# NON-INVASIVE QUANTIFICATION OF PHENOLIC CONTENT DURING RED WINE FERMENTATION

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## Introduction

Phenolic compounds are one of the most important red wine quality attributes as they are directly involved in colour and relevant mouthfeel properties such as astringency and bitterness (Vidal et al., 2003; Monagas et al., 2005). Despite the importance of phenolic compounds in winemaking, its analysis is still time consuming and difficult to perform for most of the wineries. This is due to the need of sampling, which requires time, trained personnel, and specialized equipment. These limitations can be overcome with the use of spectroscopic techniques. Spectroscopy allows for the indirect quantification of analytes by a single spectral measurement. Once a predictive calibration has been optimized, the spectral data acquisition is the only requirement for the phenolic quantification (Aleixandre-Tudo et al., 2018). Moreover, recent developments in technology and instrumentation are exploring the implementation of devices able to obtain spectral readings directly from the wines during the winemaking process.

Among the available techniques, fluorescence spectroscopy appears as a valid alternative for these tasks. The enhanced sensitivity of fluorescence and the fact that most of the phenolic compounds have fluorescent properties indicate that this technique could also be used for the quantification of phenolic compounds (Airado-Rodríguez et al., 2011). The final aim of this study is to develop a fluorescence device to quantify phenolic content using reflected fluorescent light during red wine fermentations, non-invasively and therefore without sampling. For this, four objectives have been identified. First, the conventional fluorescence approach needs to be modified to explore the possibility of obtaining fluorescence spectral properties from undiluted samples. This was achieved by making use of a front face approach by modifying the geometry of the measuring chamber. Secondly, the phenolic and spectral data of the selected fermenting and finished wines was acquired. Thirdly, chemometric techniques were explored to optimise the prediction calibrations. Finally, the influence of sample conditions on the predictive ability of the optimised calibrations as well as the ability of unaltered spectra on the prediction performance of the calibrations was evaluated.

## Materials and methods

Two hundred fermenting samples from the 2019 vintage and 100 wines from vintages spanning from 2007 to 2019 were included in the data set. Total phenol content (Iland et al., 2000), total tannin (Sarneckis et al., 2006, Mercurio et al., 2007) and total anthocyanin (Iland

et al., 2000) were the phenolic parameters evaluated in the study. For the fluorescence data acquisition, a benchtop fluorescence spectrometer was used. The excitation took place in the 245 to 400 nm range every 5 nm and the emitted light was captured in the 245 to 500 nm range at 0.5 nm intervals. The scanning speed was 5nm/min and the excitation/emission slit widths were 3 and 5 nm, respectively. Samples were frozen after collection, thawed and centrifuged before analysis. A machine learning pipeline was applied to the data set for model optimisation. The pipeline included a SMOTER algorithm for minority sample generation with KNN = 3, column selector, Savitzky-Golay filter, pre-processing selector, PCA for data dimensionality reduction and finally a XGBoost regressor for the regression exercise. For the second part of the project a single Cabernet Sauvignon fermentation was used to evaluate the ability of the optimised calibrations to predict samples in different formats i.e. degassed and unaltered. Moreover, models were optimised using spectral data obtained from degassed and unaltered samples. The same phenolic analysis, spectral data acquisition settings and machine learning pipeline as reported above were used. Sampling was done daily and in triplicate during the duration of the fermentation (12 days).

## Results and discussion

The data set was run through the pipeline and the best optimised model was selected and reported in Figure 1. Table 1 shows the model statistics for the optimised calibrations.

