Research Article

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Optimizing the winemaking process: NIR spectroscopy and e-nose analysis for the online monitoring of fermentation

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Abstract

BACKGROUND: In the winemaking process, the rapid determination of specific quality parameters such as sugar content, pH, acidity, concentrations of phenolic compounds, anthocyanins and volatile organic compounds is crucial for high-quality wine production. Traditional analytical methods allow for precise quantification of these parameters but are time-consuming and expensive. This article explores the potential application of non-destructive analytical technique (NDAT) (near infra-red [NIR] and e-nose), as efficient alternatives for online monitoring of fermentation working on two different winemaking tanks and applying chemometrics to develop predictive models to correlate non-destructive and analytical data.

RESULTS: NIR measurements have been used to build principal components regression models, showing good prediction capability for polyphenols, anthocyanins, glucose and fructose. Both offline and online e-nose applications demonstrate good capability of discriminating different fermentation phases, in agreement with aromatic profile changes observed via gas chromatography-mass spectrometry analysis. Moreover, correlation analysis reveals the potential of quartz microbalances, Taguchi Gas Sensors and H_2S sensors in predicting the concentration of compounds of great interest for winemaking (e.g. C6 alcohols, ketones, terpenes and ethyl esters) highlighting the robust connection between sensor data and specific chemical classes.

CONCLUSION: This research aims to showcase the potential employment of NDAT for online monitoring the evolution of must composition during fermentation. The proposed methods could potentially fulfil a longstanding requirement of winemakers, enabling them to closely monitor fermentation allowing the timely making of important technical decisions aimed at achieving oenological objectives in wine production.

Supporting information may be found in the online version of this article.

Keywords: non-destructive analytical techniques; fermentation monitoring; NIR; E-nose; QMBs

INTRODUCTION

Winemaking is a complex bioprocess that is influenced by countless factors, including the conditions in which it proceeds (temperature, pressure and atmosphere composition) and the adopted cellar practices.[1,2] These factors can greatly impact the phenolic extraction during maceration, alcoholic fermentation and the final composition of wine, in terms of volatile organic compounds (VOCs).[2,3]

As technology evolves, wineries are constantly searching for innovative approaches to improve their operating protocols keeping high quality standards. This is especially significant when considering the damaging issues of climate change, labour shortages and ever-rising production costs. On the other hand, consumers are increasingly demanding and, also given the growing competitiveness of the market, having tools that allow for winemaking process optimization is of paramount importance. Wineries require fast and economical methods to obtain timely information to effectively control all stages of the process.

The determination of specific qualitative parameters of grapes, musts and wines (e.g. sugar content, pH, titratable acidity, organic

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acids level and volatile acidity), as well as the quantification of important molecule classes constituting wine composition (e.g. phenolic compounds, anthocyanins, VOCs, etc.), is essential for optimally managing the vinification process, from harvesting to the stages of fermentation, maceration, Monitoring these quality parameters represents a key factor for ensuring the production of chemically and biologically stable high quality wines. The analytical methods traditionally used to determine these parameters often do not correspond to the needs of modern wine production in a global market. While they provide a precise indication of wine composition, they are often too slow and expensive, requiring specialized personnel and very expensive equipment.[4] Recently, with the final aim of improving product quality and reducing analytical costs for the wine sector, different non-destructive analytical techniques (NDAT) have been developed as fast and cheap alternatives to the conventional analysis. These techniques could satisfy an old need of winemakers, in that they could be able to closely follow fermentation evolution in terms of must/wine composition, making the proper technical decisions timely to achieve their oenological objectives.

NDAT are already applied to various foods, including grapes and wine, and could represent a useful and innovative approach. The analytical assessments involve the utilization of sensors that rely on physical–chemical properties, such as irradiance, light energy, fluorescence, optics, acoustics and semiconductor resistance, etc. These sensors operate by interacting with organic molecules.[5,6] Through chemometrics, it is possible to combine non-destructive data with destructive features of foods to develop predictive models linking sensor signal to molecule concentration.[6] A number of previous studies report examples of the different NDAT applications aiming at monitoring winemaking processes and profiling wines. For example, surface acoustic waves have been tested to automatize polyphenols measurement during the extraction process.[7] Moreover, different spectroscopy technologies have been used for grapes and wines analysis, such as near infra-red (NIR) or medium infra-red (MIR) spectroscopy, as well as Fourier Transformed (FT)-NIR/FT-MIR. These applications have primarily focused on measuring different wine components, such as ethanol, sulfur dioxide, acetic acid, organic acids and reducing sugars.[8-11] Additionally, the electronic nose (e-nose) has gained significant attention due to its high operational flexibility. It is particularly valuable to differentiate wines based on their aromatic fingerprint.[12-14] This technology is constituted by an array of different sensing tools, in most cases comprising metalloporphyrins-based sensors, but similar results can be obtained using arrays of metal oxide-based sensors, as demonstrated by several studies.[15-18]

In this context, the present study explored the use of different NDAT specifically, NIR- acousto-optic tunable filter (AOTF) spectroscopy and e-nose based on both metalloporphyrin and metal-oxide sensors, in combination with chemometrics with the final aim of (i) monitoring fermentation process; (ii) characterizing fermenting musts in terms of technological parameters, phenolic and aromatic fingerprint; and (iii) using the chemometric approaches to develop predictive models for correlating non-destructive information with data obtained from traditional destructive analyses. To achieve these goals, two different winemaking tanks were monitored throughout the fermentation process analysing the must/wine in parallel with the mentioned sensors and destructive analyses. The obtained dataset has been explored applying chemometrics and correlation analysis.

MATERIALS AND METHODS

Must/wine maceration, fermentation and sampling

In 2022 vintage grapes belonging to the variety Sangiovese and characterized by regular phytosanitary conditions were hand-picked at their optimal ripening stage, followed by the addition of 5 g hL^{-1} of SO_2, and separately processed at the winery cellar Cantina Tuscania s.r.l. (Sambuca-Tavarnelle V.P., Florence, Italy) through two innovative soft maceration techniques of red grape vinification both patented by Parsec s.r.l. (Florence, Italy): (i) NECTAR-ADCF™ system in tank V58, where the overpressure as a result of the fermentation gas development is exploited to keep the cap submerged, favouring its disruption by the carbon dioxide escape through a valve, and (ii) the M.I.AIRMIXING™ system in tank V79, avoiding the cap formation through resonance waves obtained using a fixed sequential small injections of compressed air from the bottom of the tank.[19] The employed tanks had a volume of 10 hL. Alcoholic fermentation was carried out at controlled temperature using a dry commercial yeast (*Saccharomyces cerevisiae* EnartisFerm ES454; Enartis, San Martino, Italy). From both tanks, which have been employed for an identical number of days, 13 samples were collected during the whole fermentation process, each one taken immediately after wine mixing in the tank to ensure their representativeness.

