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Partial Least Squares calibrations and batch statistical process control to monitor phenolic extraction in red wine fermentations under different maceration conditions.

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ABSTRACT

The extraction of phenolic compounds during maceration is of utmost importance in red winemaking. However, the monitoring of phenolic extraction is often hampered by analytical and statistical constraints. The aim of this study was to monitor phenolic extraction kinetics with the use of PLS phenolic calibrations and batch statistical process control. Eight batches of Cabernet Sauvignon and Shiraz grapes during alcoholic fermentation under different maceration conditions (pressing at 1/3rd, 2/3rd and end of fermentation) and punch down regimes (low vs. high frequency) were evaluated in the study. Cabernet Sauvignon appeared to be a more suitable cultivar for longer maceration conditions with increased tannin extraction observed. Similar trends were observed for punch down for both cultivars. The use of PLS calibrations and batch level modelling provided an enhanced interpretation and understanding of phenolic extraction during red wine fermentations.

KEYWORDS

PLS regression, batch statistical process control (BSPC), batch level modelling (BLM), red wine phenolics maceration, cap management practices.

INTRODUCTION

Phenolics are plant-derived compounds present in a variety of food and beverage products (Cerpa-Calderón & Kennedy, 2008; Teixera, Eiras-Dias, Castellarin, & Gerós, 2013). Phenolic compounds in red wine have been investigated for their important role and contribution towards sensorial and chemical properties as well as possible health benefits (Koyama *et al.*, 2007; Harbertson *et al.*, 2009; Monagas *et al.*, 2005a). Phenolic compounds found in grapes and wine have been classified as non-flavonoids (hydroxycinnamic acids, hydroxybenzoic acids and stilbenes) and flavonoids (anthocyanins, flavan-3-ols and flavonols) *(*Downey *et al.,* 2006; Teixera *et al.*, 2013; Lerno *et al.*, 2015). Anthocyanins are known as the red pigments located in the vacuoles of the berry skin which are responsible for red wine's colour. Proanthocyanidins or tannins are located in both skins and seeds of grape tissue and contribute to wine structure and mouth feel attributes (Monagas *et al.*, 2005b; Kelebek *et al.*, 2006; Gonzáles-Neves *et al.*, 2012; Hernández-Jiménez *et al.*, 2012). Since the desirable phenolic compounds are located in the berry skins and seeds, red wine fermentations are conducted with skin contact to favour their extraction (Bindon *et al.,* 2010; Bautista-Ortín *et al.*, 2016).

The influence of different winemaking practices on the phenolic profile of red wines has been investigated in several studies (Sacchi *et al.,* 2005; Casassa and Harbertson, 2014; Smith *et al.*, 2015). The extent of the maceration and the conditions during this period influence the extraction of phenolic compounds and the subsequent reactions they are involved in, which also influences the sensorial properties of the wine (Kelebek *et al.,* 2006; Koyama *et al.,* 2007; González-Neves *et al.,* 2008; Vazquez *et al.,* 2010). Since maceration is a selective extraction process, the time of maceration combined with variables such as different ethanol levels and temperature influences the rate and extent of diffusion of specific phenolic compounds (Gómez-Plaza *et al*., 2001; Romero-Cascales *et al*., 2005; Sacchi *et al.*, 2005; Koyama *et al.*, 2007; Smith *et al.,* 2015).

Contact between the solids and must is therefore a crucial factor to enhance extraction of desirable phenolic compounds (García-Beneytez *et al.*, 2002; Harbertson *et al.*, 2009; Nel *et al.*, 2014; Lerno *et al.*, 2015). Cap management is an

important winemaking practice applied to prevent oxidation and bacterial growth, while facilitating the contact time between the must and skins and seeds (Ichikawa, Ono, Hisamoto, Matsudo, & Okuda, 2012). Few studies have investigated different cap management techniques to increase extraction of phenolic compounds during red wine fermentations (Marais, 2003; Sacchi *et al.,* 2005; De Beer *et al.*, 2006; Ichikawa *et al.*, 2012). The results obtained (punch down, pump over, submerged cap) were found to be dependent of the grape variety itself (Fischer *et al*., 2000; Chittenden *et al.*, 2015).

