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Investigating the Relationship Between Skin Cell Wall Composition, Phenolic Extractability and Spectrometric Reflectance of Intact Grape Berries

By

ALBERTO ARDUINO

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Viticulture and Enology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2024

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It really takes a village. It took a village!

Abstract

Grape phenolics represent a class of compounds of primary interest for winemaking, as they are responsible for main sensory characteristics and important physiochemical interactions with other chemicals. These compounds are extracted from the solid phase of the berry, such as skin and seeds, throughout the alcoholic fermentation and can be adsorbed by insoluble solids, such as plant cell walls (CW) and on the surface of seeds and yeast cells. The extent and rate of these molecules' extraction strongly depend on temperature, alcohol concentration, and juice-mixing procedures. CW composition of grape skin has also been shown to play a key role in determining the extractability of grape phenolics.

In this study we investigated the relationship between grape skin cell wall composition and the extraction of grape phenolic compounds. While doing so, we evaluated the opportunity of applying spectral methods to quickly and non-destructively predict cell wall composition and/or extractability of phenolics. Grapes were collected and micro-fermentations carried out at standardized conditions. Grape phenolic composition was analyzed by exhaustive extraction and high-performance liquid chromatography (HPLC). Wine phenolic profile was characterized by HPLC. CW material was isolated from berry skins as alcohol-insoluble residue and characterized. Protein, phenolics and pectin (analyzed as uronic acid) were determined applying colorimetric procedures. Klason lignin was measured gravimetrically. Intact berries were then scanned with Ocean Insight spectrometers, working on a wavelength bandwidth range from 350 to 2,200 nm.

Grape phenolics and their extractabilities were found to mainly depend on the variety, the site and its climate. The relative amount of isolated cell wall material was found to follow a variety-dependent trend. A similar effect was found for CW proteins and phenolics, whose content negatively impacted anthocyanin and phenolic extractability. Isolated cell wall, CW lignin and pectin did not seem to correlate with phenolic extractability. With the data analysis carried out, wavelengths in the range 1004.7-1007.0 nm were found to be successful candidates to predict polymeric pigments content and extractability in wine.

Table of Contents

Introduction

Purpose of the Work. Phenolic extraction in wine processing is complicated and is dependent on grape berry characteristics and fermentation physical and chemical parameters, such as temperature and ethanol concentration (Boulton et al. 2014). Recently, researchers have found a correlation between plant cell wall composition of grape berries and the final phenolic profile extracted into wine (Medina-Plaza et al. 2021). Traditional methods for analyzing the cell wall composition are time consuming and include the gravimetric quantification of lignin as an acid-insoluble residue (González- Centeno et al. 2010) and colorimetric methods to determine uronic acid (Melton and Smith 2001), total phenolics (Singleton et al. 1999), and proteins (Bradford 1976). These procedures are very reproducible and generate accurate results, but they can be quite costly and time-consuming. These two factors make these analyses not applicable in most commercial wine production scenarios where winemaking decisions on cap management have to be made within hours of grape delivery and cannot wait for days of sample preparation and subsequent analytical measurements. Additionally, these methods inevitably lead to the physiochemical modification of the fruit sample analyzed, not allowing a throughput and/or a real-time implementation of these methods in the wine production flow. While the grape skin cell wall composition itself is not fundamentally useful information for winemaking, this parameter was found to be highly correlated with the extractability of phenolics during winemaking and, particularly, anthocyanins (Medina Plaza et al. 2021). Because of the importance of these compounds in wine, engineering models were developed to accurately estimate phenolic extractability over the alcoholic fermentation (Miller et al. 2019a, Miller et al. 2019b, Miller et al. 2020). In the circumstances just presented, we believe that a rapid and uncomplicated measurement for cell wall composition and/or phenolic extractability should be researched and made available to the grape and wine production industry to apply for monitoring of phenolics throughout grape maturation, thereby allowing for cap management decisions to be made that will result in the phenolic profile desired by the winemaker.

We identified UV-VIS-NIR spectrometry as a potentially successful technology to accomplish this goal.

Grape Phenolics and Their Extractability. Grape phenolic compounds represent an extensively studied group of grape chemicals, both for their importance in winemaking and for their beneficial role in human health. Phenolics have indeed a significant effect on the color, taste, and mouthfeel of the produced wine, affecting the overall sensory experience during wine consumption. The accumulation of these compounds in the grape berry is mainly confined to the skin and the seeds (Waterhouse et al. 2016). Two subgroups of phenolics – anthocyanins and condensed tannins – are the most abundant in red grape berries (Downey et al. 2003).

Anthocyanins are responsible for the color of red wines (Boulton et al. 2014). These compounds have a substantial qualitative and commercial importance, as the color of a wine can deeply bias the consumer and modify their impression or expectation towards the product (Lawless and Heymann 2010). Anthocyanins are located in the vacuole of skin cells, where they begin to accumulate at veraison and increase in concentration throughout ripening to eventually get degradated when high levels of ripening are achieved (Boulton et al. 2014). The anthocyanin content in the berry varies, depending on the variety (Theodorou et al. 2019), the site (Pérez-Álvarez et al. 2019, Mansour et al. 2022), the climate (Mori et al. 2007) and the applied farming procedures, such as the chosen trellising system or the adopted irrigation regime (Yu et al. 2022).

Other phenolics commonly found in grapes and wines are polymeric phenols. These compounds are also commonly known as tannins. Synthesized in the fruit, they are accumulated mainly in the tissues of the skin and the seed. When polymerization reactions occur between an anthocyanin and other phenolic compounds, the product is a polymeric chromophore called polymeric pigment. These reactions occur in wine during and after the primary fermentation (Boulton et al. 2014, Waterhouse et al. 2016). Throughout the alcoholic fermentation with maceration, anthocyanins and tannins are extracted from the skins (and seeds, in the case of tannins) into the liquid portion of the

juice and the formation of polymeric pigments can be enhanced (Boulton et al. 2014). Many authors have proven how a higher accumulation of anthocyanins in the grapes does not always result in a high concentration of the same compounds in the wine (Romero-Cascales et al. 2005a, Romero-Cascales et al. 2005b). The localization and nature of these chemicals make their final concentration in wine to be a function of many factors. Firstly, these compounds need to be extracted from the solid parts of the grapes and released into the liquid fraction (Miller et al. 2019a). This process is highly dependent on the grape variety (Medina-Plaza et al. 2021, Río Segade et al. 2008, Romero-Cascales et al. 2005b) and the growing site (Medina-Plaza et al. 2021). Grape skin cell wall materials (CWM) play a crucial role in the overall kinetics of anthocyanin extraction from the vacuole of skin cells (Medina-Plaza et al. 2021, Medina-Plaza et al. 2022, Rumbaugh et al. 2023) and also on the adsorption back onto the cell wall. The physical and chemical conditions to which maceration is carried out are also fundamental in determining the extent and kinetics of the extraction. In particular, the temperature at which the must is macerated and the concentration of alcohol produced during the fermentation have a direct positive effect on anthocyanin extraction (Lerno et al. 2015, Miller et al. 2019a, Romero-Cascales et al. 2005a). Anthocyanins are mostly extracted in the first phase of the alcoholic fermentation, when an equilibrium is reached and their concentration is mostly unvaried thereafter (Romero-Cascales et al. 2005a), while polymeric phenols start to get extracted later in the fermentation, with alcohol concentration and temperature being key parameters to influence their diffusion into the liquid portion (Lerno et al. 2015, Mayen et al. 1994). Anthocyanins can then decrease in concentration by undergoing chemical reactions leading to either their degradation or polymerization (Waterhouse et al. 2016). Lastly, anthocyanins are subject to adsorption onto suspended solids present in the medium, such as yeast, plant cell walls (Miller et al. 2020), and seeds (Giacosa et al. 2023).

Grape CWM therefore affects the final wine anthocyanin concentration in multiple ways, by influencing their extraction and being among the main solids adsorbing these pigments (Beaver et al. 2020).

Grape Cell Wall and Its Impact on Phenolic Extractability. Cell walls are essential features of the plant cells that ensure their proper functioning. These structures are indeed responsible for cells' tissue-specific shape and function. They partake in intercellular communication and interactions between plants and microbes, including defense mechanisms against pathogens. Cell walls are formally categorized into primary and secondary, although each differentiated cell has a specific wall composition falling in between the two. The primary cell wall is thinner and characterizes expanding and growing cells. The secondary is instead found in those tissues with limited or no growth and it provides mechanical strength. (Keegstra 2010, Keller 2020). The main components of the cell walls of grape berries skin are polysaccharides, lignin, phenolics, lipids and proteins. Polysaccharides account for about 50% of the cell walls' weight (Lecas and Brillouet 1994). Cellulose, hemicellulose (also referred to as cross-linking glycans), pectic substances are the main polysaccharides. Cellulose – a β 1-4 glucose polymer – is the basic unit of microfibrils. These structures are interknitted and held together by cross-linking glycans, attaching multiple fibrils by hydrogen bonds (Keegstra 2010, Keller 2020). Pectic substances are heterogeneous polysaccharides rich in galacturonic acid, which accounts for approximately 70% (Albersheim et al. 1996). These polysaccharides are amorphous and provide gel-like consistency to the cell wall. Lignin can be found in secondary cell walls, where it acts as a binder between cellulose and hemicelluloses, it enhances rigidity and strength and represents an extra barrier against pathogens and other stress factors (Liu et al. 2018, Sharma et al. 2018). Phenolic components and proteins can be found in this complex matrix. Phenolics are usually found in cell walls as tannins, in their polymeric form. Proteins have multiple functions, being mainly involved in the structure of the cell wall and in the signaling between cells (Keegstra 2010).