Chemical characterization of musts and wine

The chemical characterization of musts and wines was carried out using a calibrated Fourier transform infrared analyzer Winescan FT 120 (Foss Analytics, Hillerod, Denmark). The following oenological parameters were determined (three replicates): pH, titratable acidity, hexoses, ethanol, glycerol, methanol, volatile acidity, malic acid, lactic acid, total extract, total anthocyanins and total polyphenols. The accuracy of the Winescan analyzer has been validated by destructive analyses performed with official methods during the calibration activity before starting the analyses, following the OIV methodologies.[20,21] Colour intensity (absorbance at 420 + 520 + 620 nm) and Hue (absorbance at 420/520 nm) were obtained (three replicates) with a commercial spectrophotometer (Varian Cary 100/300 UV-VIS; Varian Medical Systems, Inc., Palo Alto, CA, USA).

NIR-AOTF

Must samples were non-destructively analyzed by detecting Near Infrared (NIR) spectra using a Luminar 5030 AOTF-NIR Analyzer (Brimrose, Baltimore, MD, USA). Analyses were conducted using the transmittance method of detection with 2-nm wavelength increments working in the range 1100–2300 nm. Four different spectra were taken for each sample and then averaged to obtain a representative spectrum. Raw spectra were transformed in absorbance (log1/*R*) using SNAP! 2.03 software (Brimrose).

E-nose

Two different types of e-nose equipment were employed for offline and online monitoring of VOCs, respectively.

Offline must VOC monitoring

A mass based (MS) e-nose was used for offline musts monitoring. This equipment is based on an array of 12 quartz microbalances (QMBs) and was designed, developed, and assembled at the University of Rome Tor Vergata.[22] The QMBs are respectively functionalized by Mg, Co, Cu, Zn, Fe, Mn, Sn, free base (H2) of 5, 10, 15, 20-tetrakis-(4-butyloxyphenyl) porphyrin, free base (H3),

copper, phosphorus and manganese complexes of 5,10,15-triphenylcorrole; the control of the instrument and data collection are managed by custom software in MATLAB.[22] The sampling protocol for offline must measurement includes the following steps: 10 mL of must was incubated in closed vials at 30 ± 1 °C for 20 min. The equilibrated headspace was extracted for 90 s using a stream of filtered air and delivered into the electronic nose sensor cell. After each measurement, the e-nose system was cleaned with a pure airstream for an additional 300 s. Sensor signals were calculated as the resonant frequency shift between the two steady conditions (i.e. sensors exposed to pure air and the sample). Four different vials were analyzed for each sample, the four values were then averaged to have two values per sample. In the following sections, this device is referred to as *e-nose_A*.

Online must VOC monitoring

For the online monitoring of must VOCs the employed e-nose device is composed of an array of different metal-oxide semiconductor (MOX) sensors. This e-nose has been installed directly in the vinification tank, allowing the online monitoring of the head-space. Tank V79 only has been equipped with this sensor array as a result of the simultaneous vinification in the two tanks. In more detail, five Taguchi Gas Sensors (TGS), as well as two H_2S (hydrogen sulfide) and three SO_2 (sulfur dioxide) sensors, have been installed on this e-nose device. These sensors were chosen based on the compound detection technology and intrinsic characteristics related to cross-sensitivity/selectivity and composition of the sensing part. The device is physically made up of a modular cylindrical chamber in which the vapors to be analyzed are conveyed into the device with a flow rate of approximately 100 mL min^{-1} using a pump (see Supporting information, Fig. S1). A specific valve allows the measurement phases to be alternated with cleaning phases exerted via fluxing clean air through the device. This procedure allows to prevent bias due to corruption of sensing capability of the different hardware. Data acquisition is entrusted to an single board computer, which, with a sampling time of 1 min, acquires data from the sensors. In the following sections, this device is referred to as *e-nose_B*.

Headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS)

The VOCs profile of must samples produced during the different experiments in tank V58 and V79 has been investigated via head-space solid-phase microextraction coupled to GC-MS analysis. For both fermentation tanks, three different technical replicates were analyzed for each sample. The employed VOCs sampling protocol, the analytical equipment and settings, as well as the identification and quantification procedures have been previously reported by Modesti *et al*[23]

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-polyphenol profiling

The phenolic profile of must samples produced from both tank V58 and V79 has been investigated via LC-MS/MS, analysing each sample in three technical replicates. The employed phenolic extraction protocol, the analytical equipment and settings, as well as the identification and quantification procedures have been previously reported by Shiriaev *et al*[24] SRM transitions and the corresponding compound parameters are reported in the Supporting information (Table S1).

Statistical analysis

The transformed NIR spectra have been first tested with different pre-treatments (i.e. Savitzky–Golay filter for first and second derivatives at 15 and 25 points of smoothing, standard normal variate) and used for different principal component analysis (PCA) models. The best modelling performance was obtained with simply absorbance-transformed spectra. The spectra were also used to build a linear regression model using a principal components regression (PCR) with the NIR information as predictor variables and destructive analyses as response variables ($n = 13$). Both datasets were first auto scaled and venetian blinds with blind thickness = 1 was used as a cross-validation method. Finally, the two datasets were integrated into a single data matrix to ensure a higher variability ($n = 26$) and used for the construction of an additional model.

E-nose numerical data were pre-treated with two normalization filters (mean centering and autoscaling) and then analyzed with PCA using venetian-blind as validation method (with a blind thickness = 1). To verify the capacity of e-nose to correctly identify different samples based on the aromatic composition, a PCA model was built, with the same modelling conditions, with GC-MS data.

For the cross-validation strategy, the Venetian blinds method was selected because of its suitability for small sample sizes. The blind thickness of 1 implies that each spectrum/e-nose signals was included in a separate validation set, whereas the remaining data were used for model calibration. This approach helps mitigate the risk of overfitting and provides a robust estimate of the model performance on independent datasets. It is important to note that the chosen cross-validation method aligns with best practices in chemometric modelling, considering the specific characteristics of the dataset. This rigorous validation process enhances the reliability and generalizability of the developed model, ensuring that it is well-suited for predictive applications in the context of our study. Multivariate analyses were performed separately for the two vinification tanks (V58 and V79) using Matlab R2013a (MathWorks, Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA).

RESULTS AND DISCUSSION

Winemaking and fermentation kinetics

As previously reported,[19] the two maceration techniques employed ensure uniform conditions in the tank in terms of temperature and gas distribution, favouring the yeast development. The results confirmed the correct choice of the yeast strain used for the fermentation because it was suitable for must with a high sugar content, high glycerol producer and guaranteed for low volatile acidity. Both the fermentations, indeed, showed a continuous consumption of sugars, reaching an ethanol content of 14.4 and 14.6% vol, respectively (see Supporting information, Figs S2 and S3), with less than 3 g L^{-1} of residual sugar and a volatile acidity lower than the sensory threshold of acetic acid (Table 1). Nevertheless, a higher value was observed in V79 wine, likely attributable to the impact of micro-oxygenation applied by the air-mixing system.