The use of spectroscopy applications is a suitable approach that could be used to measure and monitor phenolic compounds during alcoholic fermentation since it is a relative simple, cost effective and a rapid procedure (Harbertson and Spayd, 2006; Ivanova *et al*., 2012; Cozzolino, 2015). Phenolic compounds have different spectral properties with characteristic features dependent on the specific phenolic class (Harbertson & Spayd, 2006). Although each phenolic class has different absorption features, phenolic compounds have a characteristic phenol ring with the ability to absorb light in the ultraviolet (UV) region. In addition, the coloured nature of some phenolic compounds allows the absorption of visible light. Phenolics are therefore suitable to be quantified with spectrophotometric measurements (Aleixandre-Tudo *et al.*, 2017). Some studies have reported the effectiveness of UV-Vis spectroscopy calibrations to monitor phenolic compounds during fermentation (Aleixandre-Tudo *et al.*, 2018; Aleixandre-Tudo and Du Toit, 2019). However, the monitoring of a wide array of phenolic compounds on a regular basis during several different red wine fermentations have not been performed extensively, which might be due to limitations in both analytical and statistical processing methods. Batch statistical process control could also be applied to monitor red wine fermentations, however limited research is currently available on this topic (Aleixandre-Tudo and du Toit, 2019).

The aim of the study was thus to monitor phenolic extraction kinetics using UV-Vis based partial least squares (PLS) spectroscopy calibrations for the quantification of phenolic levels and BSPC (batch statistical process control) of Cabernet Sauvignon and Shiraz grapes during alcoholic fermentation under different maceration conditions (skin contact length i.e. presence/absence of skins) and punch down

strategies (low versus high frequency, performed at different times during fermentation).

MATERIALS AND METHODS

Reagents

Ethanol (96%) was obtained from Merck (Merck & Co., Darmstadt, Germany). Sodium Hydroxide (0.333N) and Potassium iodate (N/64) was obtained from Cameron Chemicals (Cameron Chemical Consultant, Cape Town, South Africa). Hydrochloric acid (HCl)) was obtained from Sigma-Aldrich Chemie (Sigma-Aldrich Chemie, Steinheim, Germany).

Experimental design

The study was conducted with four Shiraz and four Cabernet Sauvignon grape batches sourced from different vineyard blocks located in the Western Cape, South Africa (**Table 1**). The grapes were harvested in 2017 with °Brix levels ranging from 23-26. The grapes of each batch (vineyard) were randomly divided into 12 crates, with each crate containing 20 kg of grapes, at the experimental cellar of the Department of Viticulture and Oenology (University of Stellenbosch, South Africa). After the grapes were cooled in a 4 °C room, the 12 crates of each vineyard batch were randomly marked according to the experimental design**.** One crate of grapes was thus used per vinification. Berry sampling was then conducted by randomly selecting 100 berries from different clusters of each crate. Grapes were kept frozen at -20°C. Grape samples were thawed at room temperature prior analysis

As shown in **Table 2** three different pressing times (1: skin maceration until 1/3rd of alcoholic fermentation, 2: skin maceration until 2/3^{rds} of alcoholic fermentation, 3: skin maceration until the end of alcoholic fermentation) during alcoholic fermentation were investigated at two different levels of punch downs (i.e. low vs high punch down frequency). The treatments (12 punch downs per day, T) and controls (3 punch downs per day, C) of the three different pressing times were conducted in duplicate for each grape batch. Punch downs only occurred at specific times as indicated in Table 2 to assess the punch down effect at different stages of the fermentation process. Samples were collected twice a day (morning and afternoon) in 2 mL tubes

after crushing and destemming until the end of fermentation. All the must and wine samples were collected and analysed on the same day after sampling.

