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Polyphenolic compounds in Sauvignon blanc—from grapes to wine

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ABSTRACT

At harvest, the metabolic composition of wine grapes reflects the accumulated effect of the environmental conditions, the stresses endured, and viticultural manipulations applied during the growing season. The role of the winemaker is to extract and nurture this "metabolite potential" throughout the winemaking process. However, it is typically difficult to relate this grape potential to that of the eventual wines. In this study, a holistic view of Sauvignon blanc grape and wine polyphenolic compounds was attempted by measuring these compounds from different matrices, from ripe grape tissues up to the final wine, including the submatrices, such as juice, pomace and sediment. Sauvignon blanc vines from one vineyard block were manipulated to yield berries with distinctly different phenolic potentials by creating a high light (HL) or a low light (LL) microclimate in the fruiting zone of the canopy during the growing season. The analyses of the HL and LL berries and wines, as well as concomitant analyses of the phenolic compounds in the submatrices, allowed their tracing as they were (i) transferred from one matrix to another, (ii) lost as waste products, or (iii) affected by different winemaking practices (skin contact and/or fermentation in contact with the juice sediment) implemented in the experimental design. In berries, flavonols showed the largest increase due to sun exposure (HL treatment) but were absent from the juice samples at all juice processing stages. They were however detected in the juice sediment, together with high concentrations of organic acids and sugars. Juice processing was noted for dramatic fluctuations in metabolite concentrations suggesting intense metabolic activity in this pre-fermentation matrix. Both skin contact and sediment contact treatments delivered wines with higher concentrations of coutaric acid (the ester formed from coumaric and tartaric acid) and the flavanol catechin while epicatechin concentrations was unaffected. The higher catechin concentrations did not lead to increased perceived bitterness in the wines, except in the sediment contact treatments. The total phenolic compound concentration of wine from LL (low phenolic potential) grapes was comparable to wine from HL (high phenolic potential) grapes, when skin contact, or sediment contact treatments were employed. From a sensory perspective, the sediment contact decreased the fruity aromas of Sauvignon blanc, while the skin contact treatment enhanced the sensorial properties of the wines made from the LL grapes, allowing increased extraction from the skin-accumulated impact compounds.

KEYWORDS: Sauvignon blanc, polyphenolic compounds, high light exposure, grape-to-wine analysis, juice processing

INTRODUCTION

Grape phenolic compounds contribute to the quality, stability and complexity of white and red wine due to their mouthfeel attributes and their involvement in oxidation, browning and protein haze formation (Fulcrand *et al*., 2006; Oliveira *et al*., 2011; Van Sluyter *et al*., 2015). The grape skin is a primary source of phenolic compounds as well as aroma compounds and precursors (Gomez *et al*., 1994; González-Manzano *et al*., 2004). Viticultural practices and environmental factors such as water deficit, high temperatures, exposure to sunlight, or soil nitrogen deficit can all modulate the synthesis and levels of these metabolites in the grape berry (Poni *et al*., 2018). Sauvignon blanc vines are very responsive to environmental impacts (phenotypical plasticity) and previously we showed how sun exposure impacted the metabolite potential of the Sauvignon blanc grapes by primarily increasing metabolites with antioxidant and sun-protective activity such as phenolic compounds (Du Plessis, 2017; Joubert *et al*., 2016; Young *et al*., 2016).

This led us to the following questions: (i) How much of the enhanced phenolic potential of the sun exposed grapes is transferred to the wines; (ii) How would different wine making procedures such as pre-fermentative skin contact influence the transfer/sustainability of the phenolic potential to the wine; (iii) How does the magnitude of the increase obtained in the vineyard compare to that obtained by oenological treatments such as skin contact; (iv) Can we trace the "lost" metabolites and can some of the "lost" metabolite potential be reintroduced into the wine by applying specific wine making procedures (*i.e*., sediment contact during fermentation) and (v) What are the impacts of the different vineyard and wine making manipulations on the wine sensory attributes?