Throughout ripening, grape cell walls undergo important modifications in their composition and structure that lead to a softening of tissues (Nunan et al. 1998). Many authors describe a decrease in total cell wall materials, together with an increase in pectin degradation, demethylation and deacetylation happening after veraison (Ortega-Regules et al. 2008, Vicens et al. 2009). This is the

result of an increased hydrolytic activity of the enzymes associated with cell wall metabolism and negatively affects the mechanical strength of skins (Ortega-Regules et al. 2008). Our research group has intensively investigated the relationship between the changes in cell wall composition of grape skins and the extractability of aromas and phenolics (Beaver et al. 2020, Medina-Plaza et al. 2021, Miller et al. 2019a, Miller et al. 2019b, Miller et al. 2020, Rumbaugh et al. 2023). Medina-Plaza et al. (2021) found the uronic acid content to be correlated with an increase in all phenolics extractability. On the other hand, phenolic extractability showed to be negatively correlated with higher contents of lignin and proteins in cell walls and, to a smaller extent, by the relative quantity of cell wall material in the skins. Similarly, Rumbaugh et al. (2023) noticed that virus-diseased grapes had significantly lower concentration of both anthocyanins and phenolics in general. Together with a higher protein and pectin CW content, this effect resulted in a lower relative extractability of phenolic compounds in wine.

As aforementioned, cell walls from plants and yeast can also negatively impact the phenolic extractability from grapes to wine by being an important site of adsorption for this group of molecules (Medina-Plaza et al. 2020, Razmkhab et al. 2002). Osete-Alcaraz et al. (2019) showed how the elimination of suspended CWM during the maceration resulted in a substantial enhancement of the phenolic content of the wines and improved their color attributes. Some experiments even evaluated the possibility of using purified grape cell walls to reduce wine phenolic content and found that this procedure had a more impactful outcome than the use of commercially available protein-based fining agents (Jiménez-Martinez et al. 2017).

UV-VIS-NIR Spectrometry and Its Applications to Characterize Cell Wall Materials. Recently, many research groups have investigated the possibility of characterizing cell walls of different crops using spectroscopy in the ultra-violet (UV), visible (VIS) and near infrared (NIR) bandwidth ranges. Yang et al. (2021) successfully applied hyperspectral imaging to nondestructively quantify pectin polysaccharides in mulberry fruit. Other researchers used NIR

spectroscopy to analyze lignin in pears, avoiding destruction of the sample (Wu et al. 2021). Similarly, Badaró et al. (2020) used NIR hyperspectral imaging to determine pectin content in orange peels. In grapes, such technologies have been applied to estimate berry basic chemistry, such as soluble solids (Gutiérrez et al. 2019, Xu et al. 2022, Xu et al. 2023), pH (Fernández-Novales et al. 2021), titratable acidity (Fernández-Novales et al. 2021, Xu et al. 2023), tartaric and malic acid (Fernández-Novales et al. 2021), anthocyanin (Chen et al. 2015, Gutiérrez et al. 2019), total polyphenols (Fernández-Novales et al. 2021) and even the phenolic composition of seeds (Jara-Palacio et al. 2016). However, to date, no research is available on the opportunity to apply spectroscopic methods or spectrometry to predict the concentration of grape cell wall constituents or phenolic extractability.

Outline of the Work. With this experiment we are extending the work previously done by this research group (Miller et al. 2020, Medina-Plaza et al. 2021, Medina-Plaza et al. 2022) and aiming to investigate the possibility of collecting information about the cell wall composition or the phenolic extractability of grapes with a non-destructive and rapid procedure. To do so, we collected ten grape samples from a variety of commercial vineyard sites in California, belonging to four relevant cultivated varieties in this region. The grapes were crushed and the must was adjusted to standardized chemical properties, before being fermented at a controlled temperature in 300-gram batches. Phenolic composition of grapes and wines were analyzed, CWM of grape skins isolated and characterized and the reflectance of intact berries was measured in the range of 350-2100 nm, through our spectrometry apparatus.

Materials and Methods

Reagents. Bovine albumin standard solution (2.0 mg/mL), Coomassie protein assay reagent, phosphoric acid (88%) (HPLC grade) and sodium hydroxide (ACS grade) were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, USA). Malvidin-3-O-glucoside (95%) was purchased from Extrasynthese (Extrasynthese, Genay, France). Acetone (reagent grade), acetonitrile (HPLC grade), methanol (reagent grade), hydrochloric acid (37%, reagent grade), trifluoroacetic acid (HPLC grade), sulfuric acid (96% reagent grade), diethyl ether (ACS reagent, 99%), phenol (reagent grade), L-ascorbic acid (molecular biology grade), potassium bitartrate (99%), Folin-Ciocalteau reagent, sodium carbonate, (+)-catechin hydrate (98%), gallic acid monohydrate (99%), 3-phenylphenol (85%), sodium tetraborate (99%), and D-galacturonic acid (97%) were purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). Koptec brand ethanol (95%) was purchased from Decon Laboratories (Decon Laboratories Inc., King of Prussia, PA, USA). Deionized water was prepared in-house to a final purity of 18.2 MΩ.

Plant Material. Four winegrape (*Vitis vinifera* L.) varieties, Cabernet Sauvignon (CS), Pinot noir (PN), Zinfandel (ZN) and Petite Sirah (PS) were harvested at commercial ripeness. Clonal selection, geographical coordinates and basic chemical composition of grapes (Brix, pH, Titratable Acidity) can be found in Table 1. Two sites in the Napa valley floor, in the Oakville (CS-NAPA-VF-1) and Yountville (CS-NAPA-VF-2) AVAs, and one in the mountains (CS-NAPA-MNT-1), in the area of Pritchard Hill, were studied for the CS. The PN grapes were harvested from one site in Yolo County (PN-YOLO) and two sites in Monterey County (PN-MRY-1, PN-MRY-2). For ZN two sites were selected in the Napa valley mountains in the area of lake Berryessa (ZN-NAPA-MNT-2, ZN-NAPA-MNT-3), while PS was harvested from two sites in the Napa valley floor, one in the Oakville (PS-NAPA-VF-1) and one in the Oak Knoll (PS-NAPA-VF-2) AVA. Fertilization, irrigation, and canopy management were appropriately performed throughout the growing season

based on the growing region and variety. Vineyard location, sample coding, clones and grape basic chemistry are defined in Table 1.

Table 1. Vineyard coordinates, sample codes, location, and basic chemical analysis for all grape samples. TA: titratable acidity.

Variety/Code*	Clone	Latitude	Longitude	Vineyard Site	pH	°Brix	TA (g/L)
Cabernet Sauvignon							
CS-NAPA-VF-1	FPS 04	38.435644	-122.371712	Napa Valley Floor 1	3.45	22.80	6.07
CS-NAPA-VF-2	FPS 07	38.4274883	-122.358071	Napa Valley Floor 2	3.50	24.60	6.28
CS-NAPA-MNT-1	FPS 04	38.4697364	-122.3504337	Napa Mountain 1	3.88	25.30	4.91
Pinot noir							
PN-YOLO	FPS 09	38.531507	-121.753318	Yolo County	N/A	30.60	N/A
PN-MRY-1	FPS 23	36.090507	-121.025418	Monterey County 1	3.74	25.50	5.78
PN-MRY-2	FPS 23	36.088788	-121.016159	Monterey County 2	3.64	25.30	6.09
Petite Sirah							
PS_NAPA-VF-1	FPS 03	38.4348128	-122.3734343	Napa Valley Floor 1	3.58	23.50	6.72
PS_NAPA-VF-3	FPS 08	38.3419731	-122.3052767	Napa Valley Floor 3	3.31	23.90	7.15
Zinfandel							
ZN-NAPA-MNT-2	FPS 13	38.451542	-122.202015	Napa Mountain 2	4.05	24.90	4.82
ZN-NAPA-MNT-3	FPS 13	38.451928	-122.198989	Napa Mountain 3	3.57	23.50	5.30

*CS: Cabernet Sauvignon, PN: Pinot Noir, PS: Petite Sirah, ZN: Zinfandel, NAPA: Napa County, YOLO: Yolo County, MRY: Monterey County, VF: Valley Floor, MNT: Mountain, Numbers refer to sites in the same region.