Winemaking

Twelve crates representing one grape batch were separately crushed and destemmed in 25 L plastic buckets following grape juice sampling for standard analysis. Fermentation took place at 25 °C in a temperature-controlled room. Grape juice analysis included standard measurement of soluble solids (°Brix), total titratable acidity (g/L) and pH. The grapes received 30 mg/L of sulphur dioxide (SO_2) at crushing and were inoculated with 0.3 g/L commercial yeast strain Lalvin ICD D21 (*Saccharomyces cerevisiae*, Lallemand Inc., Montreal, Canada). Pectolytic enzyme (Lafase He Grand Cru, Laffort, Bordeau, France) were also added to all the buckets following the manufacturer's instructions. A yeast nutrient (0.25 g/L Fermaid K, Lalvin ICV D21, Lallemand Inc., Montreal, Canada) was added after 2-3 °Brix drop. Alcoholic fermentation was completed in 25 L plastic buckets and finished wines pressed according to the experimental design (**Table 2**). All the vinifications were pressed in an open basket press and completed alcoholic fermentation in 20 L plastic buckets.

Analysis

Grape phenolic analyses

The method reported by Iland (2000) was used to extract phenolic compounds from the berry skin and seeds. In individual test tubes, 100 µL of the grape phenolic extract was diluted 20 times with 1M HCL and placed in a dark cupboard for a waiting period of one hour. After one hour the samples were removed from the cupboard and 200 µL of each sample were pipetted into a UV-Visible Nunc F96 MicroWell plate (Nunc, Lan- genselbold, Germany) and placed in a Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Four phenolic parameters (colour density, anthocyanin content, tannin concentration and total phenols) were quantified using PLS calibrations as reported elsewhere (Aleixandre-Tudo *et al.*, 2018).

Must and wine phenolic analysis

For the samples collected during alcoholic fermentation, phenolic levels were also quantified through PLS calibrations (Aleixandre-Tudo *et al.*, 2018). Colour density,

157 total anthocyanin content, total phenolics and methyl cellulose precipitable tannins
158 as well as 27 individual phenolics were quantified. All the must and wine samples
159 were collected and analysed on the same day after sampling.

160 General analysis

161 The soluble solids (°Brix) of the grape juice were measured with a refractometer after
162 crushing and destemming. The pH and total titratable acidity were measured with an
163 862 Compact Titrosampler instrument (Metrohm Ltd., Herisau, Switzerland).

164 **Statistical analysis**

165 Grape phenolics

166 Analysis of variance (ANOVA) and post-hoc tests were conducted using Fishers LSD
167 model $p<0.05$ to compare grape phenolic values. ANOVA and LSD post-hoc test
168 was also used to evaluate the wines at the end of the fermentation process.

169 Fermenting data

170 BSPC (Batch statistical process control) is a multivariate statistical approach to
171 process datasets generated during manufacturing processes. Batch evolution
172 modelling (BEM) provides an overview of the process progression, whereas batch
173 level modelling (BLM) provides an overview of the overall batch process behaviour.
174 The batch process data is thus condensed into a single data point in the scores
175 space. This allows for between batch comparison (i.e. the different data points in the
176 scores space correspond to the different treatments and batches) (Eriksson, Byrne,
177 Johansson, Trygg, & Vikstrom, 2013). In our study a data set was generated for
178 each grape batch (eight batches), with samples collected twice a day from twelve
179 vinifications representing the three maceration times at two different levels of punch
180 down from crushing until end of fermentation per grape batch. The completed data
181 set thus consisted of 1920 fermenting wine samples. In this study only BLM data is
182 reported.

183 Multivariate modelling

184 The phenolic levels of the samples collected from initialization (crush & destem) until
185 process completion (end of alcoholic fermentation) leaded to a three-way matrix for
186 each grape batch (batch x observation x time) (Figure 1). Each grape batch is
187 composed by 12 treatment batches as indicated in the experimental design.

Principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) models were developed during batch level modelling (BLM). PCA was used to explore differences between batches, whereas OPLS-DA modelling was used to evaluate if the different classes (treatments) can be discriminated and to investigate potential differences in the phenolic extraction within and between grape batches. The score values t represents a compression of all the variables measured during the fermentation process. The loadings plot provides information about the relationships among the variables (individual phenolic compounds) and provides a summary of phenolic extraction progression during the process as they include loading values from crushing until process completion. Scores and loadings are thus used to visualize and better understand and interpret the phenolic extraction of the different treatments or batches. SIMCA 14.1 (Sartorius Stedim Biotech, Gotinga, Germany) was used for BSPC data analysis.