A significant difference was recorded also considering the glycerol content, which was lower in V79 wine. The observed glycerol fermentation pattern could be a result of the different winemaking techniques applied in the two tanks, which conferred a different amount of oxygen to the bulk.[19]

Table 1. Chemical composition of the two Sangiovese wines at the end of fermentation period (Year 2022)

Parameter	Units	V58	V79
Hexoses	g L^{-1}	2.56 ± 0.04	2.47 ± 0.13
Glycerol	g L^{-1}	11.63 ± 0.05	9.68 ± 0.01
Ethanol	% vol	14.40 ± 0.02	14.66 ± 0.01
Malic acid	g L^{-1}	0.76 ± 0.02	0.91 ± 0.01
Lactic acid	g L^{-1}	0.48 ± 0.01	0.60 ± 0.02
pH		3.25 ± 0.01	3.43 ± 0.01
Titratable acidity	g L^{-1} tartaric acid	7.65 ± 0.06	6.56 ± 0.03
Volatile acidity	g L^{-1} acetic acid	0.30 ± 0.01	0.52 ± 0.01
Methanol	mg L^{-1}	210 ± 1	160 ± 1
Total Anthocyanins	mg L^{-1} malvidin	387 ± 2	313 ± 8
Total Polyphenols	mg L^{-1} gallic acid	2456 ± 36	2412 ± 23
Colour intensity	Absorbance 420 + 520 + 620	1.26 ± 0.02	1.33 ± 0.07
Hue	Absorbance 420/520	0.54 ± 0.01	0.53 ± 0.01

Data are expressed as the mean ± SD of three samples, each one taken immediately after wine mixing in the tank.

During both winemaking processes, the onset of malolactic fermentation (MLF) has been observed thanks to the compatibility of the employed yeast strain with MLF. However, as shown in Table 1, in both experimental tanks MLF has not yet reached completion at the end of the observation period.

The measured methanol content in wine was below the legal limit for red wines (400 mg L^{-1}) in both V58 and V79 samples. However, a higher value was recorded in the V58 wine, probably as a result of the different maceration conditions applied for this tank, which enabled a higher exposure to grape skins during fermentation.[19]

Prediction of phenolic composition and technological parameters by NIR spectroscopy

Vibrational spectroscopy has revolutionized quality assessment in winemaking, particularly regarding the crucial monitoring of phenolic compounds in grape berries for high-quality wines. For example, García-Estévez *et al.*[25] have used PCR to predict phenolic and anthocyanins composition of grapes by hyperspectral measurements. Moreover, Cozzolino *et al.*[26] used visible-NIR measurement to quantify condensed tannins in different grape varieties. In this context, the NIR measurements have been used to build a PCR model to predict the phenolic composition of fermenting musts as well as other quality parameters (i.e. total acidity, acetic acid and glucose/fructose).

Considering the tank V58, two principal components were selected to reach a cumulative explained variance of about 81%. The model shows quite good prediction capability for polyphenols. For example, on a total of 33 phenolics identified, an R^2 higher than 0.6 (in CV) was obtained for the prediction of important compounds (i.e. coumaric acid, gallic acid, resveratrol, narigenin, phloretin, catechin and epi-cathechin, quercetin, and procyanidins B1, B2 and B3) (see Supporting information, Table S2). Moreover, an R^2 of 0.79 and 0.67 was obtained for total polyphenols (Cal and CV, respectively) (Fig. 1A), 0.75 and 0.64 for total flavonoids (Fig. 1B), and 0.74 and 0.61 for total anthocyanins (Fig. 1C). Furthermore, a good prediction index was obtained for glu/fru as well (Fig. 1). On the other hand, worst performances have been obtained in accurately predicting acetic acid and total acidity (with an R^2 in Cal and CV of 0.48 and 0.07, 0.69 and 0.50, 0.31 and 0.03, respectively). As far as V79 tank is concerned, selecting two principal components (for a cumulative explained variance of 85%) relatively high prediction performance was obtained not only for total polyphenols (Fig. 2A), flavonoids (Fig. 2B) and anthocyanins (Fig. 2C), but also for glu/fru (Fig. 2D), acetic acid (R^2 in Cal and CV of 0.73 and 0.67, respectively) and total acidity (R^2 in Cal and CV of 0.62 and 0.58, respectively) (data not shown). As far as single phenolics are concerned, the models show results quite similar to what we report for V58 tank. Specifically, an R^2 higher than 0.6 in CV was observed not only for coumaric acid, gallic acid, resveratrol, naringenin, phloretin, catechin, epi-catechin, quercetin, and procyanidins B1, B2 and B3 (as in V58), but also for protocatechuic acid, luteolin, myrcetin and kaempferol-3-*O*-glucoside (see Supporting information, Table S3).

To overcome the evident challenges posed by the limited number of samples, an additional PCR analysis was conducted by integrating data from both tanks, resulting in a combined dataset of $n = 26$ samples. This approach has been used mainly to glean insights into the robustness of the approach and to ascertain whether the performance of the models remained consistent when using the aggregated dataset. Three principal components were selected to reach a cumulative explained variability of 82.15% (PC1 65.99%, PC2 13.63% and PC3 2.53%). Remarkably, our findings reveal that the models constructed using the combined dataset exhibit performance metrics that are equally commendable as those derived from the separate models reported in the initial analysis (see Supporting information, Table S4). In general, the overall accuracy of the model remains largely consistent with the previous iteration. However, a nuanced examination reveals variations in performance across specific compounds. Notably, compounds exhibit heightened model performance, showcasing an improvement in predictive capability. Conversely, a subset of compounds experiences a marginal reduction in model performance. Despite the overall stability in accuracy, the nuanced performance fluctuations among specific compounds prompt a need of using a larger-scale dataset in future studies.

Most of the research regarding the application of NIR technology in grape analysis emerged over the past decade and, in all studies employing vibrational spectroscopy, researchers consistently noted similar patterns of absorbance across the spectrum. However, because of the intricate interplay of absorption

