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ORIGINAL RESEARCH ARTICLE

Characterisation of stem extracts of various grape varieties obtained after maceration under simulated alcoholic fermentation

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ABSTRACT

The removal of stems prior to maceration during red winemaking is generally associated with an improvement in wine quality. However, in recent years, increased interest in the use of stems in winemaking has been observed among winegrowers. Different advantages of this practice have been listed in the literature. In order to better understand their role during winemaking, stems of ten red grape varieties from different winegrowing regions of Switzerland were studied to determine which minerals, acids and polyphenols are extracted under simulated alcoholic fermentation. The composition of the extracts revealed differences between the grape varieties. In addition, the growing conditions and the terroir seem to have an influence on the compounds extracted from the stems, especially the mineral composition such as potassium and copper. Among the extracted polyphenols, phenolic acids and proanthocyanidins were mostly found in the stem extracts, especially caftaric acid, catechin and procyanidin B1. Their concentrations were significant compared to the average values found in wines.

KEYWORDS: grape stem extract, polyphenolic compound, anthocyanin, flavan-3-ol, whole bunch fermentation

INTRODUCTION

The stem is the structure of a grape bunch that bears the berries. Its final size is reached around veraison and accounts for 3-7 % of the fresh weight of a bunch (Pascual *et al*., 2016; Foulonneau, 2014). For white winemaking, stems are generally kept for pressing and removed with the pomace, their presence facilitating the juice flow. For red winemaking, stems are usually removed before vatting, as their removal is associated with an improvement in wine quality. However, certain winemaking traditions persist with using whole grape bunch, particularly in Burgundy for Pinot noir, in the Loire Valley for Cabernet Franc, or in Beaujolais with the methods of carbonic maceration of Gamay. In recent years, increased interest in the use of stems in winemaking has been observed among winegrowers. Several technical articles have cited the advantages of this practice, reporting better complexity, greater ageing potential, a reduction in the alcoholic degree adapted to over-ripeness due to climate change, higher freshness in the mouth and enhanced fruity (Bioteau, 2017) or floral aromas (Bazireau, 2016). A recent review summarised the impact of using stems during red winemaking according to the published data (Blackford *et al*., 2021). Of note is that the effects are not systematic and depend on several factors, such as the grape variety, the vintage or the winemaking technique. Many authors found that keeping stems during wine making induces a decrease in ethanol content and they explain this observation by a dilution phenomenon due to a release of water from the stem to the must (Pascual *et al*., 2016; Sun *et al*., 2001; Casassa *et al*., 2019). Moreover, stem maceration can increase the pH, which is generally associated with a decrease in acid concentration, especially tartaric acid (Pascual *et al*., 2016; Casassa *et al*., 2021; Casassa *et al*., 2019). The reduction of tartaric acid content has been attributed to precipitation with K^+ and Ca^{2+}, whose concentration were found to be higher in wine made with stems (Hashizume *et al*., 1998; Sun *et al*., 2001). Finally, stems may have an impact on the polyphenolic composition of the wine. Proanthocyanidins appear to increase in proportion to the amount of stems present during winemaking, regardless of the grape variety (Casassa *et al*., 2021; Suriano *et al*., 2015). Higher concentrations of catechin, epicatechin, dimer B1 and B3 were found in nearly every study (Pascual *et al*., 2016; Sun *et al*., 1999; Spranger *et al*., 2004; Sun *et al*., 2001; Suriano *et al*., 2015). A decrease in total anthocyanin content was highlighted in most of the available articles (Pascual *et al*., 2016; Spanger *et al*., 2004; Suriano *et al*., 2015; Casassa *et al*, 2021). The transfer of other polyphenolic compounds such as phenolic acids or stilbenoïds from the stem to the wine have not been well studied, despite the high concentration in these two types of compounds found in stems (Pascual *et al*, 2016; Benítez *et al*., 2005). All these impacts seem to be directly related to certain compounds extracted from the stems during maceration. However, stem composition has mainly been studied in order to valorise compounds of interest for sectors such as the chemical and pharmaceutical industries. Consequently, the compounds are generally extracted using aggressive organic solvents, with long contact times, at high extraction temperatures or with a pre-extraction treatment, such as grinding or drying. It is therefore difficult to determine the amount and the nature of grape stem extracted compounds that are actually transferred to the wine during maceration.

Currently, there is a lack of knowledge about the impact of stems in winemaking using new red Swiss varieties, such as Mara, Garanoir, Gamaret, Gamarello, Nerolo and Merello, which have a high resistance to grey rot (*Botrytis cinerea),* or Divico, an interspecific grape variety with high resistance to mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*) and grey rot (*Botrytis cinerea*) (Gindro *et al*., 2006; Spring *et al*., 2013).

Therefore, based on a previously published methodology, this study aimed to approach the extraction procedures in a similar way to alcohol fermentation and maceration processes in order to identify which stem components are extracted in significant amounts, and to determine whether there are any differences between the grape varieties. This work allows us to provide a screening of both traditional grape varieties (Gamay, Merlot, Pinot-Noir) known to be potentially macerated with stems and the previously cited grape varieties resulting from the Swiss varietal selection, which grow in Switzerland. New knowledge of these grape varieties and of the composition of their stems was also acquired.

MATERIALS AND METHODS

1. Chemicals and standards

Wine ethanol (F11®-W, > 94.0 %, w/w) was obtained from Alcosuisse AG (Bern, Switzerland). Sodium metabisulfite, butanol, hydrochloric acid, ferrous sulfate and Ca, Cu, Fe, Mg, K, Na and Mn single elements were obtained from Merck KGaA (Darmstadt, Germany). Ultrapure nitric acid 70 % (purified by redistillation, ≥ 99.999 % trace metal basis) and HPLC-grade ethanol were also purchased from Merck. Tartaric acid was obtained from VWR International, LCC (Radnor, PA). Sodium hydroxide, catechin, epicatechin, gallic acid, vanillic acid, syringic acid, caftaric acid, caffeic acid, coumaric acid and ferulic acid were purchased from Sigma-Aldrich Chemical Corp. (St. Louis, MO), protocatechuic acid from Carl Roth GmbH + Co. KG (Karlsruhe, Germany), and coutaric acid and fertaric acid from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Proanthocyanidin A1, A2, B1, B2, B3 and C1 were obtained from Extrasynthèse S.A. (Genay, France). Deionised water (> 18 MΩ) was obtained with a Millipore water purification system (Millipore Corp., Bedford, MA).

2. Stem sampling

The studied stems came from bunches of grapes harvested by hand on the different experimental vineyards of Agroscope (Leytron, Pully, Changins and Cugnasco) and of the School of Viticulture and Oenology (Gland) during the 2019 vintage. The plots were chosen for their homogeneity, the quantities of grapes to be harvested and the type of grape variety. Thirteen plots were selected for sampling. This selection corresponded

to ten different red grape varieties. The geographical distribution of the plots and the associated grape varieties are presented in Table 1. From each plot, ten whole bunches were randomly picked and refrigerated. Within 24 hours, they were weighed and then manually de-stemmed to protect the stems from mechanical alterations and possible changes in plant tissue composition before the experiment. The resulting stems were thoroughly washed in cold water and weighed. They were frozen for a week at -18 °C prior to the extraction experiment. Agroscope monitors berry maturity to determine the optimal harvest date for each plot; we can therefore assume that each plot was harvested at optimal technological maturity.