RESULTS

Grape composition

Grapes were sourced from different vineyards in the Western Cape to introduce a degree of phenolic variability in our study. These grapes for this experiment were harvested with the rest of the crop used for commercial winemaking. The Brix, pH and total acidy levels of the grapes can be seen in **supplementary information S1**. Significant differences were observed for the phenolic parameters' tannins, anthocyanins, colour density and total phenols between grape batches (**Figure 2**).

One of the main objectives of this work was to ascertain if the effect of pressing time and cap management practices was consistent, regardless of initial grape and wine phenolic content and composition. In other words, if the results observed in one batch apply to other batches regardless of its grape and wine phenolic profile. It was therefore of importance to start with grape batches with varying phenolic content and composition to assess this.

A batch level model (BLM) PCA plot was built to evaluate differences between wines made under different winemaking conditions. The BLM PCA score plot showed fermentation samples separating accordingly to the vineyard the grapes were sourced from. In the BLM PCA score plot (**Figure 3.A**) the fermentation samples separated in four groups according to the vineyard the Cabernet Sauvignon grapes

were sourced from. Batches 1 (green) and 7 (red), observed on the positive side (right side) of the PCA score plot, were associated with most phenolic compounds that showed positive p1 loading values (higher values of these measurements) (**Figure 3.B**). On the other hand, batches 5 (blue) and 8 (yellow) observed on the negative side (left side) of the BLM PCA score plot were associated with higher levels of p-coumaric acid for instance, representing negative loading values. Moreover, grape batches separated also according to the second principal component i.e. batch 1 and 7. Negative p2 loading values showed higher values of cinnamic acids, flavonols and some individual anthocyanins, whereas positive loadings in PC2 were associated with higher levels of total anthocyanins, colour, tannins or total phenols (**Figure 3.C**). Despite both batches (1 and 7) being identified as high phenolic content in PC1, phenolic differences were also observed based on p2 values.

Similar results were observed in the BLM PCA score plot of Shiraz grape batches (**Figure 4.A**). The fermentation samples separated in four groups representing the vineyards the grapes were sourced from. In the PCA score plot batches 2, 4 and 6 were observed on the negative side (left) of the PCA score plot and were associated with the majority of phenolics that showed negative loading values. For example, these batches showed higher levels of anthocyanin content, caffeic acid, catechin, colour density, coutaric acid, total phenols etc., whereas batch 3 observed on the positive side of the PCA score plot showed higher levels of B1 (dimer) content as well as tannins and polymeric phenol content (positive loading values) (**Figure 4.B**). The second principal component associated batch 6, with and intermediate position in PC1, with high levels of total and individual anthocyanins as well as tannins, polymeric and total phenols. This is depicted from the position of batch 6 in the negative part of the PC2 scores plot (**Figure 4.A**) and the negative loading values of the above-mentioned phenolic measurements (**Figure 4.C**).

The effect of pressing time (absence/presence of skins) on phenolic extraction during alcoholic fermentation

During alcoholic fermentation three different pressing times were investigated at two punch down levels (low vs. high punch down frequency). Regardless of the punch down frequency (T^a (high) vs. C^b (low)), the fermentation samples separated according to the pressing time. Therefore, the data represented in **Figure 5** and **6** is

a combination of both punch down levels (T^a and C^b) representing one of the three pressing times. The analysis per punch down frequency is however included in **supplementary information S2 and 3.**