Winemaking Protocol. The winemaking protocol followed the one described by Sparrow et al. (Sparrow et al. 2016). Three-hundreds grams of grapes were hand-destemmed and crushed, before being transferred into a Bodem Coffee Plunger (Bodum Inc., Triengen, Switzerland) with an addition of 50 mg/L of SO_2, using a 15% potassium metabisulphite (KMBS) solution. The juice was then adjusted to 25 °Brix using either sucrose or water as needed, to 6 g/L of titratable acidity using tartaric acid and to 250 mg/L of yeast assimilable nitrogen (YAN) using diammonium phosphate (DAP). The juice was inoculated at the ratio of 264 mg/L with the selected strain Lalvin EC1118 of *Saccharomyces cerevisiae* (Lallemand, Montreal, Canada), with an equal amount of the rehydration starter Go-Ferm (Scott Laboratories, Petaluma, CA, USA). Each fermentation was performed in triplicate. The temperature of fermentations was controlled by keeping the room

atmosphere at 25 °C. The skins were kept in contact with the juice by keeping the plunger pressed down. Brix and temperature were monitored by density and recorded on a daily basis. After seven days of maceration the liquid and solid fractions of the fermenting must were separated. The wine was considered dry when residual sugar (RS) concentration was below 1 g/L as determined by enzimatic analysis for detection of Sucrose, D-Glucose and D-Fructose using the automatic oenological analyser Biosystems SPICA (Biosystems, Atlanta, GA, USA). At dryness the wine was transferred into 250 ml amber glass bottles, whose headspace was purged with nitrogen, and left to settle for 7 days at 4 °C. The wine was finally racked into 50 ml Falcon tubes (Thermo Fisher Scientific, Waltham, MA, USA) and frozen at -20 °C for further analyses.

Exhaustive Extraction of Grapes. For each sample, three sets of 20 randomly selected berries were weighed and flash frozen using liquid nitrogen, before being pulverized with an A11 Basic Analytical mill (IKA® Works, Inc., NC, USA). Two grams of the powder were placed into a 50 ml Falcon tube (Thermo Fisher Scientific, Waltham, MA, USA). A 1:1 ethanol:water solution containing 0.1% w/v hydrochloric acid (HCl) and 0.1% w/v ascorbic acid was added to the pulverized frozen tissue at the ratio of 5 ml of solution to 1 g of tissue. Phenolic compounds were extracted for 20 minutes in an ultrasonic bath (Branson Ultrasonics Corp., Brookfield, CT, USA) containing ice-saturated water. The samples were centrifuged at 3,200 RPM at 4 °C for 10 minutes, and the supernatant collected and stored at -20°C. The remaining phenolic compounds in the pulverized tissue were extracted once again following the whole procedure described above for a second time. The supernatants from the first and second extractions were combined and concentrated under reduced pressure to approximately 0.5 ml at 34°C, quantitatively transferred to a 2 ml volumetric flask and made up to volume with a solution of 50% methanol containing 0.1% HCl.

RP-HPLC-DAD Analysis. Grape extracts were diluted at the ratio of 1:1 with a solution of 50% methanol containing 0.1% HCl. Wines were not diluted. Both grape extracts and wines were centrifuged for 5 minutes at 3,200 RPM before being immediately analyzed for their phenolic profile using reversed-phase high-performance liquid chromatography (RP-HPLC). The Agilent 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) used was equipped with an autosampler having controlled temperature of 8°C and a diode array detector (DAD). Two mobile phases were used in a gradient of separation as previously described by Peng et al. (Peng et al. 2002): mobile phase A (1.5% phosphoric acid v/v in water) and mobile phase B (80% acetonitrile and 20% mobile phase A). A volume of 20.0 µL of the sample was injected on an Agilent PLRP-S 100A 3µM 150x4.6 mm column (Agilent Technologies, Santa Clara, CA, USA) at 35°C and a flow rate set at 1 mL/min.

Phenolics of interest were determined at wavelengths of 280 nm (polymeric phenols) and 520 nm (monomeric anthocyanins and polymeric pigments). (+)-Catechin and malvidin-3-*O*-glucoside (Sigma Aldrich, St. Louis, MO, USA) were quantified through calibration curves produced with authentic standards with limits of quantification of 0.50 and 0.10 mg/L, respectively. Tannins were quantified as catechin equivalents, while all monomeric anthocyanins and pigmented polymers were quantified as malvidin-3-*O*-glucoside equivalents. Instrument control and data analysis were performed using Agilent ChemStation (Rev. B.04.03) software.

Phenolic Extractability of Grapes. Extractability of different classes of phenolic compounds was calculated with the formula below (Equation 1).

$$Extractability\ (\%) = \frac{Wine\ Relative\ Content\ (unit/berry\ weight)}{Grapes\ Relative\ Content\ (unit/berry\ weight)} \times 100 \quad (1)$$

Cell Wall Material (CWM) Isolation. CWM was isolated from skins as 70% alcohol-insoluble residue, following the method developed by de Vries et al. (1981). Berries were pulled from their pedicel and, using a scalpel, the skin was separated from the flesh. Ten grams of grape skins were

suspended in 15 ml of boiling water for five minutes to ensure enzyme inactivation and ground for one minute using a domestic blender (SharkNinja Operating LLC, Needham, MA, USA). One part of the homogenate was mixed with four parts of 96% ethanol and placed in a water bath (Branson Ultrasonic Corporation, Brookfield, CT, USA) at 40 °C for extraction. Alcohol-insoluble solids were separated by filtration on paper, using Whatman 1001-110 Grade 1 Qualitative Filter Paper, 97 Diameter: 11 cm, Pore Size: 11 µm (General Electric Co., Boston, MA, USA). The filtered solids were suspended again in a volume of 70% ethanol four times the initial volume of the homogenate and placed in the 40 °C water bath, before undergoing a second paper filtration. The process was repeated a third time to obtain insoluble material (IM).

Insoluble Material Mass Yield. Mass yield of IM was calculated with the formula below (Equation 2).

$$Mass\ Yield\ (\%) = \frac{IM\ (g)}{Grapes\ Skin\ Mass\ (g)} \times 100 \quad (2)$$

CWM Characterization. *Uronic acid analysis.* CWM was first hydrolyzed using sulfuric acid (Ahmed and Labavitch 1978), then pectin content was determined as uronic acid by a colorimetric 3,5-dimethylphenol assay (Filisetti-Cozzi and Carpita 1991), following the procedure described by Melton and Smith (2001). Pure galacturonic acid was used as a standard (Sigma Aldrich, St. Louis, MO, USA). Pectin content was expressed as mg anhydrous galacturonic acid/g CWM.

Phenolic content. Phenolic content of CWM was determined colorimetrically by means of Folin-Ciocalteu reagent (Singleton et al. 1999) following extraction via the saponification method (1 M NaOH, 100 °C, 10 min) using pure gallic acid as a standard (Sigma Aldrich, St. Louis, MO, USA). Phenolic content was determined at 765 nm and expressed as mg gallic acid/g CWM.

Protein analysis. The protein content of CWM was determined using the Bradford assay (Bradford 1976), after digesting the CWM sample with 1 M NaOH (10 min, 100°C). Bovine serum albumin

(BSA) solution was used as standard to calibrate the analysis with standards ranging from 0 to 2000 mg/L in concentration. Protein content was measured at 595 nm and expressed as mg BSA/g CWM. *Lignin content.* Lignin was determined gravimetrically as acid-insoluble residue (González-Centeno et al. 2010).

Spectrometry Measurement. The spectrometry apparatus was composed of two spectrometers FLAME-T-VIS-NIR-ES and NIRQUEST+2.2, working in the bandwidth ranges of 350-1000 nm and 950-2100 nm, respectively. The HL-2000-HP-FHSA halogen lamp was chosen as a light source and a 200 um reflection probe was used to convey the light from the source to the sample and from the sample to the spectrometers. A spectralon diffuse reflectance standard was used to calibrate the instruments. Oceanview was the software used to process data from the spectrometers. All the spectrometric equipment was manufactured by Ocean Insight (Ocean Optics Inc., Orlando, FL, USA). Atmospheric light was screened out to preserve replicability of measurements. For each sample fifty berries were randomly chosen and thawed at room temperature. Reflectance of light in the specific bandwidth range was then collected. Measurements were averaged for further data analysis. Reflectance was collected at 0.215 nm and 2.411 nm intervals in the UV-VIS and NIR regions of the solar radiation, respectively.