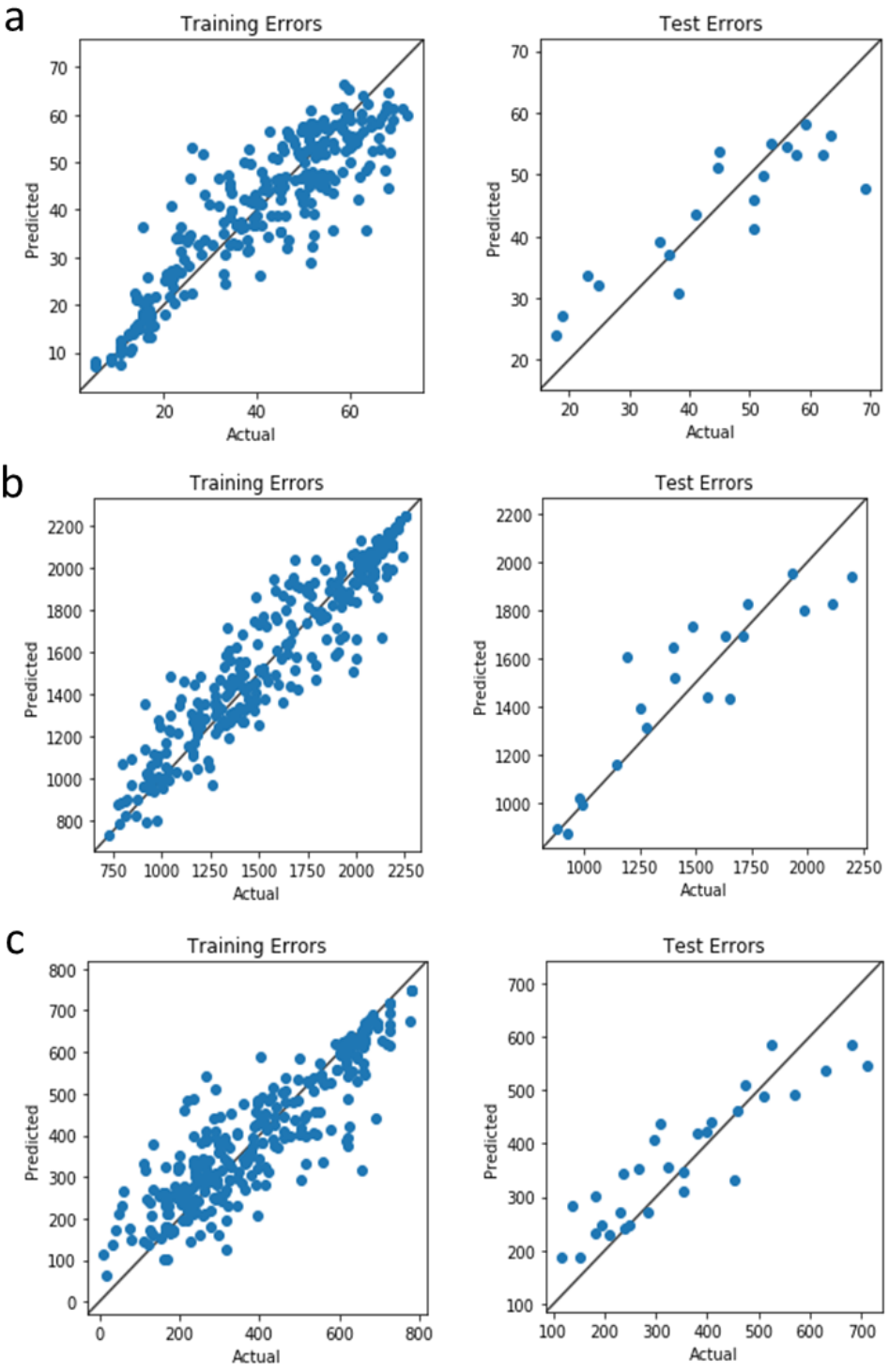


*Figure 1 Calibration and validation correlation plots for total phenol index (a), condensed tannins (b) and total anthocyanins (c)*

Correlation coefficients around 0.8 were observed for the three phenolic parameters evaluated. The most accurate models were reported for total condensed tannins (mg/L) with a $R^2$ calibration = 0.89 and $R^2$ validation = 0.8. The errors of prediction in validation were 7.16, 172.37 mg/L and 76.57 mg/L for total phenols, tannins, and anthocyanins, respectively. Mean absolute errors in validation (MAEV) were consistently lower than the root mean square errors in validation (RMSEV).