In the BLM OPLS-DA score plot (**Figure 5.A**) Cabernet Sauvignon fermentations separated in three groups representing the three different pressing times. Pressing time 1 (1/3rd fermentation) to the right side of the OPLS-DA score plot displayed a good separation from pressing time 2 (2/3rd fermentation) and 3 (end of fermentation) to the left side of the OPLS-DA score plot. A poor separation between pressing times 2 and 3 were displayed in the scatter plot. Regardless of grape variety, similar trends were observed for vinifications obtained from Shiraz grapes. The fermenting samples separated accordingly to the three different pressing times, however overlapping of fermentation samples were observed to the left side of the OPLS-DA score plot representing pressing times 2 and 3 (**Figure 5.C**). The corresponding loadings plot (**Figure 5.B** and **D**) of the different OPLS-DA models provided information about phenolic extraction kinetics during alcoholic fermentation as they show loading values from the initial starting point (day 0) to the completion of fermentation. The loadings plot also revealed differences in phenolic content between the three pressing times. For example, a high tannin content (coloured light blue) in **Figure 5 B** and **D** was at first associated with pressing time 1 with positive loading values for the first days of the fermentation, indicating higher values in those wines located in the positive part of the scores plot (pressing time 1 wines). However, as alcoholic fermentation progressed higher content of tannin was associated with pressing times 2 and 3 (negative loading values correlating with higher levels in the wines located in the negative side of the scores plot (pressing time 2 and 3). This concludes that initial extraction of phenolic compounds was associated with pressing time 1 followed by mid to end extraction corresponding with pressing times 2 and 3. Overall pressing times 2 and 3 were associated with higher phenolic content.

To further evaluate pressing times 2 and 3, additional OPLS-DA models were built to investigate possible phenolic differences between pressing times. The OPLS-DA score plot showed good separation of Cabernet Sauvignon fermentation samples (**Figure 6 A**) in two groups representing pressing times 2 and 3. Pressing time 2 to the left side of the OPLS-DA score plot was well separated from pressing time 3 to the right side of the OPLS-DA score plot. Various phenolic compounds were

responsible for the separation as indicated by the corresponding loadings plot. Pressing time 3 to the right side of the OPLS-DA score plot (positive side) were associated among others, with high levels of dimer B1, catechin, gallic acid, tannins, polymeric phenols and polymeric pigments (positive loadings). However, pressing time 2 to the left side of the OPLS-DA score plot (negative side) were associated with higher levels of anthocyanin content (negative loadings).

Interestingly Shiraz fermentation samples separated according to pressing times 2 and 3 in the OPLS-DA score plot, however the corresponding loadings plot revealed a more similar phenolic content between the two pressing times (**Figure 6.D**). In general, pressing time 3 showed higher levels of high gallic acid, catechin and dimer B1 content, among others. However, no clear effect was seen for polymeric phenols and tannins between pressing time 2 and 3. Interestingly, pressing time 2 seems to be associated with higher levels of colour density and total phenol content.

The effect of punch down frequency applied during maceration

Punching down is a traditional method used in the wine industry to enhance phenolic extraction and maintain enough contact between the skins, seeds and juice. As seen in the OPLS-DA score plot (**Figure 7.A**) Cabernet Sauvignon fermentation samples separated into two groups representing low (C^{b}) and high (T^{a}) punch down frequency. However, this separation was not that clear as when the effect of pressing time was evaluated, with some overlapping samples and wider scattering in the scores space. High punch down frequency to the right side of the OPLS-DA score plot was associated with higher content of dimer B1, catechin, gallic acid, tannins, polymeric phenols and polymeric pigments in the corresponding loadings plot. However, low punch down frequency was associated with, among others, high anthocyanin and phenolic acid content. Slightly different results were obtained for Shiraz vinifications produced with high punch down frequency (**Figure 7.C**). The corresponding loadings plot revealed vinifications produced with high punch down frequency were associated with high phenolic content such as anthocyanins, dimer B1, tannins, polymeric phenols and total phenols. Interestingly, Shiraz vinifications produced with low punch down frequency were associated with high catechin content.