Studies that trace the route of metabolites from the grapes through the different wine making production steps are scarce. Similarly, monitoring the proportion that is lost as waste products, or consumed/converted by the microorganisms in the must, or participate in anabolic processes or complexing reactions with other metabolites, are rarely done (Del-Castillo-Alonso *et al*., 2021), especially so in white cultivars. In this study we measured the carotenoids, chlorophylls, sugars, acids and phenolic compounds in Sauvignon blanc grapes that were grown in a high light (created by leaf removal in the bunch zone) and a low light microclimate (no leaf removal). The defoliation treatment increased the concentration of most of the measured metabolites such as phenolic compounds, sugars, and carotenoids, resulting in berries with an overall higher metabolic potential compared to the berries that ripened in the shade of the vine leaves. The fate of the phenolic compounds is of particular interest in white-wine making as very little information is currently available. These metabolites were therefore followed throughout the juice processing steps and up to the chemical and sensorial analysis of the wine after six months of bottle aging. The partial loss of the initial grape metabolite potential as the wine making process transpire was studied by measuring some of the metabolites in waste products such as the pomace and the enzyme-clarified-sediment. Furthermore, alternative wine making procedures such as incorporating a pre-fermentative cold soak or conducting the fermentation in contact with the enzyme-clarified sediment, were also investigated.

MATERIALS AND METHODS

1. Viticultural treatments and management

The experimental site was a 13-year-old, commercial *Vitis vinifera* Sauvignon blanc vineyard in the Elgin region, Western Cape, South Africa. The details of the site, the management of the vineyard and the experimental design was done as previously described in Young *et al*. (2016), except that all the vines in this study received a pre-fruit set leaf-removal treatment as well (implemented by the commercial farm), followed by the intentional leaf removal treatment in some of the vines as part of the experimental plan, as outlined next. When the berries reached the size of peppercorns a high light microclimate (HL) was created in parts of the block by total leaf and lateral shoot removal in the bunch/fruiting zone on the northeast-facing side of the vines (*i.e*., the side of the vines receiving sunlight exposure during the morning in the Southern Hemisphere). The HL microclimate was maintained throughout the season by continuous lateral shoot removal. The leaves and shoots of the vines from the low light (LL) microclimate was not manipulated, which resulted in more shaded/less exposed fruiting zones. The leaf removal treatment was alternated per panel, in 13 adjacent vineyard rows (four panels per row and four vines per panel), thus creating a checkerboard plot layout (with control vines adjacent to exposed vines across the entire experimental plot) to factor in any potential geospatial heterogeneity in the vineyard. Grape bunches were harvested at 19-21 °Brix from the northeast-facing side of the canopy only, and bunches from all 13 rows were pooled according to the treatment (HL or LL). Thereafter three biological repeats were randomly formed per treatment for subsequent analyses.

2. Grape sample preparation

Berry samples were collected (50 berries per sample) from each biological repeat and samples were kept on ice in the vineyard prior to their partitioning with scalpels into skin, pulp, and seed in the laboratory. The partitioned tissue was flash-frozen with liquid nitrogen and stored at −80 °C until further analysis. Ripe grape Whole Berries [WB], skin, pulp and seeds from each biological repeat were kept separate, frozen with liquid nitrogen and homogenised (Retch Mixer Mill MM 400, Germany) at 30 Hz for 1 min until a fine powder was obtained. Whole berries were homogenised with the seeds. The powder of each biological repeat was collected in a single container per sample type and mixed thoroughly. The dry weights of the whole berry, skin and pulp samples were determined by allowing moisture to evaporate at 80 °C until the weight stabilised.

3. Winemaking

The grapes that were harvested for winemaking were transferred to the cellar and stored overnight at 4 °C. The grapes from the HL and LL microclimates were crushed and destemmed, keeping the three biological repeats separate. A summary of the winemaking process, outlining the respective treatments, and sampling points for further analyses, is provided in Figure 1. Standard winemaking procedures entailed the addition of sulphur dioxide (SO_2, 2.5 % w/v) after crushing, followed by pressing using a basket press (1.5 kPa). The free SO_2 of the pressed juices were adjusted to 30 ppm, treated with Rapidase Clear (1 ml/hL, Oenobrands, Montpelier) and clarified during overnight storage at 4 °C. The clear juice was siphoned off and inoculated with the commercial wine yeast, Cross Evolution (30 g/hL, Lallemand). Three winemaking treatments were applied: skin contact (Sc), sediment contact (Sed) and skin contact combined with sediment contact (ScSed). Skin contact was conducted after crushing, for 24 h at 4 °C, followed by pressing, enzyme clarification and fermentation of the clear juice as described in the standard winemaking procedure. For sediment contact (Sed) or sediment contact with skin contact (ScSed), the pressed juice was treated with clarifying enzyme, but instead of selecting only the clear juice, all the sediment was resuspended before yeast inoculation. Dry ice was used throughout to keep the system as reductive as possible. Fermentations were conducted in triplicate in 4.5 L bottles with fermentation caps at 15 °C. The progress of the fermentations was followed by measuring the CO_2 weight loss, and the SO_2 of the wine at the end of alcoholic fermentation was adjusted to 50 ppm followed by cold stabilisation for 2 weeks at –4 °C. The cold stabilised wines were removed from the lees, free SO_2 was adjusted to 40 ppm and the wines were bottled and stored.