*Table 1 Model statistics for the optimised calibrations*

| | $R^2$cal | $R^2$val | RMSEC | RMSEV | MAEV |
|---|---|---|---|---|---|
| Total Phenols | 0.81 | 0.77 | 5.71 | 7.16 | 5.39 |
| Total Condensed Tannins (mg/L) | 0.89 | 0.80 | 104.03 | 172.37 | 129.14 |
| Total Anthocyanins (mg/L) | 0.80 | 0.77 | 60.67 | 76.57 | 61.57 |

*$R^2$cal: coefficient of correlation in calibration; $R^2$val: coefficient of correlation in validation; RMSEC: root mean square error of calibration; RMSEV: root mean square error of validation; MAEV: mean absolute error of validation.*

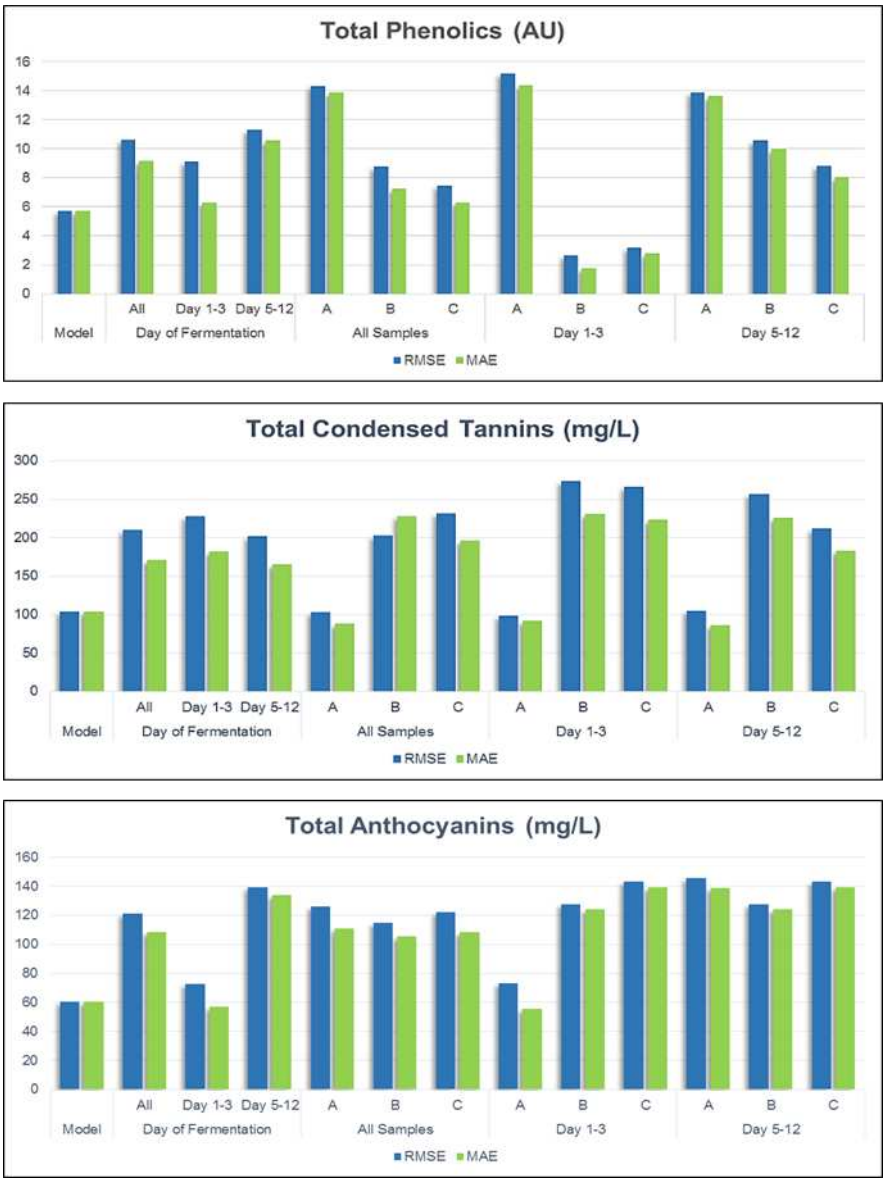


*Figure 2 Model performance with clean (A), degassed (B) and unaltered samples (C) used for model validation.*

For the second part of the experiment, the influence of sample conditions on the predictive ability of the optimised calibrations as well as the ability of unaltered spectra on the prediction performance of the calibrations were evaluated and are reported in Figure 2 and Table 2.

