5.600	3.764
Catechin	19.898	10.639	6.587
Procyanidin trimer C1	0.000	0.000	0.000
(-)-Epicatechin	19.435	8.872	5.607
Procyanidin B1	2.983	1.788	0.805
Procyanidin B2	0.000	0.000	0.000
Procyanidin B3	0.000	0.000	0.000
Epicatechin 3- <i>O</i> -gallate	0.000	0.000	0.000
Total flavonols	42.316	21.299	13.000
<i>t</i> -rasveratrol	1.422	0.610	0.325
Piceatannol	0.000	0.000	0.000
(+)- Epsilon Viniferin	1.302	0.640	0.455
Resveratrol	0.354	0.110	0.054
Total stilbene	3.079	1.360	0.834
Vanillic acid	0.000	0.000	0.000
Protocatechuic acid	14.567	6.897	4.683
Syringic acid	0.000	0.000	0.000
Gallic acid ethyl ester (Ethyl gallate)	26.167	13.305	9.559
4-Hydroxybenzoic acid	0.000	0.000	0.000
Gentisic acid	0.000	0.000	0.000

Gallic acid	71.922	35.183	23.980
Total hydroxybenzoic acids	112.657	55.385	38.222
Caffeoyl tartaric acid	0.825	0.000	0.000
p-Coumaric acid	0.000	0.000	0.000
Ferulic acid	0.110	0.049	0.026
Caffeic acid	6.377	2.917	1.899
Total hydroxycinnamic acids	6.487	2.967	1.926

Table S4. Polyphenolic profile of Sensi Bio-Veg v63 (B0, B1 and B2) wine obtained by HPLC analysis: monomer and concentration (mg L⁻¹) (mean ± SD). The displayed concentrations represent the mean of three replicates.

Monomer	Concentration mg L ⁻¹		
	Sensi Bio-Veg v63		
	B0	B1	B2
Cyanidin 3- <i>O</i> -glucoside	0.126	0.060	0.045
Delphinidin 3- <i>O</i> -glucoside	0.951	0.693	0.641
Peonidin 3- <i>O</i> -glucoside	0.547	0.232	0.180
Malvidin 3- <i>O</i> -glucoside	6.400	2.950	2.486
Petunidin 3- <i>O</i> -glucoside	0.461	0.212	0.183
Total anthocyanins	8.486	4.147	3.536
Myricetin	0.529	0.058	0.049
Quercetin 3- <i>O</i> -glucoside	4.080	2.176	1.528
Quercetin 3- <i>O</i> -rutinoside	1.720	0.666	0.283
Quercetin	1.121	0.434	0.185
Kampferol	0.000	0.000	0.000
Quercitin 3- <i>O</i> -ramnoside	1.696	0.828	0.512
Quercetin flavonols	9.148	4.162	2.558
Catechin	22.697	11.725	7.636
Procyanidin trimer C1	0.000	0.000	0.000
(-)-Epicatechin	26.427	11.710	6.933
Procyanidin B1	24.509	13.439	9.652
Procyanidin B2	29.437	13.164	8.085
Procyanidin B3	2.787	1.254	0.673
Epicatechin 3- <i>O</i> -gallate	0.000	0.000	0.000
Total flavonols	105.857	51.292	32.979
<i>t</i> -rasveratrol	1.521	0.804	0.381

Piceatannol	0.000	0.000	0.000
(+)- Epsilon Viniferin	0.000	0.000	0.000
Resveratrol	0.369	0.100	0.031
Total stilbene	1.890	0.904	0.412
Vanillic acid	0.000	0.000	0.000
Protocatechuic acid	5.426	2.254	1.683
Syringic acid	0.000	0.000	0.000
Gallic acid ethyl ester (Ethyl gallate)	17.485	9.184	6.547
4-Hydroxybenzoic acid	0.000	0.000	0.000
Gentisic acid	0.000	0.000	0.000
Gallic acid	53.880	26.473	17.894
Total hydroxybenzoic acids	76.791	37.912	26.125
Caffeoyl tartaric acid	0.000	0.000	0.000
<i>p</i> -Coumaric acid	0.000	0.000	0.000
Ferulic acid	0.261	0.113	0.073
Caffeic acid	6.820	3.739	2.432
Total hydroxycinnamic acids	7.081	3.851	2.505

5. Chapter V – General conclusions and future perspectives

Prototype non-destructive sensing represents a highly promising frontier in modern research applied to oenology, due to its ability to provide agile, economical, and efficient tools for monitoring key parameters throughout the production chain. However, the emerging nature of these technologies necessitates an initial phase of extensive applicability studies, essential for verifying not only the validity of measurements but also their reproducibility, robustness, and adaptability to real-world production contexts.