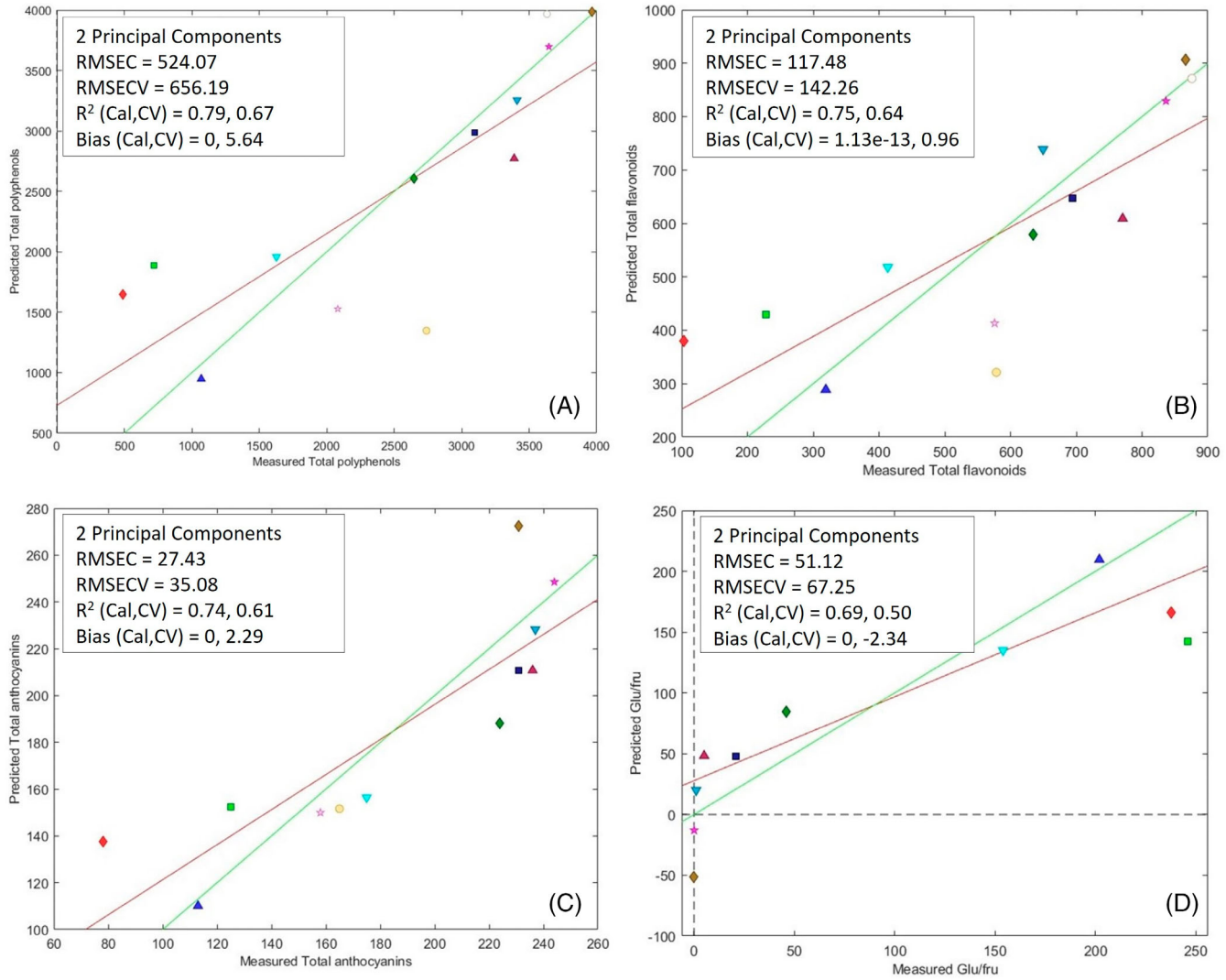

Figure 1. Score plots of the PCR model of V58 must during alcoholic fermentation built with NIR spectra (as predictor variables) and total polyphenols (A), total flavonoids (B), total anthocyanins (C) and glucose fructose ratio (D) (as response variables). RMSEC, root mean square error in calibration; RMSECV, root mean square error in cross-validation; R^2CAL, correlation coefficient in calibration; R^2CV, correlation coefficient in cross-validation.

mechanisms and overlapping bands, establishing a direct link between the concentration of phenolic compounds and the size of these bands represents a challenge.[27] Despite that, phenolic compounds tend to display consistent bonding patterns with minor deviations, and the significant bands that are identified as the main responsible for polyphenols identification also remain consistent. Specifically, C—H bonds display stretching at third (928 and 940 nm), second (1148 nm) and first overtones (1620 and 1652 nm).[28] The wavelength region between 1100 and 1300 nm corresponds to a combination band involving symmetric and antisymmetric stretching vibrations of O—H, as well as a combination band related to the second overtone of C—H aromatic vibrations and the third overtone of C—H vibrations.[29] These distinctive traits can be attributed to the chemical structure of phenolic compounds. Finally, the range around 1600 nm appeared to be linked to condensed tannins.[26,27] As mentioned earlier, considering the significance of phenolic compounds in shaping the sensory attributes of wine, novel approaches involving chemometric analysis and vibrational techniques such as NIR proposed here, represent a good opportunity for quickly gaining good results also reducing analytical costs. Our findings highlight the stability of NIR technology in discriminating different concentrations of specific, and important, phenolic molecules in both the analyzed fermentation tanks. Therefore, these results confirm the feasibility of developing models that, when properly trained and validated via destructive analyses, can potentially allow for the online monitoring of these molecules during the fermentation process. However, it is essential to emphasize the need for deploying models on a larger dataset characterized by increased variability. The present study lays the foundation, although the true test of models practical applicability lies in their performance on a more extensive dataset, ideally including unknown samples. This step is crucial to validate models efficacy in real-world scenarios and production-scale applications.

VOC evolution during must fermentation

The results of GC-MS analysis of must samples throughout the two monitored fermentation processes have been used for building two PCA models, one for each vinification tank (Figs 3 and 4 for V58 and V79, respectively). In total, 67 compounds were identified