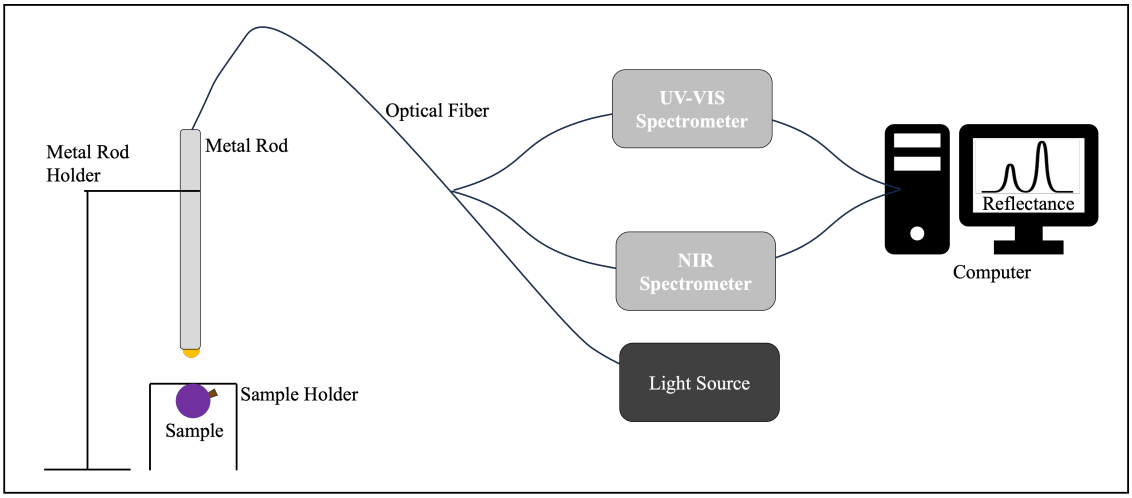

Figure 1. Spectrometry apparatus including sample holder, metal rod and its holder, optical fiber cable, UV-VIS and NIR spectrometers, light source and computer.

Statistical Analysis. Analysis of variance using Tukey's test for comparison (ANOVA, $p < 0.05$), multifactorial analysis (MFA), and principal component analysis (PCA) were carried out through XLSTAT, version 25.1.6 (Addinsoft, Paris, France).

Results

Grape Phenolics and Their Extractability. Grapes were harvested and fermented in triplicates under standard conditions to produce wines. The phenolic composition of grape exhaustive extracts and wines were then determined by means of RP-HPLC-DAD. Extractability was calculated by applying equation (1). Figure 2 shows the grape and wine contents for three different classes of phenolic compounds (anthocyanins, polymeric pigments and polymeric phenols) together with their net relative extractability achieved throughout the alcoholic fermentation.

Focusing on anthocyanins, the content in grape berries covered a wide range for the tested samples (Figure 2a). Grape variety was the main parameter affecting their accumulation in the fruit. Petite Sirah (PS) grapes had significantly higher anthocyanin contents, followed by Cabernet Sauvignon (CS), Pinot noir (PN) and Zinfandel (ZN). PN and ZN samples had very similar concentrations of berry anthocyanins and showed no significant difference among their results (Figure 2a). Interestingly, an even stronger variety effect was noticed for the anthocyanin content of wines, that showed instead no significant difference between sites, with the exception of the Petit Sirah. Within the same variety, wines showed a statistically similar – or at least comparable – content in anthocyanins, regardless of the starting concentration in the grapes. In other words, in our experiment the site had no effect on wine anthoycanins content. The only exception to this trend was represented by the wines made from Petite Sirah (PS) grapes that were also the ones with the highest concentration of anthocyanins, reflecting the grape anthocyanin content for the two PS samples. Pinot noir and Zinfandel wines fell in the same statistical grouping (Figure 2a).

Polymeric pigments results showed similar trends to anthocyanins. Site and variety were noticed to be the main drivers for polymeric pigment content in grapes and wines. In agreement with anthocyanins, their content in wine was mostly similar across samples within the same variety and the extractability of these compounds did not show a clear pattern or trend (Figure 2b). Grape polymeric phenols and their extractability seemed instead to be fairly similar across all samples, showing no evident trend with respect to the variety or the site (Figure 2c).

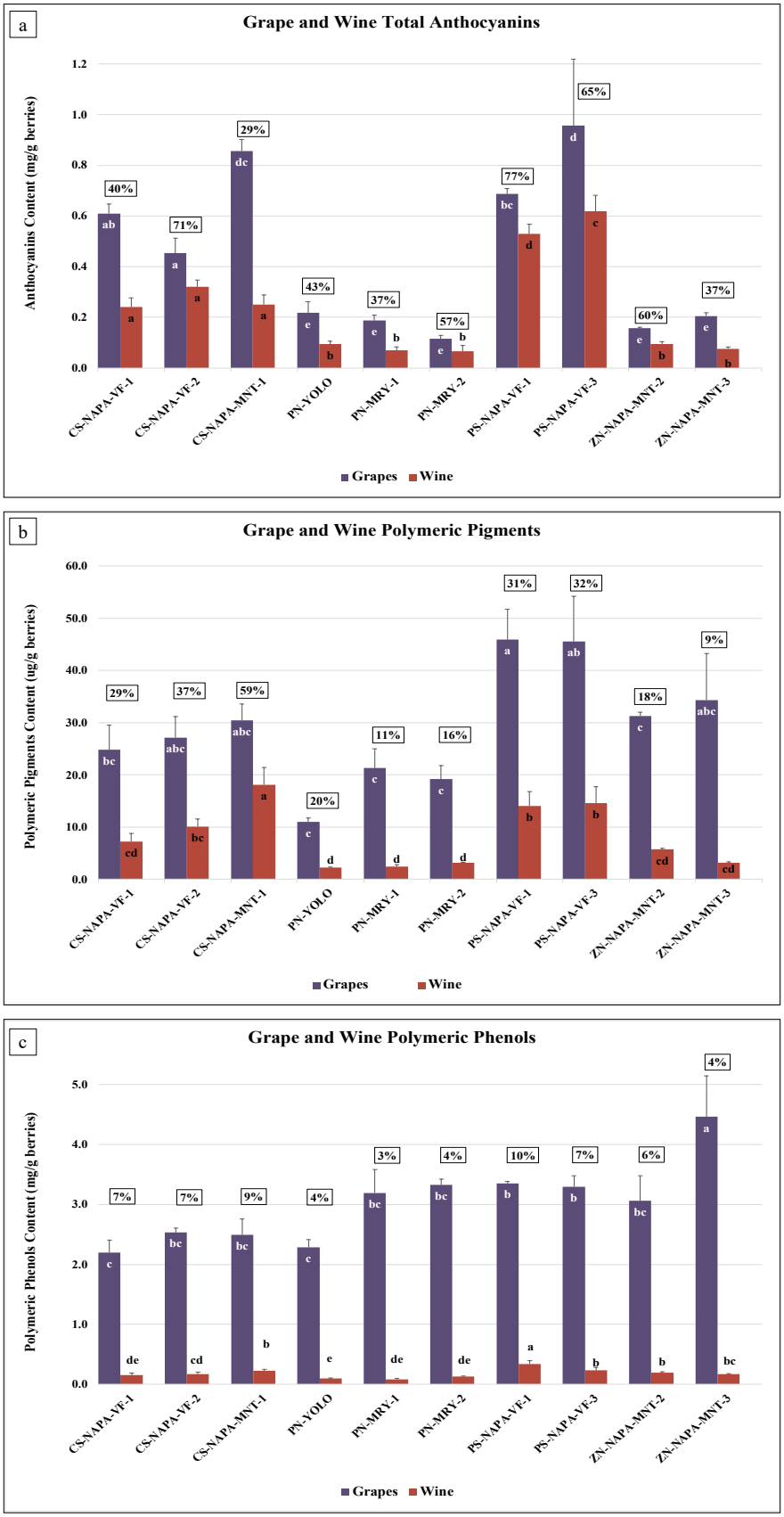

Figure 2. Average of triplicates for grape and wine **(a)** total anthocyanins, **(b)** polymeric pigments and **(c)** polymeric phenols. Error bars represent standard deviation. Significance grouping (Tuckey's HSD) and relative extractability (%) are shown. CS: Cabernet Sauvignon, PN: Pinot noir, PS: Petite Sirah, ZN: Zinfandel. VF: Valley Floor, MNT:Mountain. Numbers refer to different sites in the same region.

Grape Cell Wall Yield and Composition. Cell wall material (CWM) was isolated as alcohol-insoluble residue from grapes skins. The IM mass yield was calculated as the ratio between the weight of isolate IM and the weight of the processed skins, by applying equation (2). Table 2 presents the data for IM mass yield. It can be noticed how the results for this parameter spanned across a wide range from one sample to another and showed a variety-dependent trend (Table 2). IM yields for Cabernet Sauvignon spanned from 10.90% to 13.10%. Similar values were registered for Petite Sirah, whose IM ranged between 11.23% and 12.49%. IM tended to be higher for Pinot noir (16.62% up to 22.79%), while Zinfandel showed the biggest variability between samples, having IM that fluctuated from as low as 13.00% to as high as 21.25%, despite the proximity of the two sites where the grapes were grown (Table 1, Table 2).