Figure 2 shows error results when the models previously developed and reported in Figure 1 and Table 1 were used to predict the phenolic levels of the samples obtained from a single Cabernet Sauvignon fermentation. Samples were obtained in three formats i.e. cleaned (frozen and centrifuged) (A), degassed (B) and unaltered (C). Only the models including the entire sample set (All samples) are discussed below. In the left part of the plot the model errors in calibration (RMSEC) are shown for the three phenolic parameters evaluated (Model). As it can be observed, certain model depreciation was observed for clean samples (A) for total phenols and anthocyanins, although this was not observed for tannins with similar error values for the predicted samples. Interestingly, increased error values were observed for degassed (B) and unaltered samples (C), with this increase being less intense for total phenols. However, when the errors reported in Figure 2 are compared with the validation errors of the original models (Table 1), minimal error increase was also observed for tannins and anthocyanins.

*Table 2 Summary model statistics for calibration optimised using clean (A), degassed (B) and unaltered (C) fluorescence spectra.*

| | Treatment | $R^2$cal | $R^2$val | RMSEC | RMSEV | MAEV |
|---|---|---|---|---|---|---|
| Total Phenols Index | A | 0.90 | 0.97 | 1.53 | 1.22 | 0.96 |
| | B | 0.89 | 0.87 | 1.72 | 2.27 | 1.73 |
| | C | 0.94 | 0.96 | 1.26 | 1.38 | 1.12 |
| Total Condensed Tannins (mg/L) | A | 0.89 | 0.94 | 51.70 | 41.30 | 34.89 |
| | B | 0.86 | 0.96 | 57.36 | 34.44 | 27.55 |
| | C | 0.86 | 0.94 | 48.42 | 42.84 | 35.95 |
| Total Anthocyanins (mg/L) | A | 0.85 | 0.87 | 16.35 | 19.19 | 14.66 |
| | B | 0.89 | 0.91 | 13.90 | 18.40 | 15.09 |
| | C | 0.93 | 0.89 | 14.34 | 20.53 | 15.17 |

*R2cal: coefficient of correlation in calibration; R2val: coefficient of correlation in validation; RMSEC: root mean square error of calibration; RMSEV: root mean square error of validation; MAEV: mean absolute error of validation.*

In Table 2, calibrations were optimised using fluorescent spectral properties of clean (A), degassed (B) or unaltered samples (C). This experiment is trying to answer the question whether spectra from non-processed samples could be used to obtain accurate prediction calibrations. As can be observed $R^2$ values around 0.9 in both calibration and validation were reported for both treatments B (degassed) and C (unaltered). This high $R^2$ values are in line with the low errors in calibration and validation observed. Despite these promising model performance statistics, the results of this second part of the research should be considered with caution as only a single Cabernet Sauvignon fermentation was evaluated.

## Conclusions

The results observed in this study showed that reflectance fluorescence spectroscopy of undiluted samples could be used as an alternative to quantify phenolic content during the fermentation process as well as in finished wines. In addition, the developed calibrations seem to be suitable to predict phenolic content in minimally to non-treated samples (degassed and unaltered). Calibrations developed using fluorescence spectra from degassed and unaltered samples proved to also be accurate. The question therefore remains whether calibrations should be optimised using clean samples or on the contrary using spectra obtained from samples in unaltered format as in real fermentation conditions. Despite this, the results obtained point towards the possibility of quantifying the phenolic content directly from a fermenting tank with absence of sampling or sample preparation using reflectance fluorescence spectroscopy.

## Abstract

Phenolic compounds are responsible for the most important red wine quality attributes. Anthocyanins and tannins play crucial roles in color and mouthfeel properties of red wines. Phenolic analysis in the winery is hindered by analytical constrains. The possibility to quantify phenolic content non-invasively from a fermenting tank will provide phenolic data in real time and with absence of sampling. This could be achieved by making use of the fluorescence properties of phenolic compounds. Front-face fluorescence was in this case used to obtain fluorescence spectral properties of wines directly during the fermentation tank. Adapting the sample geometry, direct measurement from a fermenting tank through a crystal window can be obtained. Moreover, the fluorescence spectral properties were correlated with phenolic levels using machine learning techniques and accurate spectral calibrations were obtained for total phenol content, anthocyanins (mg/L) and tannins (mg/L). A prototype device for the measurement of fluorescence spectral properties was developed. The fluorescence spectrometer showed the ability to quantify phenolic content during red wine fermentations with the absence of sampling and in a non-invasive manner.

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