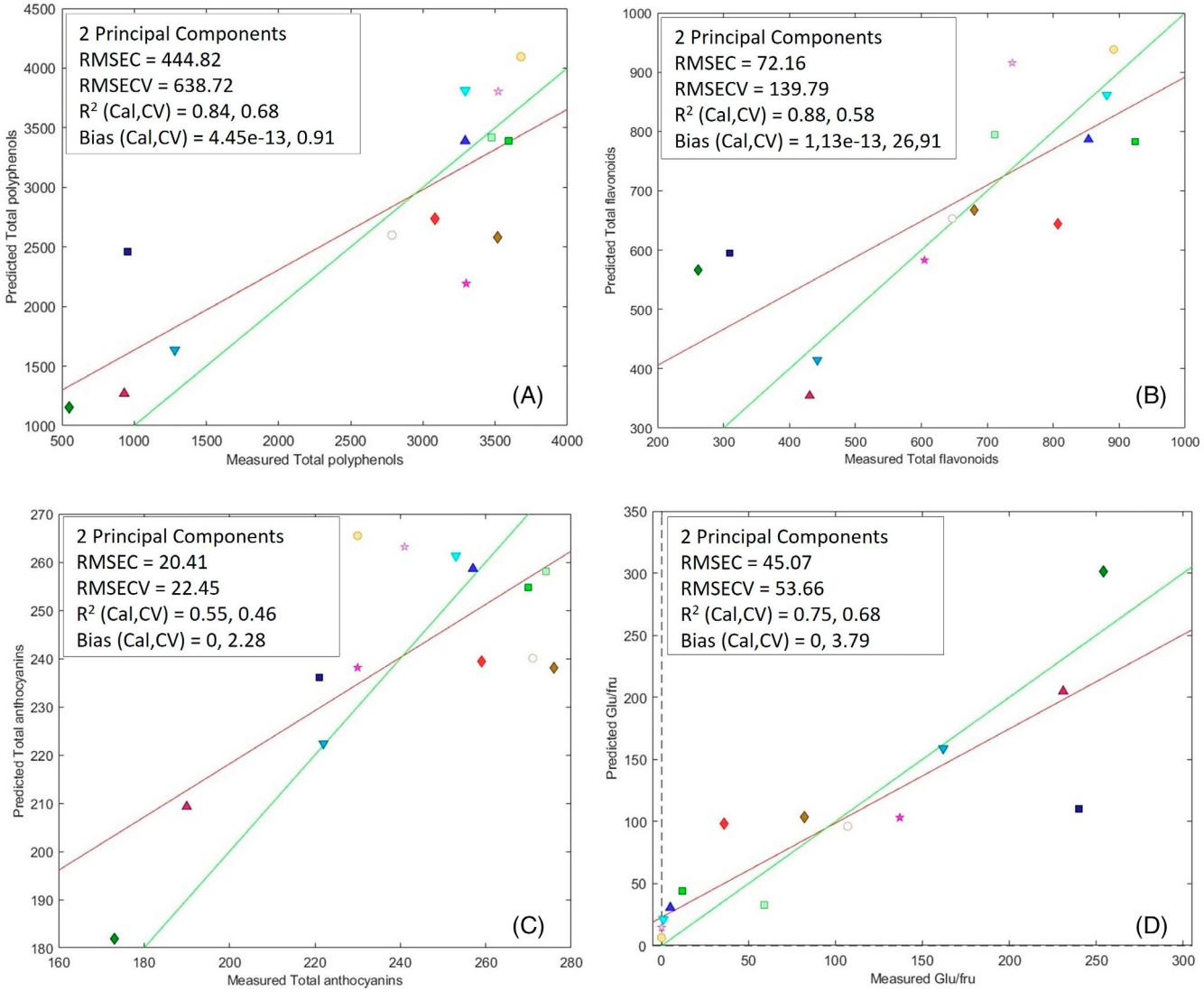

Figure 2. Score plots of the PCR model of V79 must during alcoholic fermentation built with near infra-red (NIR) spectra (as predictor variables) and total polyphenols (A), total flavonoids (B), total anthocyanins (C) and glucose fructose ratio (D) (as response variables). RMSEC, root mean square error in calibration; RMSECV, root mean square error in cross-validation; R^2CAL, correlation coefficient in calibration; R^2CV, correlation coefficient in cross-validation.

belonging to alcohol, aldehyde, ketone, ester, organic acid and terpenoid classes.

As far as the tank V58 is concerned, PCA model describes about 94% of the total variability considering together the first two principal components (Fig. 3). The spatial distribution of the different sampling times and detected VOCs provides significant insights regarding aroma evolution during fermentation. The first three sampling times (musts after 1, 3 and 4 days of fermentation) tend to separate from the rest of the samples (5–17 days), with the first day of fermentation clearly separated from all the other sampling times. By contrast, musts from the fifth day of fermentation onwards sit close to each other and are linearly distributed along PC2. Regarding the disposition of the different VOCs on the plot, there is a clear spatial distribution along the PC2 and the formation of two main clusters on PC1. A first cluster that characterizes musts at the beginning of fermentation (1–4 days) and the other that correlates with the remaining samples (5–17 days). A clear accumulation of phenylethyl alcohol, ethyl alcohol and ethyl esters along fermentation time, flanked by the decreasing of other compounds, such as terpenoids, other alcohols and aldehydes, and hexyl derivatives, is evident in the dataset.

Figure 4 reports a second PCA model built using GC–MS data from V79 tank. Overall, this model describes about 84% of the overall variance. The results of this second analysis are very similar to that reported for the V58 tank, both in terms of sample clustering and volatile distribution. Also in this case, it is possible to appreciate a clear separation, mostly along PC1, between the first stage of fermentation (1–3 days), again with the first day well segregated from the others, and the rest of the samples (6–17 days), which are vertically distributed along PC2. On the other hand, volatile evolution mirrored the one observed for V58, with the different classes of volatiles showing a similar trend over time in both tanks.

Notably, certain compounds of significant relevance in Sangiovese grape-derived wines, such as the norisoprenoid β-damascenone, along with other terpenes such as nerol, dihydromyrcenol, β-linalool, terpinen-4-ol and α-terpineol, decreased as fermentation progresses in both tanks. Terpenes are molecules of interest because they hold great organoleptic significance due to their

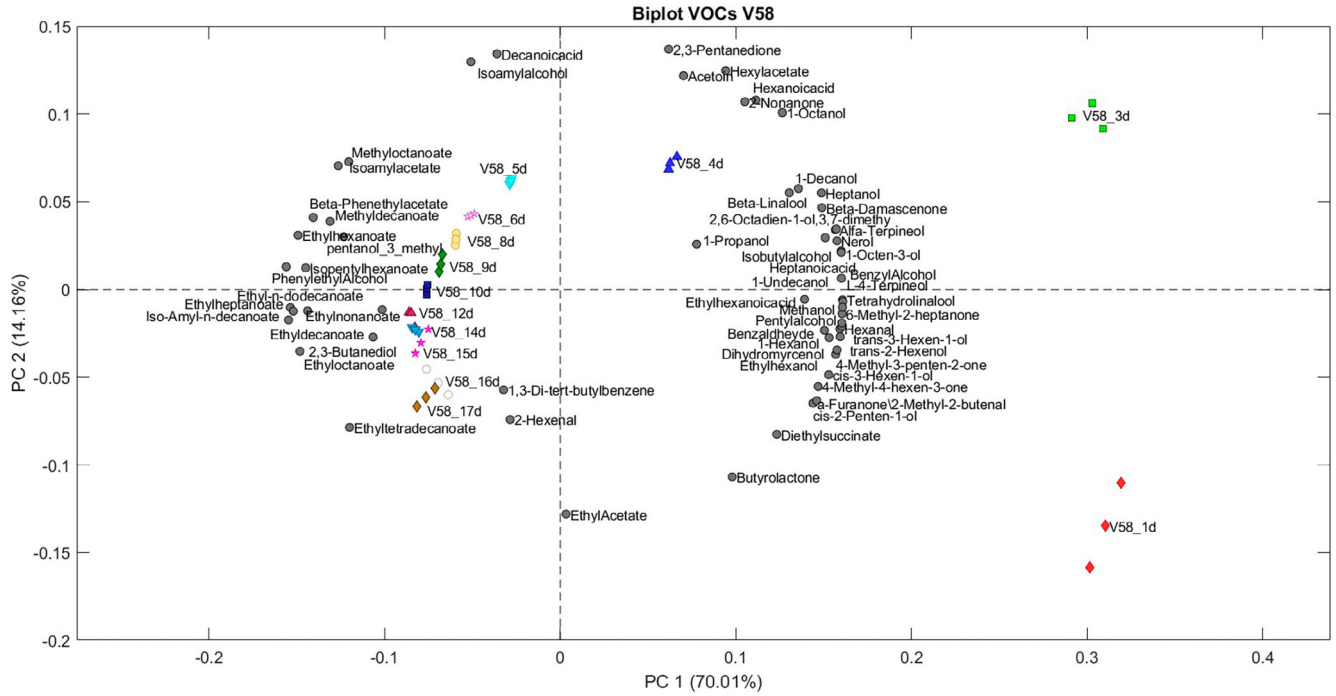

Figure 3. Biplot (PC1 *versus* PC2) of sample scores and variable loads obtained by PCA performed on VOCs detected in musts from V58 during alcoholic fermentation.