3. Wine-simulated maceration

The stems were macerated in a simplified model solution, mimicking a red wine in fermentation or maceration conditions. The protocol used had been adapted from two previously published studies focusing on the analysis of grape skins and seeds (Allegro *et al*., 2016; Del Llaudy *et al*., 2008). To avoid gas exchanges and the development of microorganisms, hermetic bottles with a septum cap were used. Seven grams of stems were introduced into 80 mL of model solution. The model solution was prepared by dissolving 6 g/L of tartaric acid in Milli-Q® purified water, adding 0.1 g/L of sodium metabisulfite and adjusting the pH to 3.53 with a sodium hydroxide (1 M) solution. Three replicates were performed for each stem sample. Three bottles filled with 80 mL of model solution without stems were used as controls. To simulate alcoholic fermentation, ethanol was raised from 0 % to 13 % (v/v) in the first eight days, adding 3 mL of ethanol every two days with a syringe through the septum cap. Total maceration duration was 11 days; after that time, the extracts were filtered (0.45 µm PET filter) and divided into two parts. One part (20 mL) was frozen in three aliquots for further analysis. The other part was used immediately for the following analyses: pH, tartaric acid, mineral composition, total polyphenol index (TPI) and extract colour measurements.

4. Extract analysis

Mineral transfers between the stem and matrix were measured with an Agilent microwave plasma spectrophotometer (4200 MP-AES, Agilent Technologies, Inc., Santa Clara, CA) equipped with a standard torch, an inert OneNeb nebuliser and a double-pass glass cyclonic spray chamber (Agilent Technologies) after dilution in a 2 % nitric acid solution (Drvodelic and Cauduro, 2016). The detection wavelength was 396.847 nm for Ca, 327.395 nm for Cu, 371.993 nm for Fe, 383.829 nm for Mg, 769.897 nm for K, 589.592 nm for Na and 403.076 nm for Mn. Three control samples with known element composition at different (low, intermediate, high) concentrations were included in each analysis as a quality control. The recovery values of these controls had to be in a range of between 80 % and 120 % of the theoretical concentration. For each sample, the results are expressed in mg/L within the limit of quantification thresholds.

The pH was measured manually at room temperature (22 °C) using a 691 pH meter electrode (Metrohm Ltd., Herisau, Switzerland). pH variation was calculated as the difference between the beginning and the end of the maceration period.

Tartaric acid variation was measured by high-performance liquid chromatography (HPLC) using a 1260 Infinity Agilent HPLC system consisting of a G4225A degasser, an isocratic G1310 pump system, a GT329B autosample injector, a G1316A column oven and a G1314F UV-detector (Agilent Technologies). Samples were pre-treated by solid-phase extraction using Waters Oasis HLB 6 cm^3 (200 mg sorbent) cartridges (Waters Corporation, Milford, MA), then filtered through 0.2 mm nylon filters (Millipore), and 20 µL were directly injected onto an Aminex HPX-87H HPLC column 300 × 7.8 mm, 9 µm particle size (Bio-Rad Laboratories, Hercules, CA). Separations were performed under isocratic conditions at 80 °C using a 0.65 mmol sulfuric acid solution

TABLE 1. Grape varieties (red), origins and code names of the stem sample.

Grape variety	Species	Origin	Canton	Code
Divico	interspecific	Leytron	Valais	Di-Le
Divico	interspecific	Pully	Vaud	Di-Py
Gamarello	*Vitis vinifera*	Changins	Vaud	Gamar-Ch
Gamaret	*Vitis vinifera*	Gland	Vaud	Gam-Gl
Gamay	*Vitis vinifera*	Changins	Vaud	Ga-Ch
Gamay	*Vitis vinifera*	Pully	Vaud	Ga-Py
Garanoir	*Vitis vinifera*	Changins	Vaud	Gar-Ch
Mara	*Vitis vinifera*	Changins	Vaud	Ma-Ch
Merello	*Vitis vinifera*	Changins	Vaud	Mer-Ch
Merlot	*Vitis vinifera*	Cugnasco	Ticino	Me-Cu
Nerolo	*Vitis vinifera*	Pully	Vaud	Ne-Py
Pinot noir	*Vitis vinifera*	Changins	Vaud	PN-Ch
Pinot noir	*Vitis vinifera*	Pully	Vaud	PN-Py

as the mobile phase at a flow rate of 0.5 mL/min. Tartaric acid was detected at 210 nm.

5. Extract phenolic composition analysis

5.1. Total polyphenol index

The absorbance of the stem extracts was measured at 280 nm using an Agilent Cary 60 spectrophotometer (Agilent Technologies). The absorbance values correspond to the TPI.

5.2. Total proanthocyanidin content

The total proanthocyanidin content (TPC) was estimated by acidic butanolysis. This method is based on the Bate-Smith reaction: coloured anthocyanidins are released from proanthocyanidins when heated in acidic conditions (100 °C for 30 min in a mixture of 50 % w/v butanol, 19 % w/v [6N] hydrochloric acid and 150 g/L ferrous sulfate). After the reaction, the absorbance is measured at 550 nm (10 mm optical path). A non-heated sample prepared with all reagents stored at room temperature in darkness was used as a control. TPC is estimated by the difference in absorbance between the sample kept at room temperature and the heated one (Equation 1).

where *DF* is the dilution factor of the sample and 0.1736 the multiplication factor related to the standard curve of leucoanthocyanidol. Each extract was diluted with Milli-Q purified water (factor of dilution $F = 50$) prior to analysis.

5.3. Quantification of flavan-3-ols and procyanidins by ultra-high-performance liquid chromatography coupled with fluorescence detection (UHPLC-FLD)

The quantification of flavan-3-ols (catechin, epicatechin) and procyanidin dimers and trimers was performed using an UHPLC Infinity 1290 system equipped with a FLD (Agilent Technologies). The separation of the compounds was performed on an Eclipse Plus C18 column (4.6 × 100 mm, 1.8 mm; Agilent Technologies) using water as mobile phase A and acetonitrile as mobile phase B, both containing 0.1 % v/v formic acid, with a flow rate of 1 mL/min. The following gradient was applied for the separation: from 0 to 18 min 9–17 % of B, then from 18 to 25 min 17-95 % of B. The column was equilibrated for 5 min with 9 % of B between each injection. Extracts were filtered (0.45 µm PET filter) and 2 µL were injected. Compounds of interest were detected with the FLD using 280 nm and 320 nm as excitation and emission wavelength respectively. The quantification was performed with external calibration curves, which were established using commercial standards for each compound.

5.4. Quantification of phenolic acid by ultra-high-performance liquid chromatography coupled with diode array detection (UHPLC-DAD)

Phenolic acids were analysed in stem extracts by using a UHPLC Infinity 1290 system equipped with a DAD (Agilent Technologies). After filtration (0.45 µm PET filter), 5 µL of extract were injected. The separation was performed at 40 °C on an InfinityLab Poroshell 120 SB-C18 column (4.6 × 150 mm, 2.7 mm; Agilent Technologies) using 0.5 % v/v formic acid in water (mobile phase A) and 0.5 % v/v formic acid in acetonitrile (mobile phase B), with a flow rate of 1 mL/min. A linear gradient from 0 % to 21 % of B in 50 min was applied for the separation. The column was washed with 100 % of B and then equilibrated for 5 min with 0 % of B between each injection. Gallic acid, protocatechuic acid, vanillic acid and syringic acid were detected with the DAD at the wavelength of 280 nm, whereas caftaric acid, coutaric acid, caffeic acid, fertaric acid, coumaric acid and ferulic acid were detected at 320 nm. The quantification was performed using external calibration curves, which were established using commercial standards for each compound.

5.5. Total free anthocyanins and anthocyanin profile

The total anthocyanin content was determined using the Puissant–Léon method adapted to an automatic photometric analyser (A25, Biosystems S.A., Barcelona, Spain) by adding 380 µL of 1 % hydrochloric acid to 20 µL of sample and measuring the absorbance at 520 nm after 300 s (Ribéreau-Gayon *et al*., 2017). Results are expressed in mg/L malvidin-3-*O*-glucoside equivalent per litre of extract.