Juice fractions were sampled at each of the processing steps (crushed/free run, pressed and enzyme-clarified juice) and from all the treatments (Figure 1). Pomace samples (mostly skins with some residual pulp) were collected after pressing. Sediment samples were taken after the enzyme-clarified, clear juice was removed to be fermented. The solid residues in the sediment were collected with centrifugation (5 min, 5000 rpm). Both the pomace and the sediment samples were deseeded and then homogenised and stored in the same way as the berry samples. Wine samples were sampled directly after fermentation (for aroma compound analysis) and after being bottle-aged for 6 months at 15 °C.

4. Organic acids, sugars and alcohols in whole berries, juice, sediment, and wine

The organic acids and sugars of the whole berries and sediment were extracted and analysed using HPLC as described by Eyéghé-Bickong *et al*. (2012). The juice (at different processing stages, with and without skin contact) and wine (at the end of alcoholic fermentation) samples were mixed at a 1:1 ratio with ribitol (4 g/L) and adipic acid (2 g/L), the internal standards (for sugars and organic acids, respectively). Results are expressed as mg/g FW and mg/L and represent the means of three biological repeats and two technical repeats. All chemicals and standards were HPLC grade supplied by Sigma Aldrich (Steinheim, Germany).

5. Polyphenol quantification in grape tissue, juice, pomace, sediment, and wine

The individual phenolic acids and flavonoids of the whole berry, skin, seeds, pulp, pomace and sediment were extracted,

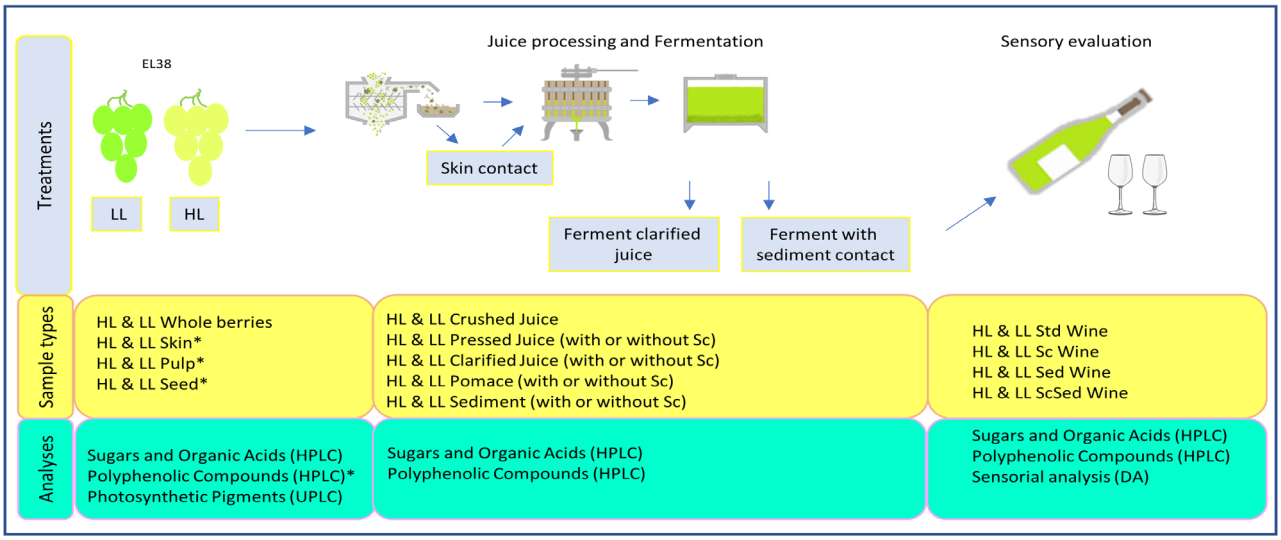


FIGURE 1. Diagram showing the high light (HL) and low light (LL) microclimate grape bunches, the winemaking process implementing standard and adjusted juice- and wine-processing steps, and the sampling and metabolite analysis layout. Asterisks (*) indicate that those samples (grape skin, pulp, and seeds) were separately analysed for polyphenolic compounds, in addition to whole berries. EL38 refers to the ripe berry samples according the EL scoring system (Eichorn & Lorenz, 1977); Sc, Skin contact; Sed, Sediment contact; ScSed, Skin and sediment contact; DA, Descriptive Analysis.