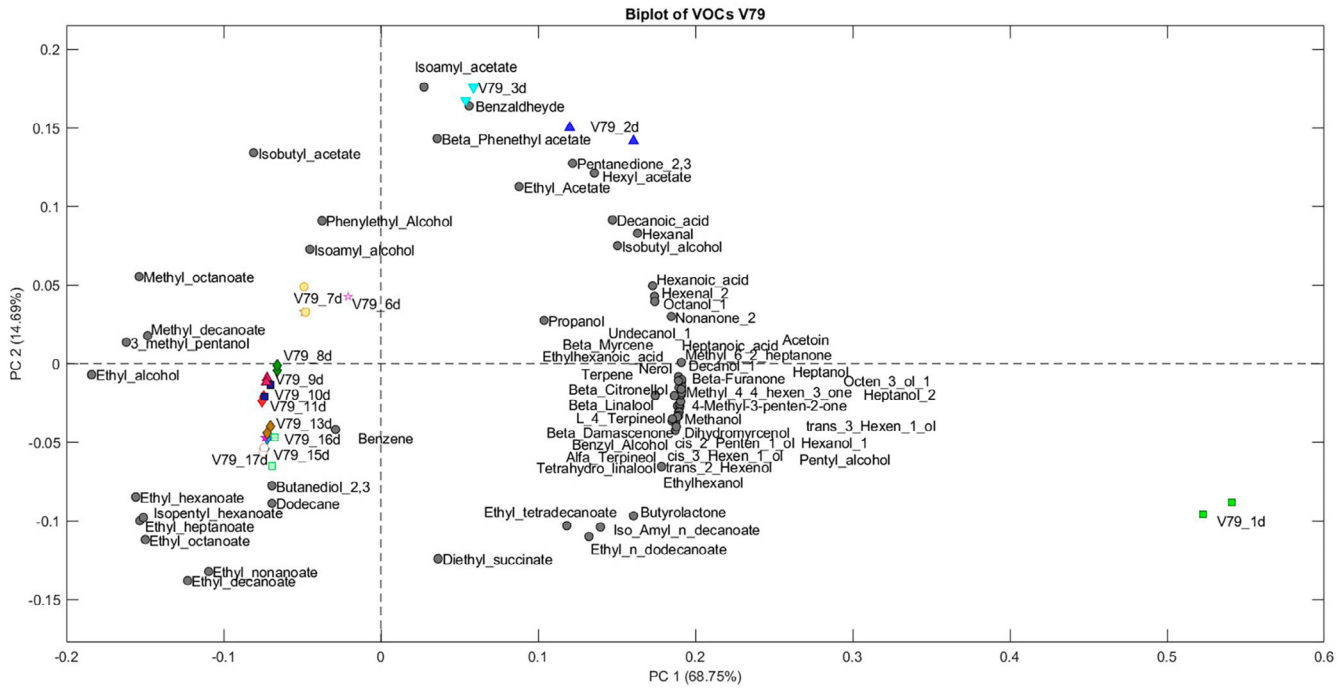

Figure 4. Biplot (PC1 *versus* PC2) of sample scores and variable loads obtained by PCA performed on VOCs detected in musts from V79 during alcoholic fermentation.

very low odour threshold (OTH) ranging from 0.3 to 100 μg L^{-1}, much lower than the ones of ester volatiles (from 5 to 7000 μg L^{-1}) or higher alcohols (from 8000 to 5000 μg L^{-1}).[30] This implies that even slight fluctuations in the concentration of terpene molecules within musts/wines can be readily discerned by consumers.

Additionally, the most of ethyl esters showed a strong increase over time. These esters originate both from esterification reactions involving must organic acids, (i.e. butanoic, hexanoic, 2-hexenoic, heptanoic and octanoic acid, etc.) ethanol and yeast activity during fermentation.[31,32] They constitute the most abundantly produced and accumulated esters in wine/must, holding

notable fruity flavour.[30] Different maceration techniques, such as post-fermentative maceration, can strongly modify wine aroma, driving to increased concentration of terpenes, norisoprenoids, as well as volatile esters, leading to a stronger flowery and spicy aroma compared to non-macerated wines.[33] Lastly, in both V58 and V79, the level of C6 alcohols such as 1-hexanol, trans-2-hexen-1-ol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol, which are characterized by green/leafy aroma, decreased along time, likely as a result of yeast consumption.

Offline VOC monitoring in must

Offline analysis of must samples has been carried out using the porphyrine-based *e-nose_A*. Two PCA models have been built using data produced analysing offline V58 and V79, respectively (Figs 5 and 6).

As far as V58 is concerned, the PCA model describes about 93% of the variability present in the dataset considering together the first two principal components (Fig. 5). In accordance with the results reported in Fig. 3, it is possible to appreciate the formation of different clusters at different sampling times. Four different clusters are evident, each one located in a different quadrant of the plot: a first cluster includes samples from 1 to 3 days; a second cluster groups musts sampled between day 4 and 8; a third cluster contains the fermenting musts sampled between day 9 and 16; and the fourth cluster includes the last sampling time alone at 17 days. Most of the loadings (*e-nose_A* channels, QMBs) are associated with the last days of fermentation (9–16 days), with QMB 10 and 11 that specifically correlate with day 17.

The results obtained by the second PCA model produced using data from the offline analysis of V79 samples are similar to those reported in Fig. 5, with QMBs being associated with late sampling times (Fig. 6). On the other hand, the separation among different stages of fermentation appears slightly different from that observed in the PCA model built using GC-MS data from the V79 tank (Fig. 4). Samples from days 1 to 10 are spatially distributed along PC2, whereas they are separated from the last sampling times along PC1. As occurred in tank V58, the different QMBs show higher signal levels at the last stages of fermentation, confirming a higher correlation with molecules which possess higher concentration in the last steps of the vinification process (e.g. ethyl esters). Notably, QMB 11 in V79 samples segregated close to musts recovered the first day of fermentation, whereas it was clustering close to day 17 in V58 samples, while QMB 10 still clustered close to the latest sampling times (Fig. 6).