The determination of the most important free anthocyanins (mono- and diglucosilated) was adapted from the OIV-MA-AS315-11 method, using an Agilent 1200 HPLC instrument equipped with a DAD (Agilent Technologies) and a data acquisition and analysis system (Agilent ChemStation, version B.04.03SP2). Two microlitres of extract were injected, and the compounds were separated on a Zorbax Eclipse Plus C18 reversed-phase column (4.6 × 100 mm, 1.8 µm) using 10 % v/v formic acid in water (mobile phase A) and 10 % v/v formic acid in acetonitrile (mobile phase B) at a flow rate of 2 mL/min. Before the analysis, the column was first equilibrated with 2 % of B at 40 °C. The following gradient was applied for the separation: from 0 to 10 min 2-9 % of B, then at 15 min decreased to 40 % of B, at 16 min decreased to 95 % of B, and at 17 min maintained at 95 % of B. Finally, 2 % of B was reached at 18 min, which corresponds to the total run time.

- **Equation 1:** $TPC\left(\frac{g}{L}eq.leucoanthocyanidol\right) = \left(A_{heatedsample} - A_{roomtemp.sample}\right) \times DF \times 0.1736$
- **Equation 2**: $I = OD_{420} + OD_{520} + OD_{620}$
- **Equation 3**: $H = \frac{OD_{420}}{OD_{520}}$

TABLE 2. Tartaric acid and pH variation in stem extracts.

Code	Δ Tartaric acid (g/L)	Tartaric acid diminution	Δ pH	pH increase
Di-Le	-0.3 ± 0.1 bc	-5 %	0.38 ± 0.05 c	11 %
Di-Py	-0.2 ± 0.2 abc	-4 %	0.42 ± 0.05 bc	12 %
Gamar-Ch	-0.8 ± 0.1 a	-14 %	0.63 ± 0.05 a	18 %
Gam-Gl	-0.5 ± 0.1 ab	-9 %	0.44 ± 0.03 bc	12 %
Ga-Ch	-0.7 ± 0.0 a	-11 %	0.44 ± 0.03 bc	12 %
Ga-Py	-0.8 ± 0.1 a	-13 %	0.54 ± 0.03 ab	15 %
Gar-Ch	-0.3 ± 0.1 c	-5 %	0.36 ± 0.05 c	10 %
Ma-Ch	-0.6 ± 0.1 abc	-10 %	0.39 ± 0.08 c	11 %
Mer-Ch	-0.9 ± 0.3 a	-15 %	0.58 ± 0.02 a	16 %
Me-Cu	-0.6 ± 0.1 abc	-9 %	0.40 ± 0.05 c	11 %
Ne-Py	-0.5 ± 0.1 abc	-9 %	0.41 ± 0.06 bc	12 %
PN-Ch	-0.3 ± 0.1 c	-5 %	0.32 ± 0.02 c	9 %
PN-Py	-0.4 ± 0.2 abc	-6 %	0.40 ± 0.03 c	11 %

Results are the mean ± SD (n = 3); different letters following the values in each column show significant differences among stem extract samples ($p \leq 0.05$).

The equilibration time between analyses was 3 min. Anthocyanins in mono- and diglucosilated form were detected at 520 nm. Instead of quantification of each anthocyanin, the profile of anthocyanins was expressed in percentage of peak area relative to the total area of peaks. Anthocyanin derivatives (acetylated, coumarylated, and others) were not given independently but as a group.

6. Extract colour measurements

The colour measurements were performed on an Agilent Cary 60 spectrophotometer using the CieLab uniform colour space (Agilent Technologies), a three-dimensional system where the L* axis ranges from 0 (black) to 100 (white) and indicates lightness, and a* and b* axes indicate chromaticity or colour. a* indicates hue on a green (−) to red (+) axis, and b* indicates hue on a blue (−) to yellow (+) axis. Following the OIV-MA-AS2-11 method, colour intensity (*I*) and hue (*H*) were calculated (Equations 2 and 3).

where OD_{420}, OD_{520} and OD_{620} (the optical density of the extract) measured at 420, 520 and 620 nm respectively. The OD_{420} stands for the yellow colour and the OD_{520} for the red one. Hue values were calculated to see if the stem extract had a redder or a yellower colour; the lower the value, the redder the extract.

7. Statistical analysis

All values presented are the mean of three replicates. The data analysis was conducted using Microsoft Excel coupled with XLStat 2019 software (Addinsoft, Paris, France). Descriptive statistics and statistical tests were applied according to the types of information collected. The normality of the data was verified by the Shapiro-Wilk test and homoscedasticity by the Bartlett test. The parametric data were analysed by a classic analysis of variance coupled with a Tukey Honest Significant Difference test to classify the different samples into groups according to their significant differences. Both the sample type and the repetition were considered in the analysis. For non-parametric data, a Kruskal-Wallis test was performed to determine significant differences between modalities. The multiple pairwise comparison was done using a Steel-Dwass-Critchlow-Fligner test. The 95 % confidence interval was used for the various statistical tests (*p*-value = 0.05). Finally, to understand the interactions between different variables, a principal component analysis was performed on the data using a Pearson correlation model. The following variables were analysed: final pH value, total free anthocyanin content, TPI, TPC, *L**, a*, b*, intensity and hue.

RESULTS

Because the stems had been harvested under the same conditions and had been de-stemmed, washed, frozen and macerated following the same protocol, it is assumed that the differences found in the stem extracts are essentially due to the intrinsic differences in the stems (grape variety, growing region, maturity, etc.).

1. Tartaric acid concentration and pH

From the initial 6 g/L in the model solution, tartaric acid concentration consistently decreased by the end of the model alcoholic maceration. Results for the different stem samples are presented in Table 2. The average decrease was of 0.5 g/L. Ga-Py and Gamar-Ch, Mer-Ch and Ga-Ch had the largest decrease in tartaric acid concentration: between 11 % and 15 %. The pH variation was calculated and the results are shown in Table 2. The control showed a pH increase of 0.22 (6 %) showing the impact of dilution by ethanol on the pH of the extracts. The increase in pH was consistently higher for the stem extracts, and significant statistical differences were found among the stem samples. Extracts of Gamar-Ch and

Mer-Ch showed the largest pH increase (18 % and 16 %), followed by Ga-Py (15 %).

As expected, pH increase and tartaric concentration decrease are closely related. A correlation analysis showed that the concentration of tartaric acid measured in the extracts explains 69 % of the variability of the pH increases recorded. The strong influence of the concentration of tartaric acid on the pH is consistent with the fact that tartaric acid is the predominant acid in the model solution.

2. Mineral composition

To our knowledge, this is the first study to quantify the mineral fraction extracted during maceration under simulated alcoholic fermentation. Calcium, copper, potassium, magnesium and manganese were measured by MP-AES (Table 3). Sodium was omitted from the results due to the artefact that sodium metabisulfite was used for the preparation of the model solution. No iron content was detectable in either the control or the samples.

Potassium was the main mineral found in the extracts, with values ranging from 492.02 to 673.89 mg/L. Ma-Ch Ga-Ch and Gam-Gl had significantly lower potassium concentrations (492.02 mg/L, 506.13 mg/L and 504.07 mg/L respectively). Conversely, Di-Py and PN-Py, showed the highest levels of potassium (> 630 mg/L). Calcium was identified as the second most extracted mineral, with measured concentrations ranging from 19.82 to 27.01 mg/L. Extracts from Ne-Py and Ga-Ch showed statistically lower calcium concentrations (under 20.24 mg/L) than extracts from Gamar-Ch, which had the highest calcium concentration (27.01 mg/L). The third most extracted mineral was magnesium, with values that ranged from 9.60 to 21.85 mg/L. For this mineral, no statistical difference was found between the samples. Manganese was also found in the extracts, but at very low concentrations (overall mean value of 0.23 mg/L). Statistical differences in manganese concentrations were found among the stem samples: Ma-Ch, Ga-Ch and Gar-Ch had low manganese concentrations (< 0.08 mg/L), whereas Gamar-Ch and Mer-Ch and Me-Cu had the highest concentrations (> 0.41 mg/L). To our knowledge, this is the first time that manganese content in stem extracts has been reported. Most of the stem extracts showed copper concentrations of between 0.05 and 0.62 mg/L, with an overall average concentration of 0.18 mg/L. Ga-Py was the only extract that did not have a quantifiable copper concentration. Me-Cu had the highest copper concentration (0.62 mg/L).