Table 2. Cell Walls

Variety/Code*	Mass Yield (%)
Cabernet Sauvignon	
CS-NAPA-VF-1	12.31%
CS-NAPA-VF-2	10.90%
CS-NAPA-MNT-1	13.10%
Pinot noir	
PN-YOLO	19.85%
PN-MRY-1	16.62%
PN-MRY-2	22.79%
Petite Sirah	
PS_NAPA-VF-1	12.49%
PS_NAPA-VF-3	11.23%
Zinfandel	
ZN-NAPA-MNT-2	13.00%
ZN-NAPA-MNT-3	21.25%

*CS: Cabernet Sauvignon, PN: Pinot Noir, PS: Petite Sirah, ZN: Zinfandel, NAPA: Napa County, YOLO: Yolo County, MRY: Monterey County, VF: Valley Floor, MNT: Mountain, Numbers refer to sites in the same region.

Isolated CWM was then characterized to determine its content in phenolic, protein, uronic acid and Klason lignin. Phenolic, protein and pectin were determined by colorimetry, while lignin was quantified gravimetrically. The concentration of these macromolecules in the berry was expressed

as µg uronic acid/g CWM, mg gallic acid/g CWM, mg BSA/g CWM and mg/g CWM, respectively and it is reported in Figure 3.

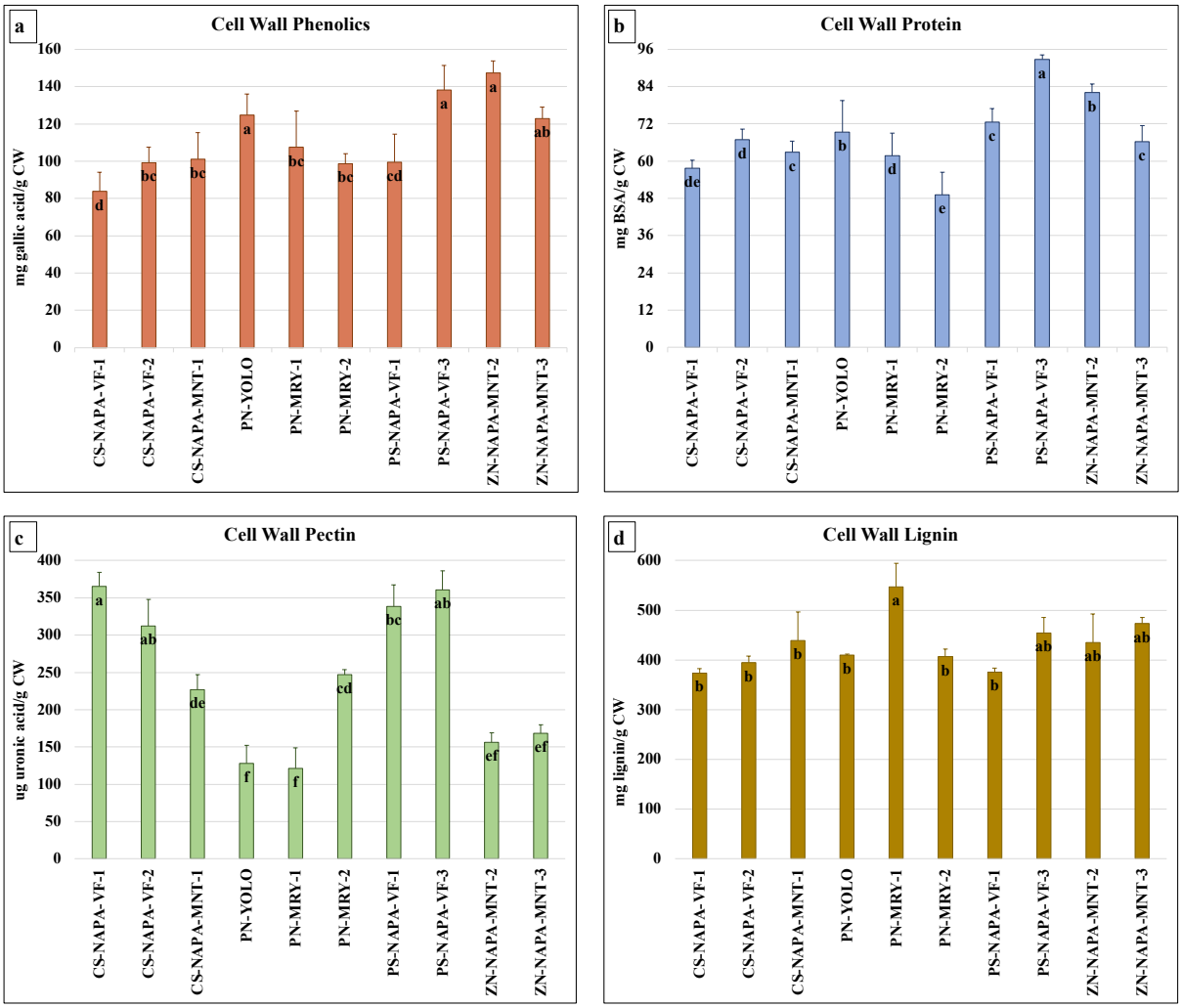

Figure 3. Average of replicates (n=3) for cell wall (**a**) phenolics, (**b**) protein, (**c**) pectin (analyzed as uronic acid) and (**d**) lignin. Error bars represent standard deviation and significance grouping (Tuckey's HSD) is shown. CS: Cabernet Sauvignon, PN: Pinot noir, PS: Petite Sirah, ZN: Zinfandel. VF: Valley Floor, MNT:Mountain. Numbers refer to sites in the same region.

CW phenolic content showed a variety-dependent trend, with the samples of Cabernet Sauvignon registering the lowest (84 ± 10 to 101 ± 14 mg gallic acid/g) and Zinfandel the highest values (123 ± 6 to 147 ± 6 mg gallic acid/g). Pinot noir CW phenolics were in the middle of the range (99 ± 6 to125 ± 11 mg gallic acid/g CW), while Petite Sirah was somehow an outlier to this pattern and showed great variability between the two samples studied (99 ± 15 mg gallic acid/g CW for PS-

NAPA-VF-1 and 138 ± 13 mg gallic acid/g CW for PS-NAPA-VF-2). Cell wall protein showed a very similar pattern to CW phenolics, suggesting a correlation existing between the two constituents of the cell wall. The CS-NAPA-VF-1 grape sample had both the lowest CW protein and phenolic contents. Similarly, PN-MRY-2 was within Pinot noir the one with least CW proteins and phenolics (Figure 2a, 2b). PS-NAPA-VF-2 and ZN-NAPA-MNT-2 were the samples amongst all to contain the most protein (93 ± 2 and 82 ± 3 mg BSA/g CW, respectively) and phenolics (138 ± 13 and 147 ± 6 mg gallic acid/g CW, respectively) in their cell walls. The two PS samples, if compared to each other, had very different results in terms of both CW proteins and phenolics (Figure 2a, 2b). This discrepancy between the two Petit Sirah's was also noticeable for grape anthocyanins (Figure 2a).

Pectin ranged widely among all samples and showed an intersting site-specific pattern. Cabernet Sauvignon samples grown in the Napa Valley floor (CS-NAPA-VF-1 and CS-NAPA-VF-2) were statistically similar to each other, but the same variety grown in the Napa Valley mountain (CS-NAPA-MNT-1) showed lower values. This variety and site specificity was confirmed by the samples of Zinfandel (ZN-NAPA-MNT-2 and ZN-NAPA-MNT-3) and Petite Sirah (PS-NAPA-VF-1 and PS-NAPA-VF-3). Belonging to the same variety and being grown in proximity, these samples fell in the same statistical grouping. Even if most of the varieties returned comparable results for CW pectin when grown in proximal vineyards. Pinot noir showed a strong inconsistency in this sense. The two samples from Monterey County (PN-MRY-1 and PN-MRY-2) had significantly different CW pectin, while the one from Yolo County (PN-YOLO) fell in between the two and in the same statistical group as PN-MRY-1. This led to the uncertainty of establishing if a clear pattern exist between the site, the variety and the CW pectin content. However these results, even with some exceptions, create an insight on the site-specificity of cell wall pectin content. Lastly, lignin showed no dependency with the site and/or variety and the results were overall very similar across all samples, with only PN-MRY-1 returning more than 500 mg lignin/g CW, but still not differentiating from the rest of the samples. This result might suggest lignin to be higly affected by

ripening, as our samples were all harvested at similar levels of technological ripeness, with a density ranging from 23 to 30 °Brix.