To unravel possible linear correlations between QMBs and the distinct class of aroma compounds, we have produced two correlation tables, one for each tank, showing the relation detected with the corresponding values of R and R^2 (see Supporting information, Table S5 and S6). In the analysis of V58 musts, significant R^2 values were observed, especially in relation to QMB1. Specifically, R^2 values of 0.78 and 0.76 were recorded for the esters ethyl octanoate and ethyl tetradecanoate, respectively. Additionally, an R^2 value of 0.81 for hexanoic acid was registered. Esters for which the concentration tends to increase during fermentation exhibit positive correlations with all QMBs, whereas esters for which the concentration tends to decrease, specifically hexyl acetate and diethyl succinate, show negative correlations along with other classes of compounds like the majority of alcohols, ketones and terpenes (see Supporting information, Table S5). On the other hand, for V79 samples no strong correlations have been observed (see Supporting information, Table S6).

The results of the offline e-nose analysis have emerged as a very rapid and potentially useful tool, demonstrating prowess in effectively distinguishing between the different stages of the fermentation process. QMBs based e-nose technology has been used to discriminate between wines from different varieties and vintages,[12] identify off-flavours attributed to undesirable microorganisms,[13] differentiate a panel of sweet wines,[14] and discriminate wines produced using various maceration techniques.[21] Nevertheless, our approach reveals that the application of e-nose extends beyond a binary distinction, capturing the intricate interplay of diverse compounds. Its sensitivity enables detection and

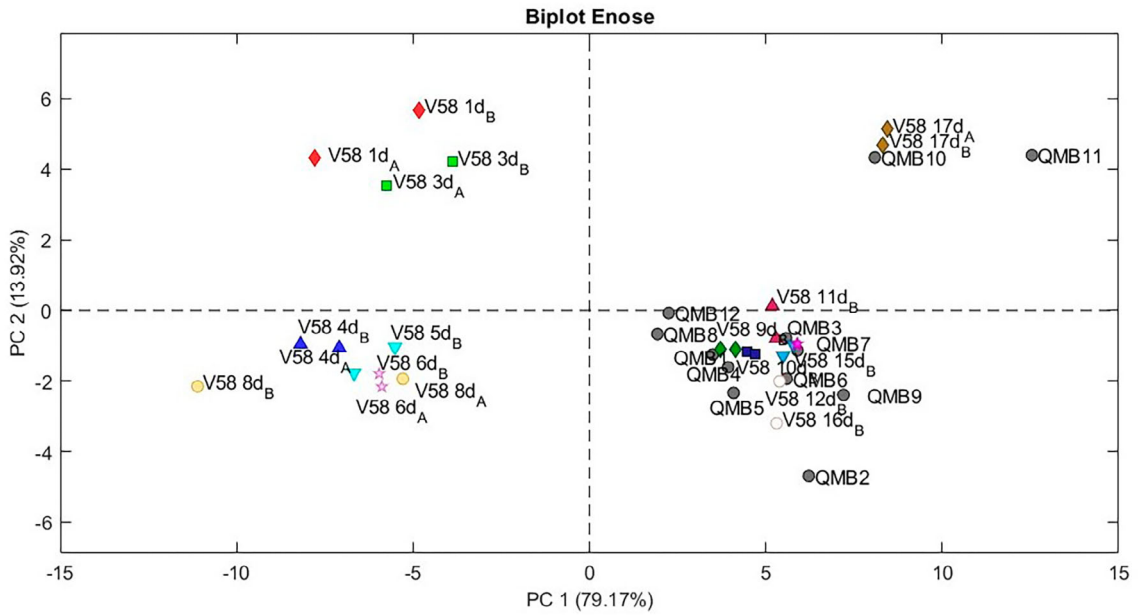

Figure 5. Biplot (PC1 *versus* PC2) of sample scores and variable loads obtained by PCA performed on e-nose_A data of musts from V58 during alcoholic fermentation.

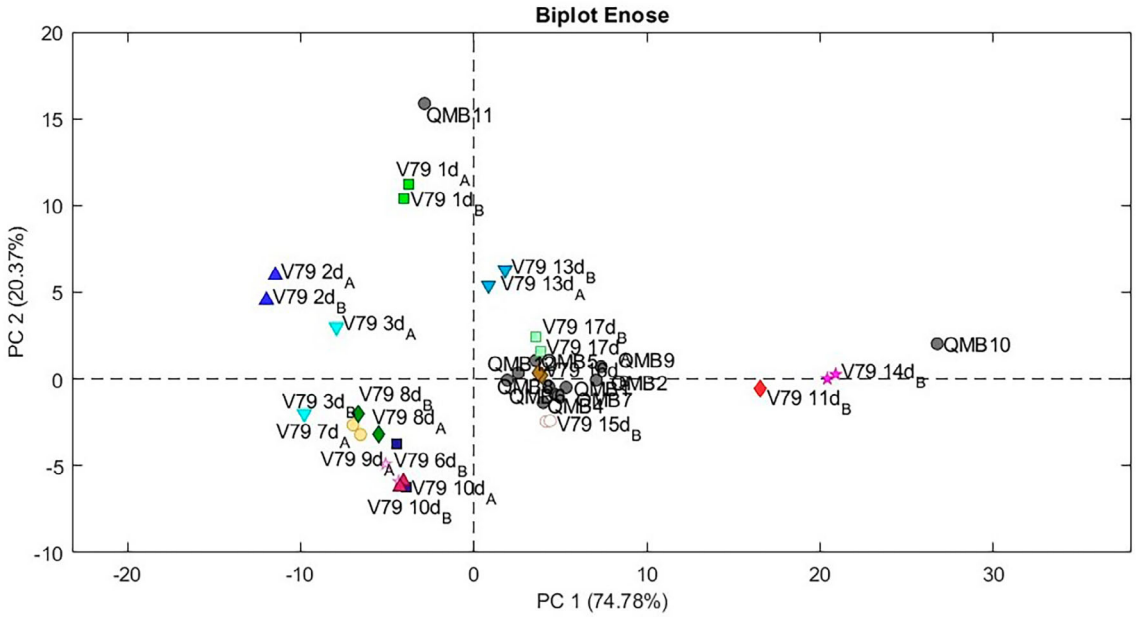

Figure 6. Biplot (PC1 *versus* PC2) of sample scores and variable loads obtained by PCA performed on e-nose_A data of musts from V79 during alcoholic fermentation.

response to the complex and dynamic chemical changes in progressing fermentation, providing a comprehensive understanding of the process dynamics. However, further evaluations with larger dataset are essential to fine-tune correlations between e-nose QMBs and the evolution and detection of specific molecules during fermentation. A common approach in e-nose technology often involves the utilization of MOX-based sensors, a methodology that has yielded promising results and that is the subject of the next section.

Online VOC monitoring in must

To evaluate the possibility of directly monitoring the evolution of must aroma during fermentation, e-nose_B has been installed on tank V79 only and has been used for online recording of sensors signals continuously. Figure 7 reports a PCA analysis performed using e-nose_B data: considering together the first two components, approximately 92% of the total variability is described by the model. TGS signals clustered in the bottom right quadrant of the plot together with must samples from 2 and 3 days. By contrast to that reported for QMBs, a noticeable decline in signal strength is evident over time for TGS. This decline well aligns with the level of volatile compounds for which the concentration similarly diminishes as time progresses, underscoring a robust correlation between TGS signals and the concentration of important molecules in the must, such as terpenes. On the other hand, other sensors that were installed on e-nose_B, namely H_2S_1 and H_2S_3, positioned diametrically opposite to the TGS sensors. This observation implies an increasing signal of these sensors along with the days of fermentation, potentially underlying good correlations with important molecules that increased over time, such as ethyl esters.