3. Phenolic composition

In red winemaking, phenolic acids, anthocyanins, flavanols and proanthocyanidins are mainly extracted from the berry skins and seeds during maceration. This section aims to determine the extraction of these compounds from the stems under simulated alcoholic fermentation.

3.1. Total polyphenol index and total proanthocyanidin content

The TPI was used to evaluate the total content of phenolic compounds. This measure is based on the fact that benzenic structures absorb at 280 nm. The TPI values for the stem extracts ranged from 17 to 39 (Table 4). Large variations were observed between the grape varieties: Ne-Py and Ma-Ch stem extract having TPI values above 30, and Di-Le, Ga-Py and PN-Py and Gar-Ch having values under 20. TPC values from 1.6 to 3.2 g/L were measured in the stem extracts with an average value of 2.1 g/L (Table 4). For both TPI and TPC, no phenolic compounds were detected in the control, which confirms that these compounds were released from the stems during maceration.

TABLE 3. Mineral composition of the stem extracts.

Code	Ca	Cu	Fe	K	Mg	Mn
Di-Le	20.73 ± 2.33 ab	0.06 ± 0.01 d	< LOQ	577.38 ± 26.97 abcde	12.11 ± 2.68	0.25 ± 0.03 bcde
Di-Py	23.96 ± 1.86 ab	0.29 ± 0.07 b	< LOQ	673.89 ± 39.39 a	25.19 ± 1.91	0.28 ± 0.12 bc
Gamar-Ch	27.01 ± 0.68 a	0.24 ± 0.02 bc	< LOQ	619.83 ± 13.35 abc	14.95 ± 3.15	0.43 ± 0.04 ab
Gam-Gl	21.62 ± 2.99 ab	0.06 ± 0.02 d	< LOQ	506.13 ± 27.18 de	19.13 ± 1.84	0.11 ± 0.03 cde
Ga-Ch	20.24 ± 2.21 b	0.05 ± 0.00 d	< LOQ	504.07 ± 18.20 de	15.09 ± 0.76	0.06 ± 0.01 e
Ga-Py	23.47 ± 1.31 ab	< LOQ	< LOQ	536.22 ± 25.93 bcde	12.63 ± 1.36	0.20 ± 0.01 cde
Gar-Ch	24.08 ± 1.32 ab	0.11 ± 0.03 cd	< LOQ	553.50 ± 30.08 bcde	13.06 ± 2.38	0.08 ± 0.03 de
Ma-Ch	25.47 ± 2.03 ab	0.15 ± 0.05 bcd	< LOQ	492.02 ± 38.45 e	21.85 ± 10.68	0.07 ± 0.02 e
Mer-Ch	21.96 ± 2.01 ab	0.14 ± 0.06 cd	< LOQ	604.38 ± 30.18 abcd	17.69 ± 4.36	0.41 ± 0.10 ab
Me-Cu	23.06 ± 0.36 ab	0.62 ± 0.07 a	< LOQ	581.37 ± 22.77 abcde	16.89 ± 7.55	0.52 ± 0.14 a
Ne-Py	19.82 ± 1.19 b	0.07 ± 0.01 d	< LOQ	516.37 ± 34.12 cde	18.33 ± 6.05	0.14 ± 0.02 cde
PN-Ch	24.01 ± 1.29 ab	0.11 ± 0.01 cd	< LOQ	577.28 ± 18.18 abcde	10.54 ± 1.90	0.18 ± 0.02 cde
PN-Py	24.04 ± 4.52 ab	0.21 ± 0.09 bc	< LOQ	632.73 ± 79.46 ab	9.60 ± 1.63	0.26 ± 0.09 bcd
Control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

Results are the mean ± SD (n = 3) in mg/L within the limit of quantification (LOQ) thresholds; different letters following the values in each column show significant differences among stem extract samples ($p \leq 0.05$).

TABLE 4. Total polyphenol index (TPI), total proanthocyanidin content (TPC) and total free anthocyanins measured in the stem extracts.

Code	TPI	TPC (g/L)	Total free anthocyanin (mg/L)
Di-Le	17 ± 4 d	1.8 ± 0.4 bc	8.4 ± 0.3 a
Di-Py	27 ± 3 bcd	3.0 ± 0.9 ab	4.8 ± 2.6 abc
Gamar-Ch	27 ± 2 bcd	2.0 ± 0.2 abc	1.4 ± 0.3 def
Gam-Gl	30 ± 2 abc	2.1 ± 0.2 abc	3.0 ± 1.2 bcd
Ga-Ch	22 ± 4 bcd	1.8 ± 0.4 abc	2.6 ± 0.4 bcd
Ga-Py	19 ± 2 d	1.6 ± 0.2 c	0.7 ± 0.1 ef
Gar-Ch	18 ± 2 d	2.6 ± 0.5 abc	1.7 ± 0.3 cde
Ma-Ch	34 ± 9 ab	2.2 ± 0.8 abc	2.0 ± 0.5 cde
Mer-Ch	25 ± 4 bcd	2.1 ± 0.2 abc	0.6 ± 0.2 f
Me-Cu	27 ± 4 bcd	2.3 ± 0.7 abc	0.6 ± 0.4 f
Ne-Py	39 ± 4 a	3.2 ± 0.2 a	3.6 ± 0.6 abcd
PN-Ch	26 ± 1 bcd	2.4 ± 0.2 abc	5.8 ± 0.2 ab
PN-Py	19 ± 2 cd	2.6 ± 0.6 abc	5.2 ± 3.4 abc

Results are the mean ± SD (n = 3); different letters following the values in each column show significant differences among stem extract samples ($p \leq 0.05$).

3.2. Phenolic acid content

Phenolic acids were analysed and the results are shown in Table 5. Caftaric acid was the main phenolic acid found in the extracts with value ranging from 10.4 mg/L for Gar-Ch to 28.5 mg/L for PN-Py, with an overall mean value of 20.2 ± 5.4 mg/L. The following two main phenolic acids to be extracted from the stems were gallic acid and *trans*-coutaric acid. Gallic acid content ranged from 2.6 mg/L for Di-Py to 12.8 mg/L for Ne-Py, with and mean value of 5.6 ± 2.8 mg/L and *trans*-coutaric from 2.5 mg/L for Me-Cu to 13.0 mg/L for Di-Py, with an average value of 5.7 ± 2.7 mg/L. In addition to the highest concentrations of gallic acid, the Ne-Py extract had the highest concentrations of vanillic, caffeic, syringic, *trans*-p-coumaric and *trans*-ferulic acids. It also had the lowest concentration of protocatechuic acid.

3.3. Flavanols and proanthocyanidins

The UHPLC analysis of the stem extracts allowed the identification and quantification of catechin, epicatechin and several procyanidins: dimers A, B1, B2 and B3 and trimer C1 (Table 6). Procyanidin A was not found in the stem extracts in our study, nor was it found in other published work (Blackford *et al*., 2021). Among the flavan-3-ol monomer contents, catechin was the major compound, with concentrations between 17.3 and 82.5 mg/L, where those of epicatechin were between 6.3 and 10.3 mg/L. Procyanidin dimers and trimers were found in higher concentrations than catechin. Procyanidin B1 was the main one found in the stem extracts, with concentrations ranging from 54.5 to 104.6 mg/L.

Significant differences were observed in the flavan-3-ol and procyanidin content between the grape varieties. As in the case of the TPI and TPC, the highest concentrations of flavan-3-ol monomers were measured in the extract of Ne-Py and the lowest in the extract of Di-Le.