separated and quantified using the method described in (Du Plessis *et al*., 2017). Juice and wine samples were diluted (1:1) in 70 % acidified methanol, filtered through a 0.22 µm cellulose acetate membrane and analysed using the same HPLC method as above mentioned. Three samples were prepared for each biological repeat. A serial dilution (0 – 250 mg/L) of authentic standards, gallic acid, caffeic acid, trans-caftaric acid, (+) – catechin, (-) – epicatechin and quercetin-glucoside was prepared to generate calibration parameters for quantification. Phenolic compounds were identified by comparing the retention times with those of the standards listed above and quantified accordingly. Phenolic compounds without corresponding standards were identified from their spectral data and elution orders and compared with MS data from a corresponding LC-MS method according to literature (Díaz-García *et al*., 2013; Figueiredo-González *et al*., 2012; Pires *et al*., 2018). The following standards were used for quantification: catechin for all flavan-3-ols, caftaric acid for all hydroxycinnamates and quercetin-glucoside for all flavonols. The concentrations of phenolic compound in solid tissues (grapes, pomace, and sediment) were normalised against the fresh or dry weight of the sample tissue and expressed as µg/g DW. In juice and wine, it was expressed as mg/L. The values presented are the averages of the biological and technical repeats. The total flavan-3-ols, flavonols and hydroxycinnamates (the hydroxycinnamic acid caffeic acid with its esterified form, i.e. caftaric acid and trans-coutaric acid, the tartaric acid ester of p-coumaric acid) were calculated as the sum of all compounds in each group. The magnitude of the response of a specific compound or compound group to a treatment was determined with the standard log2 fold change equation in Microsoft Excel (Microsoft Corporation). The fold change was the ratio between two values.

6. Photosynthetic pigment quantification in grape berries

Sample preparation and extraction of grape berry pigments, including carotenoids (β-carotene), xanthophylls (zeaxanthin, violaxanthin, neoxanthin, antheraxanthin, lutein and lutein epoxide) and chlorophylls (chlorophyll a and chlorophyll b) were done according to Lashbrooke *et al*. (2010). The analysis with UPLC (ultra-performance liquid chromatography), data acquisition and processing were previously described by Young *et al*. (2016). The relative concentration of the pigments was expressed as ng/g FW and is presented as the means of three biological repeats. The photosynthetic pigment profiles were used to confirm the success of the treatments in the vineyards linked to increased sun-exposure.

7. Wine sensory analysis

Descriptive sensory analysis (Lawless & Heymann, 2010) was conducted in three steps by a Sauvignon blanc trained panel that consisted of 10 females and 2 males, between the ages of 25 and 45 years. During the first step, panel training occurred where panellists identified descriptors to describe the orthonasal, retronasal, in-mouth taste and tactile properties of the wine from the study. Thereafter aroma standards were provided for each aroma descriptor and serial dilutions of mock samples for each taste descriptor. The ability of panellists to correctly identify reference standards, their repeatability and consensus when rating the intensity of the wines for each descriptor was tested to determine when the panel was trained sufficiently. During the evaluation phase, after completion of the training, wines were evaluated during a blind tasting session. All wine samples were presented in black glasses and given a 3-digit code, and the presentation order was randomised based on the Williams Latin-square design (MacFie *et al*., 1989) across all panellists. Aroma and taste descriptors were rated on a 10 cm unstructured line scale ranging from "none" to "intense". Each biological repeat of the wine was evaluated in triplicate.

8. Statistical analysis

One-way ANOVA was performed on the quantified chemical compounds with treatment as factor using Statistica™ 12 (TIBCO Software Incorporated, Palo Alto, USA). When a significant model ($p < 0.05$) was obtained, the Fisher's Least Square Differences (LSD) post-hoc test was applied to determine which treatments were significantly different from each other. Sensory data was analysed during a three-step process. PanelCheck (Version V1.4.0, Nofima, Tromsø, Norway), as described by Tomic *et al*. (2010), was used to evaluate panel consensus and the repeatability of individual panellists. After confirmation of sufficient panel consensus and repeatability, 2-way ANOVA as described by Tomic *et al*. (2010) was performed to identify significant attributes. Lastly, principal component analysis (PCA) was performed on the correlations matrix of the average descriptor intensity scores to investigate correlations between treatments, and treatments and sensory descriptors using XLSTAT.