The Effect of Cell Wall Composition on Extractability of Phenolics. Multi-factorial analysis (MFA) was applied to analyze the relationship between multiple sets of data. A first MFA analyzed the data for grape and wine phenolics and grape cell wall characterization. Figures 4a and 4b show the biplot and the scoreplot for this MFA, respectively (Figure 4a, 4b). The primary axis F1 of the MFA explained 45.50% of the variability, while the secondary F2 explained 25.82%. Together, they accounted for 71.33% of the variability between samples (Figure 4a). It was observed that all the wine phenolic components closely fell in the same area of the biplot, suggesting a relationship between each other's extraction and with their content in wine. Wine polymeric pigment showed a strong correlation with grape anthocyanin. With regards to the cell wall components, we had already noticed a great similarity between the patterns of cell wall phenolics and proteins that was further supported by the results in Figure 4a. These components were closely located on the MFA biplot, confirming that a correlation between the two may exist. Proteins and phenolics also seemed to limit the phenolic extractability. CW yield and lignin seemed to be closely linked to each other as well. Finally, CW yield and CW lignin and CW uronic acid seemed to have little or no effect on the extraction of phenolic compounds from the berries (Figure 4a). Even if this trend seemed to not be relevant across all samples and not supported by the results of the MFA, we had noticed that in Zinfandel a higher yield of cell wall (13.00% for ZN-NAPA-MNT-2 versus 21.25% for ZN-NAPA-MNT-3) negatively affected the extractability of anthocyanin (60% for ZN-NAPA-MNT-2 versus 37% for ZN-NAPA-MNT-3) (Fig 1a, Table 2). As for the scoreplot (figure 4b), the samples were moderately clustered acording to their variety and origin. The two PNs from Monterey county fell on the right end of the plot, while the ZNs in the top left quadrant and the PSs and CSs scattered, but still close to each other and mostly in the bottom quadrants (Figure 2b).

A second MFA was carried out on the data for extractability of different phenolics and grape cell wall characterization. The biplot and the scoreplot can be found in Figures 4c and 4d, respectively

(Figure 4c, 4d). From this results it can be noticed how clearly the cell wall components negatively affect the extractability of phenolics. Particularly CW phenolics and CW proteins, with CW lignin having secondary effects and CW Yield and CW uronic acid being found to have almost no effect on the extractability of any of the analyzed classes of phenolics (Figure 4c). In the scoreplot for this second MFA the samples clustered very clearly mainly based on their grape variety and secondarily because of site. The Petite Sirahs were found in the top left quadrant, The Cabernet Sauvignons in the top right, the Pinot noirs from Monterey County in the bottom right and the Zinfandels in the bottom left, together with the Pinot noir from Yolo County. Interestingly this last sample, coming from a warmer climate than the other two PNs, were closer to the Zinfandels than the other two Pinot noirs, in terms of the relationship between cell wall composition and extractability of phenolics (Figure 4d).

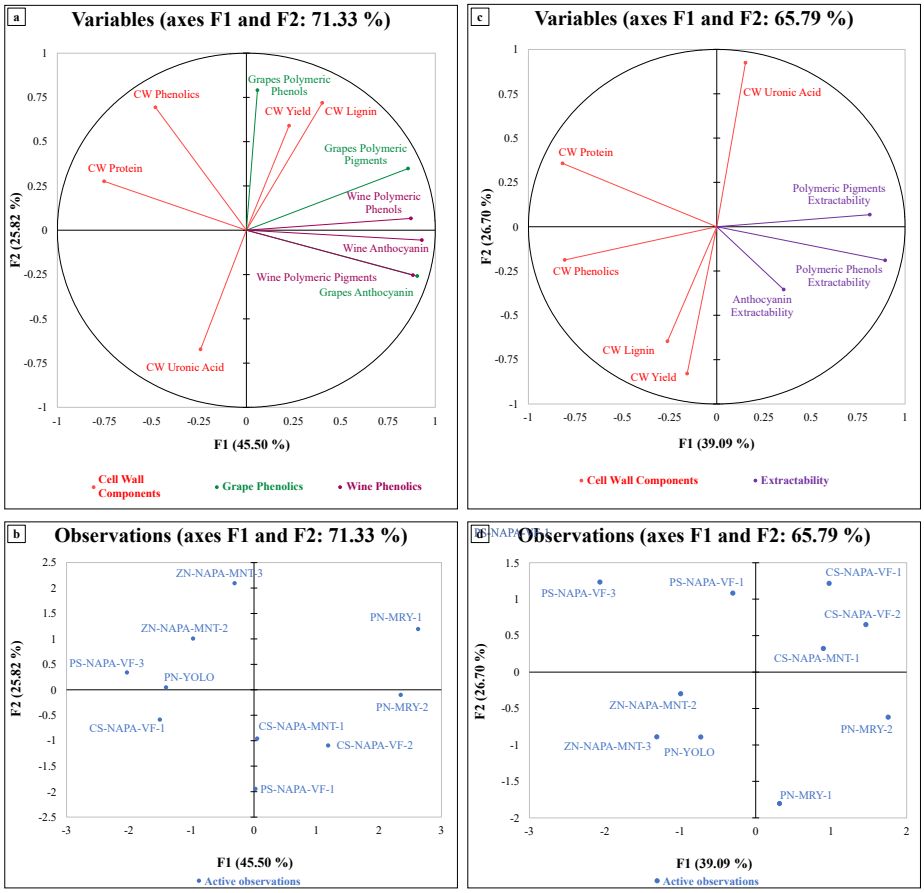

Figure 4. a) Multiple factor analysis (MFA) biplot and **(b)** its score plot for cell wall (CW) components, grapes phenolics and wine phenolics. **(c)** Multiple factor analysis (MFA) biplot and **(d)** its score plot for cell wall (CW) components and phenolic extractability. CW: Cell Wall.

Correlation of Near-Infrared Spectrometry with Cell Wall Composition and Phenolic Extractability.

The phenolic composition of both grapes and wines, their extractability calculated by applying equation (1), IM composition and yield, calculated by means of equation (2), and the reflectance of the grapes samples in the NIR region – were analyzed by multi-factorial analysis (MFA) (Figure 5a, 5b). The cumulative variance explained by the first three factors was 79.83% (Figure 5), F1 accounting for 41.44%, F2 for 20.46% and F3 for 17.93%. Few key wavelengths were significantly correlated with one or more parameters obtained by means of wet chemistry. Generally, reflectance in the spectral range between 999.8 nm and 1007.0 nm seemed to be the most correlated. In particular, reflectance at 999.8 nm was noticed to be loosely correlated with the CW uronic acid (Figure 5a and 5b). Reflectance at 1002.2 nm seemed to correlate well with cell wall phenolics in the two primary dimensions (Figure 5a), but this relationship was not confirmed in the third dimension (Figure 5b), showing a weak relationship instead. A very good correlation was noticed in all of the three primary factors between the reflectance at 1007.0 nm and the concentration of polymeric pigments in wine. Similarly, the reflectance at 1004.6 nm and the extractability of polymeric pigments were well correlated (Figure 5a, 5b).

