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Chemometric compositional analysis of phenolic compounds in fermenting samples and wines using different infrared spectroscopy techniques

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ABSTRACT

The wine industry requires reliable methods for the quantification of phenolic compounds during the winemaking process. Infrared spectroscopy appears as a suitable technique for process control and monitoring. The ability of Fourier transform near infrared (FT-NIR), attenuated total reflectance mid infrared (ATR-MIR) and Fourier transform infrared (FT-IR) spectroscopies to predict compositional phenolic levels during red wine fermentation and aging was investigated. Prediction models containing a large number of samples collected over two vintages from several industrial fermenting tanks as well as wine samples covering a varying number of vintages were validated. FT-NIR appeared as the most accurate technique to predict the phenolic content. Although slightly less accurate models were observed, ATR-MIR and FT-IR can also be used for the prediction of the majority of phenolic measurements. Additionally, the slope and intercept test indicated a systematic error for the three spectroscopies which seems to be slightly more pronounced for HPLC generated phenolics data than for the spectrophotometric parameters. However, the results also showed that the predictions made with the three instruments are statistically comparable. The robustness of the prediction models was also investigated and discussed.

1. Introduction

The highly competitive global wine market is currently demanding top quality products. Internationally wine producers are facing the challenge of an increasingly competitive international scenario [1,2]. The inclusion of state of the art analytical technologies to ensure high quality standards and process control is thus a priority [3,4]. Analytical technologies combine several analytical tools which include physical, chemical, mathematical, statistical and other analytical resources to provide a holistic insight into product properties. The information obtained can thus be beneficial for benchmarking, decision making, grading, process control, adulteration or geographical identification tasks, among others [5–7].

The use of spectroscopy with chemometrics combines several of these tools. Spectroscopy has been declared as suitable for process control and monitoring [4,8–10]. The use of infrared spectroscopy (IR) relies on the molecular overtones and vibrations of the atoms when infrared radiation is passed through a sample. The amount and frequency of the absorbed light as well as the amount of reflected or transmitted light provide information of the grape and wine biochemical components. In addition, IR has been defined as a non-destructive, fast and easy to perform analytical technique [10,11]. The fact that it can measure more than one parameter at a time makes it the analytical technology of preference in food-related and non-related industries [6,12]. In the past years an increased availability of IR instruments and applications, including quantification and discrimination tasks, have been reported [10], nevertheless its industrial implementation seems to be slow and only possible to medium and large size wineries [6].

Phenolic compounds in combination with other major wine constituents are mainly responsible for the mouth feel attributes of a red wine [13,14]. Moreover, the colour properties of a wine depend on the levels and chemical state of the phenolic compounds present at the time of evaluation [15,16]. Phenolic compounds are extracted during the fermentation mainly from the solid parts of the grape berry [17]. However, the level of these compounds is not the main factor contributing to their later presence in wine. The interactions and associations among phenolic substances, which occur as soon as the compounds coexist in the must, influence their further presence and consequently their contribution to the wine organoleptic properties [18,19].

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A number of studies aiming to monitor phenolic compounds during the fermentation process using infrared spectroscopy have been reported in the literature. Visible-Near infrared spectroscopy was explored by Cozzolino [20]. Only calibration statistics for malvidin-3-glucoside, polymeric pigments and tannins models using HPLC analysis as reference method were reported in Cabernet Sauvignon and Shiraz fermentations collected over two vintages. Additionally, Fragoso [21] also reported quantification models for fermenting samples using Fourier Transform (FT) Mid-infrared with the spectrophotometric determination of total phenolics (TP), anthocyanins (TA) and methylcellulose precipitable (MCP) tannins as reference methods. Five different cultivars were included in the trail and microvinifications (4 kg) were performed at different ripening levels. Samples were collected during fermentation for 10 days. Validation residual predictive deviation (RPD) values higher than 3 for TP and TA and lower than 2.5 for MCP tannins were reported. Finally, di Egidio [8] investigated the use of Near- (NIR) and Mid-infrared (MIR) to monitor the levels of TP, TA and total flavonols (TF) in 15 micro-vinifications during the fermentation process of Nebbiolo grapes. Good calibration models were reported, however the lack of the standardization of the predictive accuracy makes it difficult to compare the results with those reported in other studies. The combination of ultraviolet-visible and near infrared (UV–VIS–NIR) spectroscopy was also investigate for some of the most representative phenolic compounds [22]. Accurate single cultivar models were observed for catechin and malvidin, however due to the limited number of samples no validation data was reported.

NIR and IR (WineScan™, Foss Electric) spectra in transmission mode as well as attenuated total reflectance (ATR) MIR spectra in reflexion mode was collected from a large set of fermenting samples and wines. Prediction models were built for 27 individual phenolic compounds quantified using an HPLC method as well as for the spectrophotometric determinations TP, TA, MCP tannins and colour density (CD). The aim of this study was thus to provide accurate externally validated prediction models for phenolic monitoring, quantification and profiling during the winemaking process. The goal of comparing three spectroscopic techniques relies on the identified need of providing an increasing number of applications to scientists and professionals. Despite the published studies, a direct comparison between three different spectroscopic techniques has not yet been investigated. An additional statistical treatment of the predictions obtained with the different instruments is reported in this study. The suitability of each technique has been evaluated based on the results obtained from the process of model calibration and validation.