4. Colour and anthocyanin composition

At the end of the maceration time, the stem extracts showed a light brown colour with various shades depending on the stem analysed. The colour and anthocyanin composition of the stem extracts were therefore characterised.

4.1. Colour, intensity and shade

Cielab coordinates are shown in Table 7. Ne-Py had the lowest L* value of 51.4 and was significantly different from the other stem extracts that had an average L* value of 91.7 ± 2.4. The a* values ranged from 1.36 for Ga-Py to 13.39 for Ne-Py. The b* value ranged from 7.66 for Ne-Py to 28.29 for Me-Cu. For both a* and b*, significant differences were found between the stem extracts. Ne-Py had the highest a* value and the lowest b* value; therefore, it was the reddest stem extract. In contrast, the Me-Cu stem extract was the yellowest, having the highest b* value of 28.29. Similar results were found with for intensity and hue analysis. Except for Ne-Py, having a value of 1.98, the colour intensity values of the grape stem extracts were low with an overall mean value of 0.58 ± 0.44. Gar-Ch had the lowest intensity value (0.28). In terms of hue, Ne-Py had the lowest value, (1.033) and Gar-Ch the highest (2.795). The other stem extracts had quite close hue values, with an overall mean value of 2.241 ± 0.328.

4.2. Anthocyanin composition

To further understand the origin of the stem extract colour, the total free anthocyanin content was measured using the Puissant–Léon method (Table 4). The results showed significant differences between the extracts, with values

TABLE 5. Phenolic acid composition of the stem extracts.

Code	Gallic acid	Protocatechuic acid	Caftaric acid	*Trans*-coutaric acid	Vanillic acid	Caffeic acid	*Trans*-fertaric acid	Syringic acid	*Trans*-p-coumaric acid	*Trans*-ferulic acid
Di-Le	2.9 ± 0.9 c	1.3 ± 0.1 a	22.2 ± 2.2 ab	8.2 ± 1.3 b	0.2 ± 0.0 cd	0.9 ± 0.0 def	1.9 ± 0.1 a	1.5 ± 0.2 cde	1.0 ± 0.0 c	0.6 ± 0.0 bc
Di-Py	2.6 ± 0.3 c	0.8 ± 0.1 cde	28.5 ± 5.5 a	13 ± 2.6 a	0.3 ± 0.1 cd	0.8 ± 0.1 ef	1.8 ± 0.1 a	2.1 ± 0.5 abc	0.9 ± 0.1 c	0.6 ± 0.0 bc
Gamar-Ch	6.4 ± 0.9 bc	0.9 ± 0.0 bcd	23.4 ± 0.6 ab	5.3 ± 0.4 bcd	0.2 ± 0.1 cd	1.2 ± 0.1 bc	1.0 ± 0.0 bcd	1.3 ± 0.1 de	1.1 ± 0.1 c	0.6 ± 0.0 bc
Gam-Gl	4.8 ± 0.8 bc	1.2 ± 0.1 ab	13.6 ± 1.3 ab	4.5 ± 0.7 bcd	0.3 ± 0.1 bcd	1.4 ± 0.0 ab	1.1 ± 0.1 bcd	1.8 ± 0.3 bcde	1.5 ± 0.1 b	0.7 ± 0.0 abc
Ga-Ch	5.1 ± 1.6 bc	0.9 ± 0.2 bcd	20.8 ± 3.0 ab	4.5 ± 1.1 bcd	0.3 ± 0.1 cd	0.9 ± 0.1 def	1.1 ± 0.1 bcd	1.6 ± 0.2 bcde	0.9 ± 0.1 c	0.6 ± 0.0 abc
Ga-Py	4.0 ± 0.5 c	0.9 ± 0.1 bcde	19.7 ± 3.3 ab	3.6 ± 0.5 cd	0.2 ± 0.1 cd	0.8 ± 0.1 ef	1.0 ± 0.1 bcd	1.0 ± 0.1 e	0.8 ± 0.1 c	0.6 ± 0.0 abc
Gar-Ch	5.1 ± 0.5 bc	1.1 ± 0.1 ab	10.4 ± 1.5 b	3.5 ± 0.4 cd	0.1 ± 0.1 d	0.9 ± 0.1 def	0.8 ± 0.1 d	1.2 ± 0.1 de	1.0 ± 0.0 c	0.6 ± 0.0 bc
Ma-Ch	6.6 ± 4.2 bc	1.1 ± 0.1 abc	21.7 ± 6.3 ab	5.6 ± 1.9 bcd	0.4 ± 0.2 bc	1.0 ± 0.1 cde	1.1 ± 0.2 bcd	1.6 ± 0.5 bcde	1.1 ± 0.1 c	0.6 ± 0.0 bc
Mer-Ch	2.9 ± 0.5 c	1.0 ± 0.0 bcd	20.6 ± 3.5 ab	5.7 ± 0.5 bcd	0.3 ± 0.1 cd	1.1 ± 0.0 cd	0.9 ± 0.0 cd	1.5 ± 0.2 cde	1.1 ± 0.0 c	0.6 ± 0.0 c
Me-Cu	9.2 ± 2.2 ab	0.8 ± 0.1 de	13.8 ± 3.3 ab	2.5 ± 0.9 d	0.4 ± 0.1 bc	1.3 ± 0.0 ab	0.9 ± 0.1 cd	1.7 ± 0.2 bcde	1.0 ± 0.0 c	0.7 ± 0.0 ab
Ne-Py	12.8 ± 1.0 a	0.6 ± 0.0 e	16.1 ± 2.3 ab	7.1 ± 0.6 bc	0.8 ± 0.0 a	1.5 ± 0.2 a	0.9 ± 0.0 cd	2.7 ± 0.1 a	2.2 ± 0.3 a	0.7 ± 0.1 a
PN-Ch	4.5 ± 2.0 bc	0.9 ± 0.0 bcd	25.9 ± 2.6 ab	6.1 ± 0.4 bcd	0.6 ± 0.0 ab	0.8 ± 0.0 f	1.1 ± 0.0 bc	2.4 ± 0.1 ab	0.9 ± 0.0 c	0.6 ± 0.0 bc
PN-Py	5.3 ± 1.2 bc	1.0 ± 0.2 abcd	25.9 ± 14.1 a	4.7 ± 2.6 bcd	0.4 ± 0.1 bcd	0.8 ± 0.1 ef	1.3 ± 0.3 b	1.8 ± 0.3 bcd	0.9 ± 0.0 c	0.6 ± 0.0 abc

Results are the mean ± SD (n = 3) in mg/L; different letters following the values in each column show significant differences among stem extract samples ($p \leq 0.05$).

TABLE 6. Flavan-3-ol monomer and procyanidin composition of the stem extracts.