In addition, since overlapping and more scattered grouping was visible with all three pressing times combined, separate OPLS-DAs were created for each pressing time for both cultivars (i.e. $C1^b$ vs $T1^a$ etc.) to evaluate possible phenolic differences for the different pressing times or in other words to evaluate if the punch down effect was constant despite the pressing time. Overall similar results were observed for Cabernet Sauvignon as seen in the OPLS-DA score plots for the different pressing times combined (**Supplementary material S4**). T^a was associated with high content of dimer B1, catechin, gallic acid, polymeric phenols and polymeric pigments for pressing time 1, 2 and 3, whereas C^b were associated with high anthocyanin content. In general, similar results were also observed within each pressing time for Shiraz fermentations. T^a was associated with higher content of anthocyanins, dimer B1, tannins, polymeric phenols and total phenols. However, $T3^a$ was not associated with high anthocyanin content. In addition, $C1^b$ was associated with high phenolic acids, whereas control three was associated with high gallic acid content (**Supplementary material S5**).

DISCUSSION

The phenolic content of both Shiraz and Cabernet Sauvignon grape homogenates were analysed by Du Toit and Visagie (2010) and Bindon *et al.* (2014) and large variations in these were found, depending on the origin of the grapes, which corresponded with our results. The batch level modelling applied in this study falls under batch statistical process control (BSPC) strategies and can be applied for the evaluation of the process evolution of different grape batches. These techniques are also suitable to evaluate process deviations at treatment level between and within batches making use of multivariate data analysis.

Various practices and variables can have an influence on the phenolic content of red wine (Sacchi *et al.,* 2005; Smith *et al.*, 2015). Phenolic content can be enhanced or modified with different winemaking techniques such as cold maceration, thermovinification, extended maceration and must freezing. However, various studies point to the management of skin contact time as one of the most crucial factor influencing phenolic content and therefore sensory attributes (Casassa & Harbertson, 2014). However, the main aim of this study was not to use different winemaking techniques to evaluate their effect on wine phenolic composition, but to

rather validate the suitability of PLS calibrations and batch statistical process control (BSPC) to monitor phenolic extraction during red wine fermentations.

Longer maceration conditions in our study often resulted in red wines with especially higher tannin and polymeric phenol content. This is in agreement with other studies concluding longer maceration conditions resulted in wines with increased tannin and polymeric phenol formation (Gómez-Plaza *et al.,* 2001; Romero-Cascales *et al.,* 2005; Sacchi *et al.,* 2005; Casassa *et al.,* 2013; Casassa and Harbertson, 2014). Literature has highlighted that skin and seed tannins follow different extraction kinetics (Casassa *et al.*, 2013). Skin tannins are extracted during the early stages of fermentation and will reach a plateau, whereas seed tannin will increase linearly if maceration is extended (Cerpa-Calderón & Kennedy, 2008), leading to more seed tannin extraction and may contribute towards increased phenolic content (Hernández-Jiménez et al., 2012).

On the other hand, fermentations pressed at 1/3rd of alcoholic fermentation often contained lower phenolic content. Optimum phenolic extraction entails sufficient skin contact time between the skins and the juice, since the desired phenolic compounds such as anthocyanins and tannins are located in the berry skins. Romero-Cascales *et al.* (2005) reported anthocyanin extraction reaches a maximum at day seven of maceration, whereas treatments pressed at 1/3rd of alcoholic fermentation in our experiments were only in contact with the skins for two days. Lower anthocyanin concentrations would be expected from fermentations pressed at time 1. In addition, anthocyanin and tannin extraction follows similar kinetics regardless of grape variety (Bautista-Ortín et al., 2016). Similar trends were observed for both cultivars when evaluating the effect of skin contact time on phenolic content during different stages of maceration (**Figure 5.C** and **D**). The fermentation samples separated in the BLM OPLS-DA score plot accordingly to the three different pressing times 1, 2 and 3.