2. Materials and methods

2.1. Reagents and standards

Phosphoric acid and caffeic acid were purchased from Fluka (Sigma-Aldrich Chemie, Steinheim, Germany). Acetonitrile was obtained from Merck (Darmstadt, Germany). Methyl cellulose, ammonium sulphate, hydrochloric acid (HCl), gallic acid, catechin, p-coumaric acid, quercetin-3-glucoside and quercetin were obtained from Sigma-Aldrich Chemie, (Steinheim, Germany) and malvidine-3-glucoside chloride was purchased from Extrasynthese (Lyon, France).

2.2. Samples

Samples during the fermentation process were collected from 13 commercial scale vinifications at the Welgevallen cellar (Stellenbosch, South Africa) over two consecutive vintages (2015–2016). Nine different fermentations were followed in 2015 and four fermentations were sampled in 2016. Four cultivars were represented including Cabernet Sauvignon, Shiraz, Pinotage and Grenache. Samples were collected daily the first 15 days of the fermentation and every 3 days for a maximum period of two months after fermentation. Samples were passed through a kitchen sieve and frozen immediately after collection. Varying phenolic and sugar ripening levels, cold maceration, the use of different yeast strains, extended maceration, tannin addition and malolactic fermentation in barrel were some of the winemaking variables included in the sample set. Fermentations took place in different fermenters, ranging from 3.000 to 10.000 L. A total of 391 samples were collected. The day of analysis, the samples were thawed at room temperature and centrifuged in a 7366 Hermle centrifuge (Wehingen, Germany) at 3248 g for 5 min before spectra collection or analysis were performed. Additionally, wine samples (178) spanning a range of vintages (from 2005 to 2016) and cultivars (12) as well as some blends were also collected and analysed. Before analysis the samples were also centrifuged at 3248 g for 5 min. A total number of 569 samples including fermenting samples and wines were used in the calibrations.

2.3. Spectrophotometric analysis of phenolic compounds

The method reported by Iland [23] was used for the quantification of total anthocyanin and total phenolic content. The samples were diluted 50 times with HCl 1 M and kept for 3 h before the absorbance at 280 nm and 520 nm was recorded using a Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The total phenolics index was calculated as the A280nm times the dilution factor (DF = 50). The anthocyanins content (mg/L malvidin-3-glucoside) was calculated using the molar extinction coefficient (ε) and the molecular weight (MW) of the most abundant anthocyanin found in wine malvidin-3-glucoside (ε = 28.000 L/cm*mol; MW = 529 g/mol) times the dilution factor. The total tannin content was quantified using the methyl cellulose (MCP) tannin assay developed by Sarneckis [24] and later adapted to a high throughput format by Mercurio [25]. Briefly the method consists of a polymer-tannin interaction that results in an insoluble complex that precipitates and can be measured by comparing a control sample and a treatment sample (where tannins are removed by centrifugation). In a 2 mL microfuge tube, 600 μL of MCP solution was mixed with 50 μL of wine and let to stand for 2–3 min. 400 μL of saturated ammonium sulphate solution and 950 μL of distilled water were added to a final volume of 2 mL (treatment sample). A control sample with distilled water (600 μL) instead of MCP solution was also prepared. Samples were left for 10 min prior to centrifugation in an Eppendorf 5415D centrifuge (Hamburg, Germany) at 9279 g for 5 min. The difference between the control A280 nm value and the treatment A280 nm value was converted into epicatechin equivalents (mg/L) using a calibration curve. A dilution factor of 40 was used to calculate the total tannin content. The methylcellulose solution (0.04% w/v; 1500 cP viscosity at 2%) was prepared according to the method's instructions [25]. Colour density was measured according to Glories [26]. Fifty μL of wine were directly pipetted into a UV–VIS Nunc F96 MicroWell plate (Nunc, Langenselbold, Germany) and the absorbance at 420 nm, 520 nm and 620 nm were recorded. The colour density was calculated as the sum of the three wavelengths. Absorbance values were always referenced to a standard 10 mm path length.

2.4. HPLC analysis of phenolic compounds

The phenolic composition was analysed following the method initially reported by Peng [27] with some modifications. An Agilent Technologies 1260 Infinity series (Agilent, Waldbronn, Germany) HPLC system with a PLRP-S polymeric reversed phased column (3 μm particle size, 100 Å pore size, 150 mm × 4.6 mm), at 35 °C was used for the quantification of phenolic compounds. The solvents used were 100% acetonitrile (A) and phosphoric acid in water at 1.5% (B) for a gradient elution flow rate of 1 mL/min: 0 min (5% solvent B), 73 (25% solvent B), 78 (50% solvent B), 86 (50% solvent B),