Code	Catechin	Epicatechin	Procyanidin B1	Procyanidin B2	Procyanidin B3	Procyanidin C1
Di-Le	17.3 ± 1.6 e	6.3 ± 0.9 b	65.8 ± 16.9 b	5.9 ± 0.6 bcd	9.5 ± 0.1 c	9.7 ± 1.4 ab
Di-Py	37.7 ± 11.5 bcde	7.8 ± 1.8 ab	82.9 ± 12.6 ab	4.7 ± 0.3 de	16.6 ± 5.3 abc	11.5 ± 1.5 abc
Gamar-Ch	48.4 ± 5.8 abcd	8.5 ± 0.6 ab	55.1 ± 2.8 b	8.4 ± 1.1 ab	17.8 ± 2.7 abc	9.9 ± 0.9 ab
Gam-Gl	53.7 ± 1.5 abc	9.5 ± 0.9 ab	76.2 ± 9.9 ab	7.7 ± 0.8 abc	17.5 ± 0.9 abc	14.7 ± 1.0 bc
Ga-Ch	29.6 ± 1.2 cde	7.6 ± 1.8 ab	66.2 ± 4.2 b	7.5 ± 1.3 abcd	10.7 ± 2.4 bc	10.0 ± 1.6 ab
Ga-Py	24.8 ±10.2 de	6.4 ± 1.1 b	54.5 ± 1.8 b	6.7 ± 0.6 bcd	9.7 ± 4.0 c	7.8 ± 0.7 a
Gar-Ch	30.6 ± 9.3 cde	6.6 ± 1.1 b	64.5 ± 4.8 b	8.3 ± 0.5 ab	9.1 ± 2.5 c	9.8 ± 1.5 ab
Ma-Ch	65.0 ± 30.8 abc	8.0 ± 1.7 ab	75.1 ± 22.7 ab	5.0 ± 2.4 cde	24.9 ± 13.1 ab	13.3 ± 5.1 abc
Mer-Ch	61.7 ± 6.0 abc	6.9 ± 0.6 ab	62.4 ± 10.8 b	2.5 ± 0.3 e	17.9 ± 1.6 abc	12.0 ± 1.5 abc
Me-Cu	61.8 ± 11.2 abc	8.4 ± 0.1 ab	84.2 ± 8.5 ab	9.8 ± 1.0 a	29.0 ± 6.5 a	13.8 ± 1.6 bc
Ne-Py	82.5 ± 6.5 a	10.3 ± 1.7 a	85.3 ± 10.4 ab	8.3 ± 0.4 ab	24.5 ± 0.5 ab	14.4 ± 1.4 bc
PN-Ch	73.1 ± 6.2 ab	8.0 ± 0.7 ab	104.6 ± 4.0 a	8.0 ± 0.9 abc	27.1 ± 2.6 a	16.6 ± 1.1 c
PN-Py	43.0 ± 8.4 abcd	7.4 ± 0.0 ab	84.4 ± 11.5 ab	6.4 ± 0.0 bcd	14.7 ± 4.1 abc	11.6 ± 0.0 abc

Results are the mean ± SD (n = 3) in mg/L catechin equivalent; different letters following the values in each column show significant differences among stem extract samples ($p \leq 0.05$).

TABLE 7. Colour analysis of the stem extracts.

Code	L*	a*	b*	I	H
Di-Le	93.8 ± 0.1 b	1.97 ± 1.05 cd	13.64 ± 0.98 de	0.35 ± 0.01 bc	2.427 ± 0.052 bc
Di-Py	90.8 ± 1.7 b	4.79 ± 1.90 bcd	25.56 ± 0.93 ab	0.57 ± 0.07 bc	2.560 ± 0.289 bc
Gamar-Ch	93.0 ± 0.5 b	3.22 ± 0.45 cd	17.62 ± 0.56 cd	0.40 ± 0.02 bc	2.386 ± 0.117 bc
Gam-Gl	90.3 ± 3.0 b	5.52 ± 1.18 bc	13.38 ± 0.73 de	0.45 ± 0.12 bc	1.686 ± 0.179 ab
Ga-Ch	93.2 ± 1.2 b	3.14 ± 0.24 cd	13.96 ± 0.22 de	0.36 ± 0.05 bc	2.064 ± 0.165 abc
Ga-Py	90.0 ± 7.4 b	1.36 ± 0.43 d	14.32 ± 1.73 cd	0.53 ± 0.34 bc	1.908 ± 0.663 abc
Gar-Ch	95.6 ± 0.8 b	1.81 ± 1.05 cd	14.35 ± 2.27 cd	0.28 ± 0.04 c	2.795 ± 0.367 c
Ma-Ch	93.4 ± 0.4 b	3.93 ± 0.72 bcd	15.94 ± 2.22 cd	0.36 ± 0.03 bc	2.158 ± 0.030 abc
Mer-Ch	93.0 ± 1.3 b	1.49 ± 0.47 cd	18.96 ± 4.12 cd	0.46 ± 0.04 bc	2.582 ± 0.519 bc
Me-Cu	86.7 ± 0.9 b	3.21 ± 0.67 cd	28.29 ± 2.31 a	0.75 ± 0.02 b	2.332 ± 0.122 abc
Ne-Py	51.4 ± 7.1 a	13.39 ± 3.08 a	7.66 ± 2.34 e	1.98 ± 0.40 a	1.033 ± 0.059 a
PN-Ch	90.6 ± 0.9 b	7.51 ± 0.58 b	19.17 ± 1.14 bcd	0.49 ± 0.04 bc	1.902 ± 0.041 abc
PN-Py	89.4 ± 3.5 b	5.46 ± 3.14 bcd	20.76 ± 4.31 bc	0.57 ± 0.17 bc	2.096 ± 0.312 abc

Results are the mean ± SD (n = 3); different letters following the values in each column show significant differences among stem extract samples ($p \leq 0.05$).

ranging from 0.6 to 8.4 mg/L malvidin-3-*O*-glucoside equivalents. Surprisingly, the stem extract of Ne-Py had a low free anthocyanin content (3.6 mg/L) compared with Di-Ly (8.4 mg/L). This finding suggests that the observed colouration of the Ne-Py extract was not mainly due to free anthocyanins.

The individual anthocyanin profiles were also analysed by HPLC at 520 nm (Table 8). Malvidin glucosides, mono-and di- (Mv-3-glc; Mv-3,5-diglc), were the principal anthocyanidins (38–82 %) found in the extracts, followed by peonidin glucosides (Pe-3-glc; Pe-3,5-diglc) (8–21 %). In the stem of *V. vinifera* varieties, cyanidin-3-*O*-glucoside (Cy-3-glc) was more abundant than delphinidin-3-*O*-glucoside (Dp-3-glc) and petunidin-3-*O*-glucoside (Pt-3-glc). It should be noted that Pinot noir stem extracts contain no anthocyanin derivatives.

4.3. Relationship between colour and stem extract composition

To better understand which variable influences the colour of the stem extracts, a principal component analysis was performed using final pH value, total free anthocyanin content, TPI, TPC, *L**, a*, b*, intensity and hue.

TABLE 8. Profile of individual free anthocyanin content of stem extracts.

Code	Dp-3-glc	Cy-3-glc	Pt-3-glc	Pe-3-glc	Mv-3-glc	Pt-3,5-diglc	Pe-3,5-diglc	Mv-3,5-diglc	Anthocyanin derivatives
Di-Le	1.6 ± 0.7 a	0.8*	2.6 ± 0.2 a	4.6 ± 1.3	24.3 ± 1.7	1.4 ± 1.0	8.8 ± 2.0	50.5 ± 3.0	5.9 ± 0.6 a
Di-Py	0.8 ± 0.5 b		1.7 ± 1.1 b	4.8 ± 2.5	25.9 ± 13.2	0.4 ± 0.2	10.5 ± 4.5	51.8 ± 30.4	4.1 ± 2.6 b
Gamar-Ch	0.4 ± 0.6	1.2 ± 0.5 b	1.7 ± 0.4	6.1 ± 1.3 bc	58.2 ± 11.7 cd				32.4 ± 5.7 bc
Gam-Gl	0.5 ± 0.9	0.6 ± 0.3 b	1.7 ± 0.9	11.0 ± 5.7 abc	54.8 ± 11.7 cd				31.4 ± 8.8 a
Ga-Ch	1.7 ± 1.0	3.3 ± 2.0 ab	1.9 ± 0.6	8.1 ± 2.5 abc	56.8 ± 7.3 cd				28.1 ± 2.3 ab
Ga-Py	0.7 ± 1.2	1.3 ± 1.3 b	2.1 ± 0.3	6.0 ± 1.0 c	63.6 ± 17.4 cd				26.3 ± 4.0 cd
Gar-Ch	0.2 ± 0.4	1.0 ± 0.7 b	1.1 ± 0.7	13.0 ± 5.4 abc	58.2 ± 15.2 cd				26.4 ± 8.3 bc
Ma-Ch	2.0 ± 0.4	1.9 ± 0.8 b	2.5 ± 0.3	14.2 ± 1.3 abc	53.1 ± 15.0 cd				26.3 ± 8.2 bc
Mer-Ch	0.0 ± 0.0	1.4 ± 1.6 b	1.5 ± 1.4	13.0 ± 6.9 bc	52.3 ± 18.9 cd				31.7 ± 8.5 cd
Me-Cu	5.7 ± 2.6	14.0 ± 9.2 ab	3.6 ± 2.6	12.8 ± 9.4 bc	37.2 ± 21.5 d				26.6 ± 18.5 cd
Ne-Py	5.4 ± 2.6	0.8 ± 0.6 b	5.0 ± 3.6	7.0 ± 1.6 abc	59.2 ± 10.2 bc				22.6 ± 6.4 ab
PN-Ch	1.7 ± 0.4	2.7 ± 1.2 a	1.6 ± 0.0	21.2 ± 1.0 a	72.7 ± 3.8 a				0.0 ± 0.0 d
PN-Py	3.2 ± 2.8	2.0 ± 0.9 ab	2.8 ± 3.6	21.2 ± 22.4 ab	70.8 ± 36.7 ab				0.0 ± 0.0 d