RESULTS AND DISCUSSION

This study investigated the phenolic compounds of Sauvignon blanc grapes, as influenced by a high light (HL) microclimate created by defoliation in the bunch zone versus that of a low light (LL) microclimate where no defoliation took place. We investigated how these compounds were transferred to the juice and wine or retained in the winery waste matrices. Furthermore, we measured the effect of a short skin contact period or juice sediment contact during fermentation.

1. The high light (HL) microclimate in the canopy resulted in a dramatic increase in flavonols

Sugars and organic acids, photosynthetic pigments and phenolic compounds, were quantified in ripe Sauvignon blanc berries harvested from LL and HL microclimates (Table S1). The phenolic compounds were determined in the whole berries as well as in the skin, flesh and seeds of the berries while all the other compounds were determined in the whole berry. Increased light exposure in the HL environment (proof of the altered microclimates provided by Joubert *et al*., 2016; and Young *et al*., 2016) led to an increase in zeaxanthin, resulting in a larger xanthophyll pool size (Violaxanthin + Antheraxanthin + Zeaxanthin; V+A+Z) and consequently

A

	LL vs HL (log_2 fold change)							
	WB		Skin		Pulp		Seed	
Caftaric acid	0.4	*	0.4	*	0.3	*	0.0	
Coutaric acid	0.6	*	0.7	*	0.6	*		
Caffeic acid	0.4	*	0.5	*			-0.1	
Total Hydroxycinnamates	**0.4**	*	**0.5**	*	**0.3**	*	**0.0**	
Epicatechin	0.1		0.0				0.0	
Catechin	0.1		0.4	*			0.0	
Total Flavan-3-ols	**0.1**		**0.3**	*			**0.0**	
Quercetin-Galactoside	14.6	*	17.2	*			0.0	
Quercetin-Glucoside	4.1	*	3.8	*				
Keampherol-glucoside	15.4	*	17.8	*				
Isorhamnetin-glucoside	0.0		15.7	*				
Total Flavonols	**4.4**	*	**4.3**	*			**0.0**	
Total Phenolic compounds	**0.7**	*	**1.6**	*	**0.3**	*	**0.0**	

B

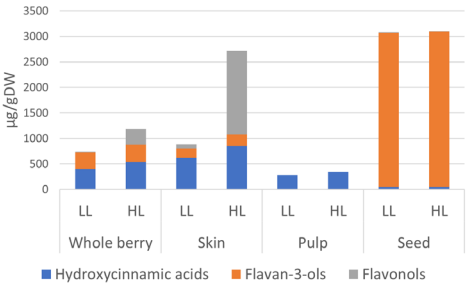


FIGURE 2. Grape berry. The magnitude of the difference between the concentration of phenolic compounds found in Sauvignon blanc HL and LL grape berry tissues (A). * Indicates statistically significant differences with P-value < 0.05. (B) The composition of polyphenolic compound concentrations per group, measured in grape berry tissues. WB refers to whole berry samples, including seeds.

a higher de-epoxidation state (DEPS) in HL samples. Furthermore, a decrease of chlorophyll a and b in HL samples was observed, but there was no significant difference in total carotenoids between LL and HL samples. These results validated previously reported data for similar experimental conditions (Joubert *et al*., 2016; Young *et al*., 2016) and confirmed that the HL conditions triggered the expected metabolic reactions. The HL berries accumulated higher levels of sugars (196 versus 172 mg/g FW) and there were also differences in the organic acid levels (10 versus 12 mg/g FW) from the two microclimates (Table S1).

Nine phenolic compounds (four flavonols, two flavan-3-ols and three hydroxycinnamates [free forms and esters]) could be quantified in the grape berries with the analytical method that was used (Table S1). The berry pulp only contained hydroxycinnamates; the seeds had the highest concentration of flavan-3-ols; whereas the skin had the highest diversity of polyphenol compounds of the three berry tissues (Figure 2). Sauvignon blanc grapes from the HL microclimate had a higher phenolic content than the LL grapes, in line with previous reports on other cultivars (Ristic *et al*., 2007; Šebela *et al*., 2017; Song *et al*., 2015). The log2 fold change was calculated for individual phenolic compounds to determine the magnitude of the response to the HL conditions (Figure 2A) and this showed a strong increase in the flavonols of the berry skin. In fact, most of these flavonols could not even be detected in the LL skin, while significant concentrations were found in their HL counterparts (Table S1).