Additionally, taking a closer look at the Cabernet Sauvignon fermentation samples pressed at 2/3^{rds} of alcoholic fermentation higher anthocyanin content compared to fermentation samples pressed near the end of alcoholic fermentation was observed. These results are in agreement with our current knowledge regarding anthocyanin kinetics during alcoholic fermentation (Bautista-Ortín et al., 2016). Anthocyanins are extracted in the first few days of maceration, however anthocyanin content can start to decrease with longer maceration times due to yeast cell reabsorption,

degradation, re-fixation on the skins or due to polymeric pigment formation (Gonzáles-Neves et al., 2012). With regards to tannin content, fermentations pressed near the end of alcoholic fermentation were associated with higher levels. Higher tannin concentrations were to be expected, since proanthocyanidin content can be modified by managing the maceration length (Casassa & Harbertson, 2014). Ivanova *et al.* (2012) concluded seed proanthocyanidin extraction were driven by maceration length and alcohol content. Increased tannin and polymeric phenol content were probably due to increased seed tannin extraction with longer skin contact time. Authors have reported seed tannins contributes towards the majority of total wine tannins in longer maceration conditions (Harbertson et al., 2009). In addition, longer maceration conditions promote hydration of the grape seeds and may cause increased gallic acid extraction from seeds as well (Lerno et al., 2015). This was observed comparing fermentation samples of pressing times 2 and 3, where the latter contained higher levels of gallic acid.

Fewer phenolic differences were observed investigating the Shiraz fermentation samples pressed at time 2 and 3 (**Figure 6.C** and **D**). However, similar trends were obtained compared to Cabernet Sauvignon fermentations pressed near the end of alcoholic fermentation. Fermentations pressed at time 3 were associated with higher gallic acid content, whereas no clear difference in terms of tannin content could be observed. Longer maceration conditions were probably favourable for seed hydration and could have possibly increased gallic acid extraction near the end of alcoholic fermentation (Cerpa-Calderón & Kennedy, 2008). However, with regards to tannin differences observed between Cabernet Sauvignon and Shiraz vinifications, tannin composition is greatly influenced by grape variety. Busse-Valverde *et al.* (2010) reported the proanthocyanidin profiles of Cabernet Sauvignon and Shiraz wines were more dependent on grape variety itself than the winemaking practices applied. It is well known that certain cultivars are richer in phenolic content compared to others (du Toit and Visagie, 2012). Cabernet Sauvignon have been characterized as a cultivar high in tannin content, whereas Shiraz is known to be high in anthocyanin content. However, tannin structure, extraction and concentration may depend on the variety, (Mattivi, Vrhovsek, Masuero, & Trainotti, 2009), or degree of ripeness of the grapes (Harbertson *et al.*, 2009; Canals *et al.*, 2005).

On the other hand, few studies have investigated the effect of cap management during fermentative maceration (Ichikawa *et al.*, 2012; Smith *et al*., 2015; Lerno *et al.*, 2018). Phenolic differences were observed between the two punch down levels during maceration for both Cabernet Sauvignon and Shiraz fermentations. High punch down frequency was associated with higher levels of phenolic compounds such as dimer B1, catechin, gallic acid, tannins, polymeric phenols and polymeric pigments for Cabernet Sauvignon. In Shiraz wines higher anthocyanin content was associated with increased punch down frequency. The ease of extractability from the grape to the must may have contributed to these results. It has been reported that anthocyanin extraction reaches an equilibrium by day six or seven of alcoholic fermentation, limiting further extraction. Bautista-Ortín *et al.* (2016) reported 80% of anthocyanins were extracted from Cabernet Sauvignon grapes at maximum extraction time point, however only 67% of anthocyanins were extracted from Shiraz grapes. Enhanced punch down frequencies could have led to mechanical disruption of the Shiraz skins, leaching anthocyanins and increasing content. Enhanced polymeric phenol, tannin and gallic acid content were to be expected, due to their localization in grape seeds. In addition, the authors Fischer *et al*. (2000) reported enhanced mechanical disruption increased seed tannin extraction.

With regards to low punch down frequency, Cabernet Sauvignon fermentations were associated with among others, high anthocyanin and phenolic acid content. In these ferments lower anthocyanin content might occur with enhanced punch down frequency, since mechanical disruption of the grape tissue could have led to re-fixation on the skins (Ichikawa et al., 2012). Loss of anthocyanin content could also be due to adsorption by yeast cells or participation in oxidation or condensation reactions (Bautista-Ortín et al., 2016). In addition, high phenolic acid content can probably be expected with low punch down frequencies, since these phenolic acids are most abundant in free-run juice (Teixera et al., 2013). However, these trends might be cultivar dependent as increased punch down frequencies led to higher anthocyanin levels in the Shiraz fermentations.