* Value found for one replicate; results expressed as % of total anthocyanin; results are the mean ± SD (n = 3) in mg/L malvidin-3-O-glucoside equivalent; different letters following the values in each column show significant differences among stem extract samples ($p \leq 0.05$); statistical analysis for the profile of individual anthocyanin content was performed separately for Vitis vinifera varieties and for Divico samples.

Stem extracts were distributed on the plane defined by the two principal components, which together explained 71.60 % of total variance (Figure 1). The first axis (PC1), accounting for 52.81 % of the variance, opposes stem extracts that have high TPI, TPC, a* and intensity values but low hue, b* and L* value. The second axis (PC2), accounting for 18.79 % of the variance, opposes stem extracts that have high final pH value with those having high total free anthocyanin content.

DISCUSSION

By studying stem extracts obtained after maceration under simulated alcoholic fermentation, with a progressive increase in ethanol content, the objective was to determine whether the quantities of the extracted compounds were negligible or not compared to the amount found in the wines. This allowed us to determine the potential effect of stems on the must and on the wine when kept during winemaking. The study of different grape varieties allowed us to determine whether the behaviour is similar for all the stems or whether parameters, such as the grape variety or the growing region, have an impact on the extracted compounds.

All the stem extracts had a higher pH value at the end of the maceration period. In order to understand the effect of the grape variety and of the growing region on the pH values, a statistical analysis was performed on the data of Gamay, Pinot-Noir and Divico stem extracts from 3 different vineyards (Pully, Changins and Leytron). The results showed a significant influence of both parameters. We saw that stem extract pH is highly related to the tartaric concentration, as it is the predominant acid in the model solution. The decrease in tartaric acid was similar for the two Gamay, the two Pinot-Noir and the two Divico stem extracts made with stems from different origins. The grape variety had a significant influence on the intensity of the decrease in tartaric acid, despite the growing region. Moreover, the acidic conditions of the extract was also highly influenced by mineral content extracted from the stems, such as potassium and calcium, which can induce tartaric acid precipitation. The potassium and calcium concentrations found in the stem extracts showed no influence of the grape variety, but a significant influence of the growing location on potassium content. The intensity of the pH variations could therefore depend on a combination of the grape variety considered and the cultivation area.

The decrease in tartaric acid concentration measured in the stem extracts has also been observed in studies that incorporated stems during the real winemaking maceration stage. They also found an impact of the grape variety on the intensity of the decrease: 4 % for Castelao wines (Spranger *et al*., 2004), 9 % for Muscat Bailey A wines and 10 % for Pinot noir wines (Hashizume *et al*., 1998). It is interesting to note that these variations are of the same order of magnitude as those found in our model extracts (values of between 4 % and 15 %). The impact of stems on the mineral composition of the matrix was also observed in real winemaking conditions. As for the stem extracts, authors highlighted significant increases in potassium, phosphorus and calcium concentrations, regardless of grape variety (Hashizume *et al*., 1998; Sun *et al*., 2001). However, no data are available on the same grape variety from different planting areas, making it impossible to confirm what we observed in the stem extracts. Finally, the decrease in tartaric acid and the increase in mineral content, such as potassium, were consistently significant when stems were added, while the increase in pH was not, which contrast with previous observations made in model conditions (Casassa *et al*., 2019; Hashizume *et al*., 1998; Pascual *et al*., 2016). Differences in terms of variations may be linked to the high buffering

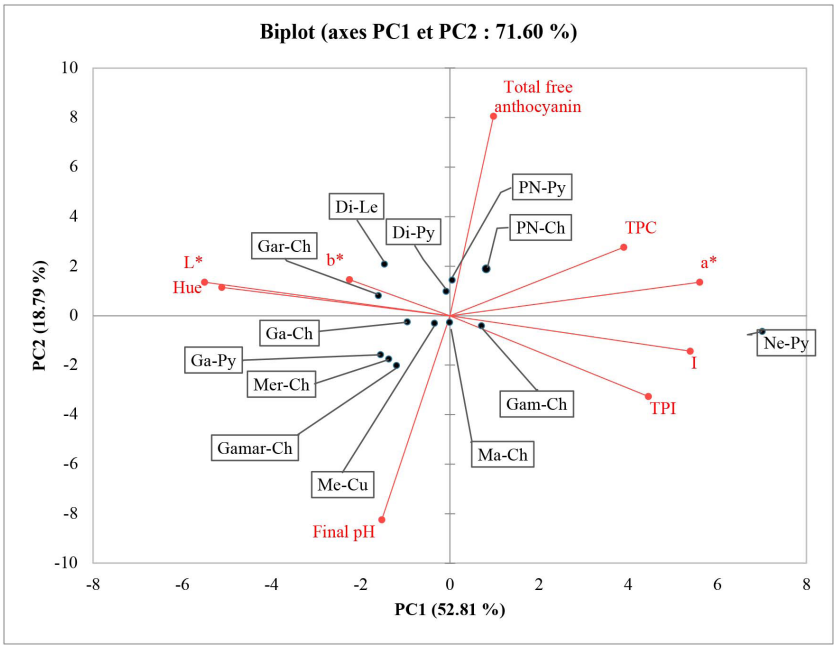

FIGURE 1. Biplot of stem extract composition and colour characteristics (correlation matrix PCA).

capacity of wine matrices over acido-basic balance, which mainly depends on the grape variety (Ribéreau-Gayon *et al*., 2017). Therefore, the study of the extract alone does not seem to be sufficient to predict the evolution of the pH in the wine made keeping stems.

Among the mineral compounds extracted from the stems, potassium was not the only mineral element to be influenced by the growing region. Statistical analysis on the copper content also showed significant differences depending on the growing location, whatever the grape variety. Extracts made with stems from Pully had significantly higher copper content than the ones from Leytron, Changins having intermediate values. The growing region, including soil composition and treatments applied on the vine, seems to have a higher impact on the amount of copper extracted from the stem than does the grape variety. In real winemaking conditions, among the three grape varieties for which the impact of stems on mineral content of final wines has been studied, only Cabernet Sauvignon wines had higher copper concentrations (Hashizume *et al*., 1998; Sun *et al*. 2001). This finding could be explained by two phenomena. The first one may be that the extracted copper content differs according to the growing conditions, as explaned above. The second one could be that a large proportion of the copper present in the must is eliminated along the winemaking process. It is therefore difficult to know the real impact of the copper extracted from the stem when considering the finished wine. One of the main risks of increasing the concentration of copper in must is a fungicidal action on the fermentative yeasts. However, the quantities extracted under model alcoholic fermentation conditions, between 0.05 and 0.62 mg/L, are low compared to the values of 20 mg/L that have been mentioned as having an impact on yeast activity (Cavazza *et al*., 2013).