As well as for the offline VOC evolution, we have produced a correlation table showing the relation between detected VOCs and sensor signals with the corresponding values of R and R^2 (see Supporting information, Table S7). These correlations show that the TGS sensors could be used as good predictors for C6 alcohols, ketones and terpenes concentration. Specifically, R^2 values for all terpenes, except for beta-citronellol, ranged from 0.80 to 0.90, and, for all ketones excluding the 2-nonanone, the R^2 values ranged from 0.85 to 0.97. By contrast, the pattern is less clear for C6 alcohols. Indeed, approximately half of the detected alcohols show good correlations with TGS sensors, with R^2 values ranging from 0.80 to 0.90, whereas the remaining alcohols fall below the threshold of R^2 equal to 0.70. These differences may be probably attributed to the various factors influencing the concentration of these compounds because their presence does not always correlate with a linear increase during fermentation. On the other hand, H_2S sensors signal may be practically linked to ester levels. Particularly, hexyl acetate and ethyl octanoate, nonanoate and decanoate exhibit the highest values of R^2 among this class equal to 0.76, 0.79, 0.74 and 0.75, respectively. This has the potential to yield significant outcomes, particularly when considering the critical need for real-time monitoring the fermentation process. The online application of e-nose for VOC monitoring during fermentation could enable timely interventions to shape wine aroma profile and prevent the formation of undesirable off-flavors. However, it is important to note that wine aroma is not solely the sum of individual molecules but rather the result of a complex interaction among them.[6] Consequently, driving the fermentation in a specific direction can result in a diverse bouquet, ultimately achieving the desired wine style.

CONCLUSIONS

Determining specific qualitative parameters of grapes, musts and wines is essential for optimizing the wine making process, as well as ensuring the production of chemically and biologically stable, high-quality wines. Recognizing the evolving needs of the modern global wine industry, this article discusses a possible application of NDAT as efficient and cost-effective alternatives to monitor the fermentation process in terms of must/wine

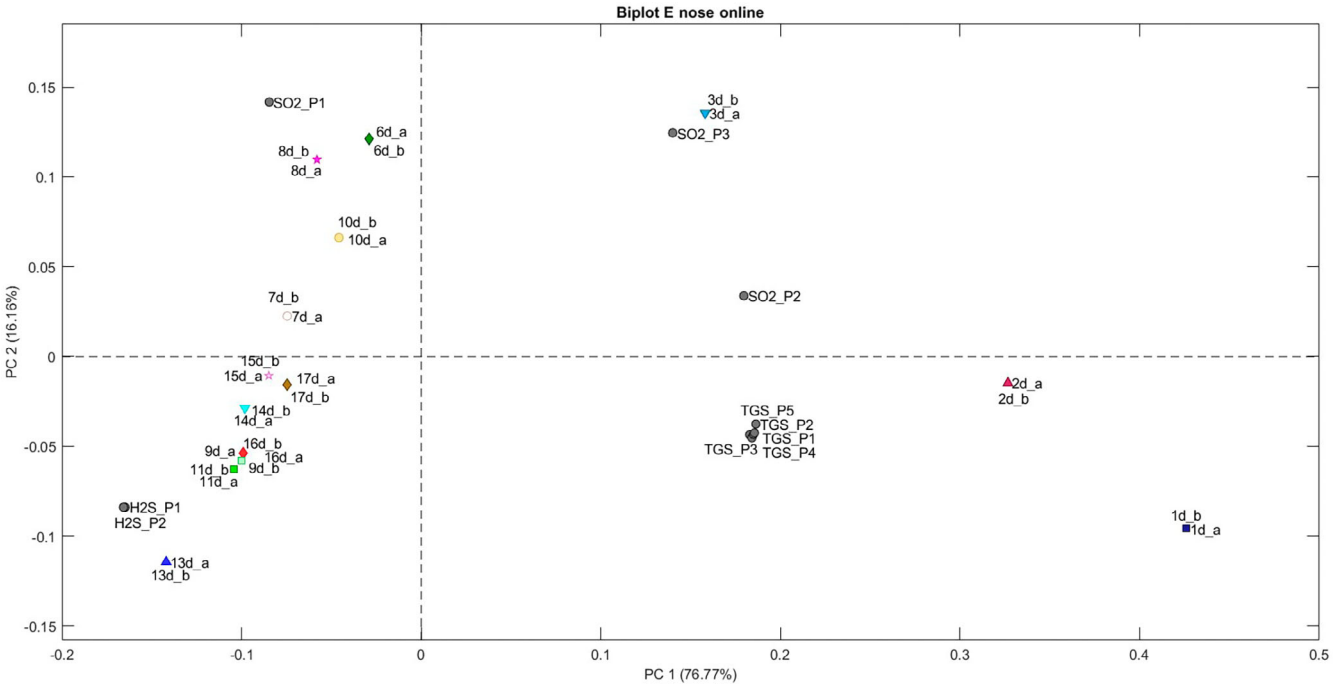

Figure 7. Biplot (PC1 *versus* PC2) of sample scores and variable loads obtained by PCA performed on e-nose_B data of musts from V79 during alcoholic fermentation.

composition. NIR measurements have been used to build PCR models to predict phenolic composition as well as technological parameters (i.e. glucose/fructose). The models showed quite good prediction capability, highlighting the potentiality of this technology. Moreover, both offline and online e-nose measurements exhibited good capability of discriminating different fermentation phases. As far as offline e-nose_A analysis is concerned, interesting correlations have been observed for QMB1 and 2 channels, showing promise in predicting ethyl esters concentration. Considering online e-nose_B monitoring, correlation analysis indicates that the TGS sensors have the potential to serve as reliable indicators for predicting the concentration of important molecules, such as C6 alcohols, ketones and terpenes, whereas the signal from H_2S sensors may be directly associated with ester levels. The produced data represent a step forward in clarifying the intricate relationship between sensor data and evolving chemical composition during fermentation. However, it is crucial to acknowledge the preliminary nature of the present study, given the constraints imposed by the limited number of samples. This relatively low sample size, although indicative of promising trends, necessitates caution in drawing definitive conclusions: the inclusion of a more extensive and diverse dataset will be imperative to capture a broader range of chemical scenarios and enhance the robustness of our models. Nevertheless, in light of recent advancements in terms of artificial intelligence (AI) and big datasets processing power, these results would suggest that a well-structured sensor array could soon fulfil a longstanding requirement of winemakers: closely monitor fermentation progress in terms of must/wine characteristics/composition in real time.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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