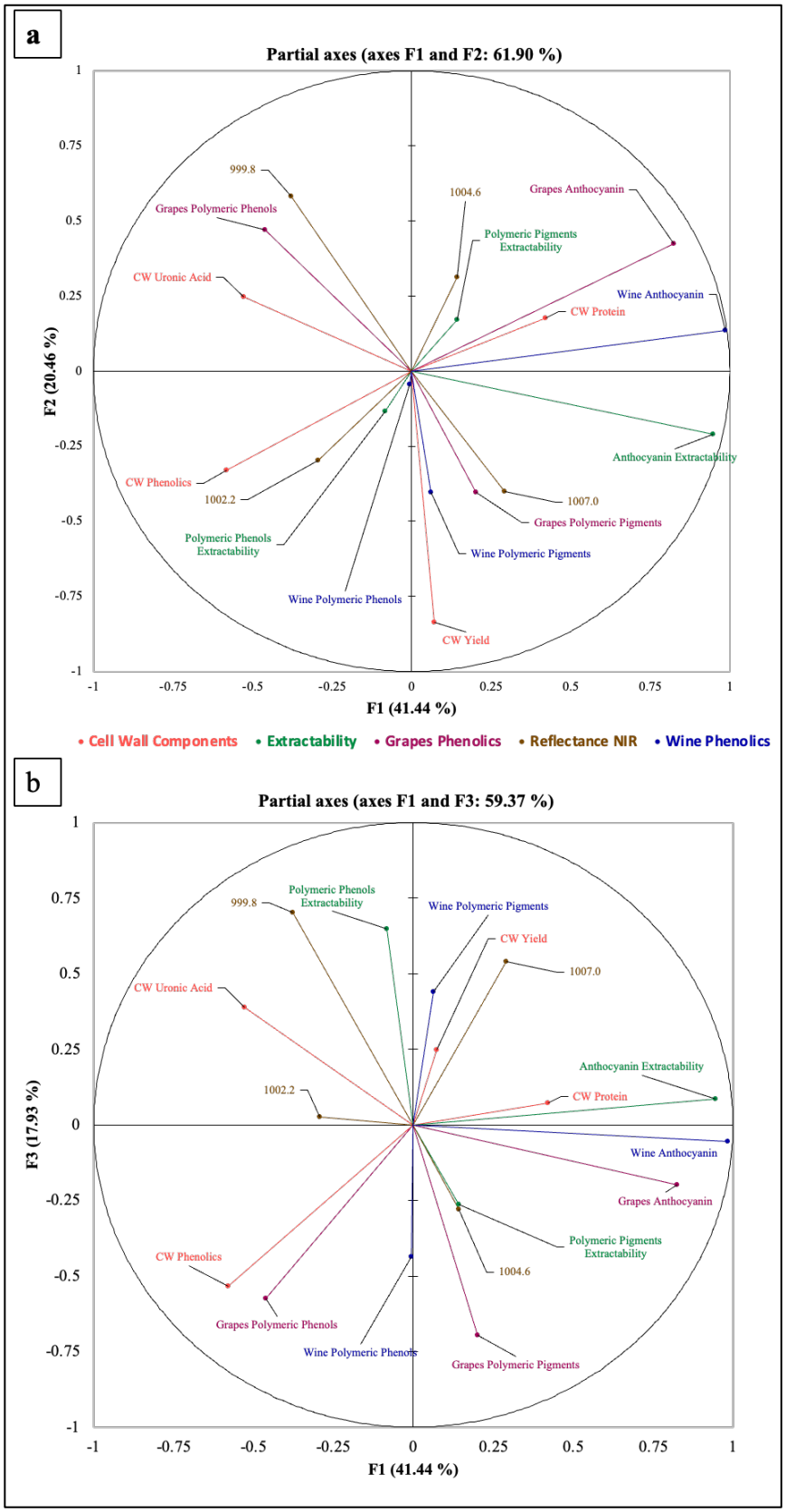

Figure 5. MFA biplots for cell wall components showing (**a**) partial axes F1 and F2 and (**b**) partial axes F1 and F3. Cell wall components (red), phenolic extractability (green), grapes phenolics (purple), NIR reflectance (brown) and wine phenolics (blue) are plotted. Percentage of variability described by factors F1, F2 and F3 is reported in brackets.

Discussion

The findings of this research deepen the understanding of the extraction of anthocyanin and polymeric phenols from grape skins into the fermenting must and the formation of polymeric pigments, investingating the relationship between these phenomena and the skin cell wall composition of the grapes. Our observations not only reaffirmed established findings on the connection between phenolic extractability and cell wall composition but also shed light on the nuanced interplay between extractability, cell wall composition, and external factors like grape variety and cultivation site. Additionally, our findings opened the door to the application of spectrometric technologies to detect cell wall components of intact grape berries and better predict fermentation outcomes. This is important, as these new spectrometric methods present a potential rapid and non-invasive method to predicting phenolic extractability outcomes in red wine processing, directly from grapes prior to fermentation and harvest.

Grape Phenolics and Their Extractability. Grape samples were exhaustively extracted and their phenolic composition was determined by RP-HPLC-DAD. The results (Figure 2a) were found to be consistent with previous work that identified variety, together with site and growing conditions to be the main drivers affecting berry anthocyanin content at ripeness (Pérez-Álvarez et al. 2019, Mansour et al. 2022, Mori et al. 2007, Yu et al. 2022). A site dependency was also observed for the accumulation of anthocyanin in the fruit. The mountain site for Cabernet Sauvignon (CS-NAPA-MNT-1) had a higher anthocyanin content compared to the valley floor samples (CS-NAPA-VF-1 and CS-NAPA-VF-2). At a higher altitude polyphenol and anthocyanin biosynthesis was found to be enhanced, because of an increase in UV-B radiation combined with a cooling effect, that together increase the production and prevent the degradation of these compounds (Mansour et al. 2022, Mori et al. 2007). Of course, a wider variety of locations, both from the valley floor and mountain regions, including sites outside of Napa, would be needed to confirm this finding. Similarly, the Petite Sirah samples differed significantly based on their location, although the two vineyards were

relatively close to each other. PS-NAPA-VF-1 was located in the Oakville AVA, while PS-NAPA-VF-3 in the Oak Knoll AVA. The latter is a cooler area compared to the first (National Center for Environmental Information, accessed on 1/20/2024). Grapes from warmer sites typically show a decreased anthocyanin content, because these compounds' biosynthesis slows down and their degradation increases when temperature exceeds 95 °F, or 35 °C (Mori et al. 2007) . A similar trend was found in Pinot noir grapes. Even if not statistically significant, the fruit picked in Yolo county had a lower anthocyanin content compared to the one coming from the cooler region of Monterey county (National Center for Environmental Information, accessed on 1/20/2024). This fruit was also picked at the high end of the ripeness range, which could have resulted in a higher anthocyanin degradation over time and thus a lower content. Lastly, such comparison between sites could not be addressed for the Zinfandel samples, as these two were picked from blocks belonging to the same vineyard and returned very similar results regarding anthocyanin contents. The site-effect is believed to be due to the soil composition and its water availability, but also on the farming procedures applied (Yu et al. 2022). As a matter of fact, the fruit sourced for this experiment was originated from commercial vineyards, each managed for different winemaking objectives and thus under different farming strategies. The farming procedures put in place may have affected the biosynthesis and storage in the berries of anthocyanins and all phenolics (Yu et al. 2022). Among the examined samples, differences in grape polymeric phenols were not highly significant, but these compounds have also been shown to be highly dependent of the vineyard management conditions (Yu et al. 2022).

Wines made from grapes belonging to the same variety showed no consistent statistical difference in anthocyanin content (Figure 2a). This translated into extremely variable values for anthocyanin extractability between vineyards, confirming the site to be the main driver for this parameter (Medina-Plaza et al. 2021). Overall, the anthocyanin extractability for individual grape samples seemed to be independent of both variety and region, showing a great variation between samples belonging to the same variety and grown in the same growing macroclimate. Previous work has

identified extractability to be a function of grape ripeness, berry sugar concentration and acidity (Hernández-Hierro et al. 2014). Cell wall composition has also been found to profoundly impact anthocyanin and phenolic extractability (Garrido-Bañuelos et al. 2019a, Garrido-Bañuelos et al. 2019b, Medina-Plaza et al. 2021, Ortega-Regules et al. 2006, Río Segade et al. 2008). This relationship will be further discussed.

Polymeric Pigments showed a strong variety and site specificity (Figure 2b) and a very similar trend to the one for anthocyanins (Figure 2a, 2b). This makes sense as polymeric pigments are the products of reactions between anthocyanins and other compounds, happening both in the grape exhaustive extraction media and in wine during the alcoholic fermentation (Harbertson et al. 2003, Waterhouse et al. 2016). Grape polymeric phenols results also showed a somehow similar pattern to the one of berry anthocyanins (Figure 2c). However this parameter was more even across all samples, showing smaller differences between varieties and sites. The extractability of these molecules was very low, which translated in even lower polymeric phenol contents in the wines (Figure 2c). Genrally, the increased contact time between wine and skins and seeds after the end of the fermentation, combined with higher temperatures, lead to an increased extraction of polymeric phenols in the liquid phase (Lerno et al. 2015, Mayen et al. 1994). These conditions were purposely avoided in our winemaking protocol, as polymeric phenols are mainly concentrated in grape seeds, rather than skins, and thus were not the focus of this study.

Grape Cell Wall Yield and Composition. CWM was isolated as alcohol-insoluble residue from grape skins and the mass yield calculated by means of equation (2). Our results were in range and comparable to the ones previously found by this and other research groups. Medina-Plaza et al. (2021) found Cabernet Sauvignon from the California North Coast had IM yields between 11% and 13%, and Pinot noir from 12.5 to 19.5%. Rumbaugh et al. (2023) found IM to yield around 12% in healthy Merlot grapes harvested at 25° Brix, while this value decreased to about 8% when grapes were at 27° Brix. Tempranillo grapes were found to yield between 2% and 5% of IM at

harvest, decreasing as the grapes were ripening and over-ripening (Hernández-Hierro et al. 2014). All the results presented showed a varietal dependency for the amount of cell wall present in grape skins, that overall decreased over the ripening.

IM were also characterized to quantify its content in uronic acid, phenolic, protein and Klason lignin (Figure 3). Cell wall phenolics and proteins seemed to be correlated with each other and to be variety-dependent. This effect and the results for this two CW constituents showed to be in range with results previously produced by this and other research groups. Similar results were found for Cabernet Sauvignon and Pinot noir CW phenolics (Medina-Plaza et al. 2021, Rumbaugh et al. 2023). Hernández-Hierro et al. (2014) found cell wall protein to be around 130 mg BSA/g CW at harvest at 24° Brix in Tempranillo grapes. On the other hand, Medina-Plaza et al. (2021) found proteins to be between 49.47 ± 1.70 and 83.01 ± 3.52 mg BSA/g CW for Cabernet Sauvignon from the California North Coast and between 34.26 ± 2.31 to 84.21 ± 5.34 mg BSA/g CW for Pinot noir sourced from the California Central Coast and Central Valley.

In this experiment CW pectin ranged between 121 ± 27 (PN-YOLO) and 366 ± 19 (CS-NAPA-VF-1) μg g uronic acid/g CW and we found some of our results to suggest that CW pectin may follow a site-specific trend. Ortega-Regules et al. (2005) found a lower value for the CW pectin of Cabernet Sauvignon grapes – about 180 mg uronic acid/g CW – although no investigation between different sites was carried out in that work. Medina-Plaza et al. (2021) had found cell wall polysaccharides such as pectin and cellulose to not have a clear connection to either the variety or the region the grapes were grown in. Lignin was also not found to be affected by any of the variables such as grape variety or site specifics. This further support the hypothesis for CW lignin to be highly dependent of ripeness (Medina-Plaza et al. 2021), as in both of these studies grape sugar concentration was used as harvest parameter and its variability minimized by design. Lignin is known to change during ripening of fruit, as cell walls lignify and its content increases (Liu et al. 2018, Zhang et al. 2021) and this effect was found to be enhanced in grapes grown under drought stress (Tu et al. 2020).