The phenolic composition of the stem extract is important as phenolic compounds can contribute to colour, tannic quality, colloidal stability and ageing ability. The influence of the grape variety on total polyphenol content of the stem extracts had already been reported in the literature for other grape varieties, and our results confirm similar behaviour for Swiss grape varieties - whether *Vitis vinifera* or interspecic (González-Centeno *et al*., 2012; Karvela *et al*., 2009; Souquet *et al*., 2000). In our study, the TPC values of the stem extracts ranged from 1.8 to 3.2 g/L, with significant differences between grape varieties. In a previously published study, the TPC of Cabernet Sauvignon stem extracts obtained under simulated alcoholic fermentation was measured (Del Llaudy *et al*., 2008). After 11 days of maceration, the TPC found ranged from 2.5 to 4.7 g/L depending on the maturity of the stems. In comparison, the amount of proanthocyanidin extracted from the berry skins and seeds ranged from 1.5 to 2.0 g/L and from 1.5 to 2.5 g/L respectively. Thus, the proanthocyanidin fraction potentially extracted from the stems during maceration seems to be non-negligible. The dominance of catechin over epicatechin and the fact that procyanidin B1 was the main dimer in Swiss grape varieties, is consistent with the data available for other grape varieties (Esparza *et al*., 2021; González-Centeno *et al*., 2012; Spatafora *et al*., 2013). It is interesting to highlight that a stem extract with a high TPC generally has particularly high concentrations of all identified flavan-3-ols and flavan-3-ol derivatives. Thus, the extraction of these compounds appears to be non-selective. Despite the amount of tannins released from the stem into the must, their quality is another great concern. These compounds influence the astringency and bitterness of wines in varying proportions depending on their concentration and size. An in-depth analysis of the quality of tannins would make it possible to better advise winegrowers in their winemaking practices.

Regarding the phenolic acid content, we found significant differences between the stem extracts. The analysis of Gamay, Pinot-Noir and Divico stem extracts from three different vineyards (Pully, Changins and Leytron) showed that both the grape variety and the growing region can influence the stem extract concentration of *trans*-coutaric, vanillic, *trans*-fertaric and syringic acids. *Trans*-fertaric acid content was only impacted by the grape variety and protocatechuic acid was only influenced by cultivation area. These results are consistent with the existing literature that found that the stem extracts composition was influenced by the grape variety and the growing region (Alonso *et al*., 2002; Anastasiadi *et al*., 2012; Spatafora *et al*., 2013). Moreover, the amount of phenolic acids of our stem extracts were non-negligible in comparison to the concentrations found in red wines (100 to 160 mg/L, Van Leeuw *et al*., 2014). Despite these high values, when stems were kept in real winemaking conditions, some research teams showed that the phenolic acids found in grape stem extracts did not seem to be significantly transferred to wine, as final concentrations did not increase (Pascual *et al*., 2016; Benítez *et al*., 2005). It would be interesting to study why these compounds are not found in the final wines. Several hypotheses can be put forward: the grape varieties studied in the literature could have a lower phenolic acid composition than the grape varieties studied in this article; under real maceration conditions, the wine matrix limits the extraction of phenolic acids; the extracted phenolic acids degrade or complex with other molecules in the must and wine. Again further investigation are needed to verify these hypotheses.

Regarding the anthocyanin content, the stem extracts of *Vitis vinifera* varieties contained only monoglucosilated anthocyanins, whereas extracts of Divico, an interspecific variety, also contained diglucosilated anthocyanins. The presence of diglucosilated anthocyanins have previously been observed in berry skin contact maceration extracts of several interspecific varieties (Roman *et al*., 2019). Moreover, the anthocyanin profiles of stem extracts are similar to those obtained with wines (Zaffalon *et al*., 2014). It should be noted that, as in wines, Pinot noir stem extracts contained no anthocyanin derivatives (Gao *et al*., 1997). The low anthocyanin content found in our stem extracts is consistent with the values in the literature (Barros *et al*., 2014; Leal *et al*., 2020). In many studies, where stems were kept during maceration, the total anthocyanin content in the wines decreased in proportion to the amount of stems in the tank (Blackford *et al*., 2021). Ribéreau-Gayon and Milhé rejected the hypothesis of molecular interactions between compounds extracted from the stems and the must, but suggested that the stem bodies may adsorb a fraction of anthocyanin molecules (Ribéreau-Gayon and Mihlé, 1970). Other authors also proposed this hypothesis more recently (Suriano *et al*., 2015). The adsorption capacity of the stems, in terms of anthocyanin compounds, seems to be stronger than the potential release of this type of compounds into the wine matrix during maceration. This observation could be interesting to exploit in the case of very coloured grape varieties, such as Divico, Gamaret or Mara, whose skins release a lot of anthocyanins (3200 mg/L, 600 mg/L and 550 mg/L respectively, average values measured in wines, unpublished data). Whole bunch winemaking could be an option for reducing total anthocyanin content while increasing the tannic structure, and could thus improve the overall balance of the wine.

The colour intensity of the grape stem extracts were low, with a mean value of 0.58 ± 0.44. For comparison, a rosé wine generally has a colour intensity of between 0.4 and 0.9 points of optical density, a clairet wine of between 1.2 and 2.5, and a red wine over 3 points of optical density. However, in order to understand the origin of the extracts' colour, we performed a PCA analysis. It is known that the colour of free anthocyanin varies depending on the pH of the solution and, as we saw in Section 1.1, the pH values of the extracts differed at the end of the maceration experiment. The PCA results showed that neither total free anthocyanin content nor the final pH value affected hue or colour intensity. This might be explained by the very low free anthocyanin content. Its influence on the colour of the extracts was not significant compared to other polyphenolic compounds highlighted by the positive correlation between a* values, the intensity, TPC and TPI.

In the first years of ageing, tannins and phenolic acids can act as co-pigments and can contribute to the colour of young wines. At the same time, interactions occur between anthocyanins and tannins to form more stable polymeric pigments during wine ageing. These reactions depend on the wine conditions (acidity, temperature and oxidation state), as well as on the type of tannins and the proportions of tannins and anthocyanins in the wine. Therefore, the use of stems may modify all these parameters, and thus may have an impact on colour and colour stability. Further study would be necessary to address this hypothesis.

Finally, it is important to note that, under our conditions, according to Fick's law of diffusion, the mass transfer of some compounds may have been favoured by the initial zero concentration in the model solution. Fermenting must is a complex matrix composed of different chemical compounds. It is therefore logical to assume that this study may have overestimated the extractability of some of the compounds of the stems. However, some of them were extracted in non-negligible quantities, suggesting that they may have an impact on the final wines.

CONCLUSION

This article provides information on the compounds extracted from grape stems under model alcoholic fermentation conditions. For the first time, the stem of grape varieties from the Swiss varietal selection were analysed. The composition of the extracts revealed differences between the grape varieties. In addition, the growing conditions and the terroir seem to have an influence on the compounds extracted from the stems, especially the mineral composition, such as potassium and copper. Among the extracted polyphenols, phenolic acids and proanthocyanidins were mostly found in the stem extracts, especially caftaric

acid, catechin and procyanidin B1. Their concentration were significant compared to the average values found in wines. A small amount of anthocyanins was also found in the extracts. In this study, the stems were collected at a date defined by the maturity of the berries. Therefore, the maturity of the stems was not taken into consideration. This could be an interesting parameter to study on the different grape varieties, as it could have an influence on the stem extracted compounds.

ABBREVIATIONS

DAD: diode array detector

FLD: fluorescence detector

HPLC: high-performance liquid chromatography

LOQ: limit of quantification

TPC: total proanthocyanidin content

TPI: total polyphenol index

UHPLC: ultra-high-performance liquid chromatography

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