In this work, the applicative potential of various prototype devices based on different technologies was explored, with a particular focus on the analysis of phenolic and volatile compounds during the winemaking process. Through the use of two spectrophotometers – one operating in the VIS region and the other in the NIR region – the monitoring of phenolic extraction during red wine fermentation was systematically addressed. Tests were conducted at laboratory scale on different grape varieties and according to various vinification protocols, in order to cover a wide range of operating conditions and analytical concentrations, thus ensuring good representativeness of the real variability characterizing the oenological sector.

The results highlighted that the first prototype, based on VIS spectroscopy, although allowing a preliminary modelling of phenolic parameters, showed limitations in terms of predictive robustness, probably due to the reduced spectral range and resolution of the instrument. A comparison with a commercial VIS spectrophotometer confirmed inferior performance, underlining the need for further technological optimizations. Conversely, the prototype operating in the NIR region demonstrated significantly improved predictive capabilities for the phenolic parameters considered. Although the results have not yet matched those of commercial devices, the observed improvement indicates promising development potential for this platform.

Looking ahead, the combination of VIS and NIR technologies into a single device would represent a particularly promising solution, capable of merging ease of use with excellent analytical coverage. Most wine phenolic compounds show characteristic absorptions in the UV-VIS region, particularly at 280 nm, 307 nm, 365 nm, and 520 nm. At 280 nm, the main absorption of simple phenols (phenolic acids and monomeric flavanols) is observed; at 307 nm, more structured flavonoids such as stilbenes prevail; at 365 nm, flavonols and maturation-derived compounds dominate; while at 520 nm, free anthocyanins responsible for the intense red color of red wines are detected. Integrating these measurement points into a VIS spectrophotometer would enable direct monitoring of the main phenolic compounds responsible for the visual, olfactory, and gustatory quality of wine.

4196 While the VIS provides specific data on pigments and phenols, the NIR offers global information on
4197 the chemical-physical structure of the wine matrix, allowing for the simultaneous estimation of
4198 parameters such as total polyphenols, residual sugars, alcohol, acidity, and colloidal structure.
4199 Therefore, a combined VIS-NIR device could significantly enhance the precision of predictive
4200 models and the reliability of measurements under winery operating conditions, representing an
4201 important step towards portable, economical, and rapid sensing systems for quality control in
4202 oenology.

4203 In parallel, the exploration of QCM-D-based sensor solutions has opened new perspectives in
4204 phenolic monitoring. In particular, a sensor functionalized with salivary proteins showed interesting
4205 selectivity towards hydroxybenzoic acids, demonstrating the concrete possibility of discriminating
4206 specific phenolic families based on their chemical structure. The sensor achieved good predictive
4207 levels for total polyphenol content, highlighting the effectiveness of the approach. However,
4208 challenges remain in the complexity of the immobilization procedure, the stability of the
4209 functionalization, and the limited range of recognizable compounds. In the future, the use of new
4210 functionalization strategies, such as engineered peptides or proteins, could increase specificity
4211 towards phenolic compounds of particular interest, such as catechins, epicatechins,
4212 proanthocyanidins, or condensed tannins. The ability to discriminate individual tannins or related
4213 phenolic categories would open new opportunities for advanced qualitative analysis, crucial to
4214 address phenolic ripeness issues linked to climate change. A new generation sensor could assist
4215 producers in evaluating grape phenolic quality, monitoring tannin extraction during fermentation, and
4216 predicting the astringency potential of wines during aging. To achieve these goals, it will be necessary
4217 to optimize the chemistry of sensor surfaces, improve operational robustness in complex matrices
4218 such as must and wine, and build advanced predictive models based on chemometric approaches.

4219 Overall, the results of this research confirm the great potential of non-destructive technologies in the
4220 oenological sector, while highlighting the need for further efforts in validation, reliability, and
4221 operational ergonomics. The future challenge will be to develop increasingly high-performing,
4222 accessible, and easily integrable tools within production protocols, without requiring high-level
4223 technical skills.

4224 In light of climate change and new production needs, the adoption of rapid, reliable, and non-
4225 destructive technologies appears not only desirable but essential. If perfected and validated, prototype
4226 sensing technologies could play a key role in promoting more sustainable, conscious, and quality-
4227 oriented viticulture and oenology. In the future, it would be desirable to extend applicability tests to
4228 industrial scale and integrate these sensors into real-time monitoring systems, in view of the

4229 digitalization and automation of processes. Only a multidisciplinary approach, combining basic
4230 research, technological development, and field validation, will complete the transition of these
4231 technologies from the laboratory to everyday operational practice.