The Effect of Cell Wall Composition on Extractability of Phenolics. All the phenolic components of the wine were relatively close to each others in the MFA (Figure 4a), suggesting similar extraction behavior for the different classes of phenolics. Wine polymeric pigment showed a strong correlation with grapes anthocyanin (Figure 4a). Wine polymeric pigments are the product of anthocyanin polymerization and this effect had been previously noticed (Giacosa et al. 2023, He et al. 2012). Content of both anthocyanins and polymeric pigments in the grapes were positively associated with the extraction of these compounds into wine, while this was not the case for polymeric phenols, whose content in the grapes did not correlate with the extraction in wine (Figure 4). This effect had been observed by others (Medina-Plaza et al. 2021, Giacosa et al. 2023), but might have been amplified by the nature of our methods. Our winemaking protocol provided for controlled temperature of 25 °C and purposely lacked a mixing regime in the vessels or an extended maceration at the end of fermentation. These factors were considered in the project design, as the focus of this work was not on seed tannins. Anthocyanins and skin tannins are extracted from the grapes in the first 4 to 7 days of the fermentation, while seed trannins extract in the the latter part of the fermentation, when more alcohol is present in the solution (Lerno et al. 2019). If an extended maceration takes place and the seed are left longer in contact with the wine (Lerno et al. 2015), or if the fermentation is carried out at higher temperatures (Mayen et al. 1994), the tannin concentration further increases. The limited extraction of polymeric phenols can be addressed by the nature of our winemaking protocol, that provided for a lower fermentation temperature, lacked of a proper mixing regime and did not contemplate any extended maceration time. On the other hand, when analyzing for grape phenolic composition, we carried out an exhaustive extractions of whole grape berries, seeds included, by increasing their surface area (through flash-freezing and subsequent pulverization) and using a high-alcohol-concentration, acidic solution. In other words, the extraction of these compounds was minimized during winemaking, but maximized when measuring berry phenolics. This discrepancy caused the results to be totally independent from one another.

The concentrations of CW phenolics and proteins showed to be correlated with each other. So did the content of CW lignin and the mass yield of cell wall, suggesting a higher level of cell wall lignification occurs when a thicker cell wall is formed (Figure 4a). We had found Zinfandel samples to show a negative effct of IM on the extractability of anthocyanins (Table 2, Fig. 2a). A similar relationship between IM and phenolic extractability was found by Medina-Plaza et al. (2021). In line with our findings (Figure 4c), Medina Plaza et al. (2021) and Rumbaugh et al. (2023) had found extractability of phenolics to be negatively correlated with cell wall components. Similarly, variety and site were found to be impactful on both cell wall composition and extractability of phenolics (Medina-Plaza et al. 2021).

Correlation of Near-Infrared Spectrometry with Cell Wall Composition and Phenolic Extractability. By MFA analysis of data for grape and wine phenolics, grape CW composition, and NIR spectral reflectance we found two main wavelenghts suitable to predict grape and wine polymeric pigments (1007.0 nm) and their extractability (1004.6 nm). These wavelengths are within the range of the solar spectrum most useful for molecule fingerprinting, ranging between 1000 and 1486 nm (Schwanninger et al. 2011). This result helps expand the knowledge on NIR spectroscopy and its application for the analysis of grape skin macromolecules concentrations, without destructing the sample. Other researchers had found NIR spectroscopy to be applicable to characterize pectin (Yang et al. 2021), lignin (Wu et al. 2021), anthocyanins and polyphenols (Chen et al. 2015, Fernández-Novales et al. 2021, Gutiérrez et al. 2018). These works deeply processed the spectral acquisition data through pre-treatment – often including smoothening, filtering and derivation – and modeling of the data (Chen et al. 2015, Fernández-Novales et al. 2021, Gutiérrez et al. 2019, Yang et al. 2021, Wu et al. 2021). Due to time limitations, this was not possible in our work. However, such analysis could uncover a higher number of connections between the data we generated for phenolic extractability, cell wall composition and NIR reflectance and should be the next step.

Industrial Application of Findings. For the first time, the relationship between cell wall composition of grapes skins, the phenolic extractability during small-scale alcoholic fermentation and the opportunity of predicting these two by spectrometric measurements was investigated. Phenolic compounds, being among the most important components of red wines, are often analyzed and tracked throughout the grape ripening, the fermentation and the wine ageing processes. Measuring grape anthocyanin and tanins, although offering relevant information for vineyard management and harvest timing decisions, does not allow to foresee the fermentation outcome in terms of the extraction of these compounds, nor it offers the opportunity of changing the conditions at which fermentation takes place to achieve a targeted extraction of these molecules.

Many works have investigated the relationship between the composition of cell walls and the extraction of phenolics. This experiments enriched the pool of data in this field and offered new insights on the effect that the site might play on the cell wall compositions and how some of it constituents affect the extractability of phenolic compounds. Isolating and characterizing grape skin cell wall, however, remains a time-consuming and complicated procedure, which hardly fits in the schedule of a commercial winery lab at harvest and risks to create rather than solve troubles for winemakers. On the other hand, whole berries can be scanned by a UV-VIS-NIR spectrometer apparatus to easily and quickly predict their cell wall components and how these will affect the phenolic extractability throughout the fermantion. In this first-of-a-kind experiment, we have shown how the reflectance at some key wavelenghts reflects the extraction of phenolics. We are confident that further research will be able to add on to our findings and potentially create a useful model for winemakers to be used for both predicting the phenolic outcome of fermentations, given set adjusments to the fermenting conditions.

Conclusion

Based on the results presented in this work, grape anthocyanins were found to vary significantly based on the variety and the site, predominantly because of the site macro- and meso-climate, its geology and topography. Wine anthocyanin content was also found to be highly specific to the grape variety, with the site being less impactful. The ratio of cell wall material per skin seemed to follow a variety-dependent trend as did both the cell wall proteins and phenolics. These two constituents were also found to be similarly accumulated in the cell wall. On the other hand, pectin content did not seem to follow any pattern with respect to the variety or the location. The same could be said for lignin, that overall seemed to be very similar across all the samples examined, suggesting a possible ripening-dependency. Anthocyanin extractability was highly variable between different varieties and within sites planted to the same variety. The extraction of anthocyanins and other phenolic compounds were found to be closely correlated, suggesting that these molecules' extraction and adsorption follow similar mechanisms. Wine phenolics were found to relate to their contents in the grapes, with the exception of polymeric phenols, whose content in wine was not related to the grape concentration. This effect might have been amplified by our grape extraction and winemaking protocols. Cell wall phenolics and proteins were found to be correlated with each other and negatively affected the extractability of phenolics. For the most part, the amount of cell wall materials isolated per unit of skin weight was found to not have a significant role on extractability, although some noteworthy exceptions were highlighted. Of minor importance was also the effect of pectin and lignin on phenolic extractability. Two main wavelengths (1007.0 and 1004.6) were found to be significantly relevant to predict grape cell wall composition and/or their phenolic extractability. These wavelengths were closely correlated to wine polymeric pigments and their extractability, respectively.

Future Work

A more extensive analysis – including smoothening, filtering and derivation – should be carried out on the collected spectral data. Such analysis, combined with multivariate analysis methods such as the ones we used in this work, would be very helpful in confirming the wavelenghts we found to be key in predicting grape cell wall composition and phenolic extractability. And it could reveal more regions of the spectrum useful to this goal.

Another experiment should be designed over two or more vintages, to consider how the climate may impact not only the compositions of cell wall and phenolic profile of grapes. But also, how the former impacts the extraction of the latter over multiple years. In this case, only few varieties could be taken into consideration. Given their commercial importance and the presence of extensive existing literature on them, Cabernet Sauvignon and Pinot noir would be the ideal candidates. A multitude of samples should be collected in a relatively close, but climatically diverse area. Similarly to what done in this work. The plant material should be collected in a large sample size, in order to allow for both development and validation of a mathematical model. The samples belonging to each variety should be collected at the exact same technological ripeness, avoiding chaptalization or watering back of musts. The sugar concentration (or must density) at harvest could be set differently for Cabernet Sauvvignon and Pinot noir, reflecting the usual ripeness at whichh these two varieties are picked for commercial use.

Grapes should be destemmed, fermented in buckets and punched down twice a day. These conditions are more true to a commercial vinification scenario. Grapes should be scanned on the spectral apparatus as fresh berries, rather than stored frozen and subsequently thawed.

If enough data are produced, this would allow for the development and validation of a mathematical model that predicts and allows to better understand the impact of cell wall constituents on the extraction of phenolics and how this can be forecasted by the use of spectral data.

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