Regarding the statistical approach, the evaluation of the phenolic extraction of many phenolic compounds and ferments has not been reported in detail yet, which could be due to phenolic and statistical analyses limitations. BSPC provides an overview of a batch process from initialization until completion (Eriksson *et al.,* 2013, Wold *et al*.,

1998). This method has been applied in different industrial processes to monitor batch evolution i.e. monitoring beer fermentations as well as Baker's yeast production (Andersen and Runger, 2011; García-Muñoz *et al.,* 2004; Kourti, 2003). Interestingly, as far as we known, only a limited number of studies have used this approach in the past and therefore the ability of the method to monitor and evaluate process progression still needs further evaluation. Moreover, BSPC, as far as we know, have not been extensively used before to monitor and evaluate phenolic evolution in red wine fermentations.

According to our findings BSPC seems suitable to be used as a tool to monitor the progression of phenolic extraction during maceration. This approach would enable winemakers to adapt the winemaking protocol, such changing mixing and pressing regimes to correct deviating batches in terms of phenolics during the fermentation process. Batch level modelling (BLM) models are built after process completion, with the aim to evaluate the performance of a single batch and compare it with other batches. In BLM data captured during the entire process is unfolded and condensed into a single observation, corresponding to a treatment batch in this case. Scores and loadings can then be evaluated to identify between and within batch process variation. The loadings plot includes loading values for the measured variables (phenolics in this study) from initialisation to process completion and can also be used to evaluate process progression, providing an enhanced interpretation and understanding of the phenolic extraction during the fermentation. A variety of statistical techniques such as PCA, PLS or OPLS and the corresponding discriminant analysis can be used to evaluate process performance. Numerous phenolic data points (n=59 520) were generated during this study, illustrating the effectiveness of spectroscopy PLS calibration models and BLM as rapid tools to measure and monitor phenolic extraction during fermentation, as well as a potentially predictive tool to modify the phenolic profile in future red wine productions.

In order to use BSPC as a monitoring and control tool, BLM needs to be complemented with batch evolution modelling (BEM) data. Batch evolution shows batch trajectory by condensing all measured variables in a score value t for every time point measured. It basically provides a PCA analysis over time with the condensation of the X variables into a t score value. This allows the visualization of the process trajectory with a quick identification of deviations from target conditions.

Contribution plots can also be evaluated to identify the reasons causing the deviations. Through this, the variables (phenolic compounds) with larger contribution scores will explain deviations from ideal conditions. BSPC is therefore suitable to be applied in real time applications thus providing an optimized monitoring tool. The raw data can also be consulted through X observation plots. However, the main aim of BSPC is to provide a condensed visualization of the process trajectory. The zoom in functionality of BSPC provides further detailed evaluation and understanding of process performance.

CONCLUSION

This study showed the suitability of batch level modelling in combination with UV-Vis spectroscopy PLS calibrations to measure and monitor phenolic extraction during red wine production under different maceration conditions. Batch level modelling (BLM) provided an overview of batch behaviour at grape and treatment batch level. In general, the fermentation samples separated accordingly to the three pressing times regardless of grape variety. Longer skin contact time proved to enhance polymeric phenol and tannin levels. However, Cabernet Sauvignon seems to be a more suitable cultivar for longer maceration conditions, since more clear effects were observed between phenolic extraction up until 2/3^{rds} of fermentation compared to skin contact time until the end of alcoholic fermentation, whereas fewer phenolic differences were observed for Shiraz. Furthermore, BLM displayed phenolic differences between low and high punch down regimes with minor differences observed between these two cultivars. Finally, the results showed in this study, illustrates the effectiveness of BLM and spectroscopy PLS calibrations to monitor detailed phenolic content in different ferments, proving to be a suitable, rapid and cost-effective method.

DATA AVAILABILITY STATEMENT

The phenolic extraction data generated and used in this study are openly available from Zenodo.org public repository at https://doi.org/10.5281/zenodo.3715104.

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