Figure 2B illustrates how the proportion of flavonols (and the total phenolic content) increased in the berry and particularly in the skin under HL conditions, while the hydroxycinnamates and flavan-3-ols in the pulp and seed respectively, showed little change. The increase in the flavonols, and to a lesser extent the hydroxycinnamates of the skin is an indication of the protective role that these metabolites play by absorbing excessive ultraviolet radiation (Gregan *et al*., 2012) and scavenging reactive oxygen species induced by photodamage (Agati *et al*., 2013). Although the seeds had the highest concentration of polyphenolic compounds, they did not react to the microclimate as was previously also shown by Ristic *et al*. (2007) in Shiraz, and seed contribution to the total phenolic content of the berry is relatively small due to their small contribution to the overall berry weight.

2. Transfer of phenolic compounds from grape to juice and throughout the juice processing stages

The concentration of the phenolic compounds measured in the juices at the different processing stages (crush/free run; after pressing and after juice clarification; and with and without skin contact treatments) are summarised in Table S2.

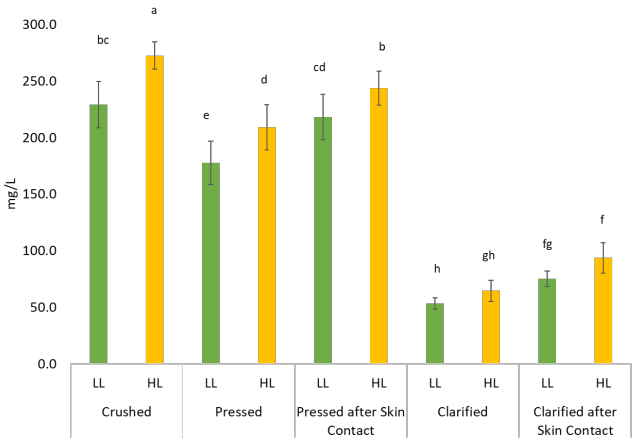


FIGURE 3. Juice. The effect of juice processing and skin contact treatment on the levels of the total polyphenolic compound concentration in Sauvignon blanc juices that originate from grapes from a High Light (HL), or Low Light (LL) microclimate in the bunch zones. The concentration of polyphenols is shown at the beginning (Crush) and the end (Clarified) of juice processing, as well as the differences in concentrations from juices that were treated with skin contact (Clarified with skin contact) or without skin contact (Clarified). Statistically significant differences were determined with ANOVA and Fisher's LSD post *hoc test* (p <0 .05) and are indicated by different letters.

Of the seven and nine different phenolic compounds that were detected in the LL and HL grapes respectively, only five were found in the juice namely, caftaric, coutaric- and caffeic acid, as well as catechin and epicatechin. None of the grape skin flavonols, that showed a dramatic response to HL conditions, were detected in the juice. The catechin and epicatechin that were present probably originated from the skins because flavonoids are more easily extracted from the skin than from the seeds, even under fermentative conditions (González-Manzano *et al*., 2004; Romero-Cascales *et al*., 2005).

During juice processing (crushing followed by pressing and clarification steps), there was a general decrease in the total/sum of polyphenolic compounds (Figure 3; Table S2), particularly during the clarification step. The compounds that decreased most were caftaric acid (Figure 4A) and catechin in both LL and HL juices (Table S2). This contrasted with coutaric acid (Figure 4C) and caffeic acid that increased from crushing to pressing, whereas epicatechin was the only polyphenol whose concentration was unaffected throughout juice processing (Table S2).

The concentration of compounds can increase during juice processing due to their continuous and gradual release from the vacuoles of skin and pulp tissue cells and extrusion in the wine press, or their formation (either from precursors, or due to other enzymatic reactions). Oxidation of polyphenols such as caftaric acid and catechin, especially in white cultivars (Ferreira-Lima *et al*., 2016; Maggu *et al*., 2007; Singleton *et al*., 1985) together with their tendency to bind to other polyphenols, or to cell wall polysaccharides, in particular pectin (Bindon & Smith, 2013; Brglez Mojzer *et al*., 2016; Renard *et al*., 2017) can account for their decrease during juice processing. The concomitant increase in coutaric and caffeic acids during the same process might be due to their liberation after enzymatic depolymerization of cell wall pectin polymers (Bautista-Ortin *et al*., 2016; Bindon, *et al*., 2010a; Padayachee *et al*., 2012).

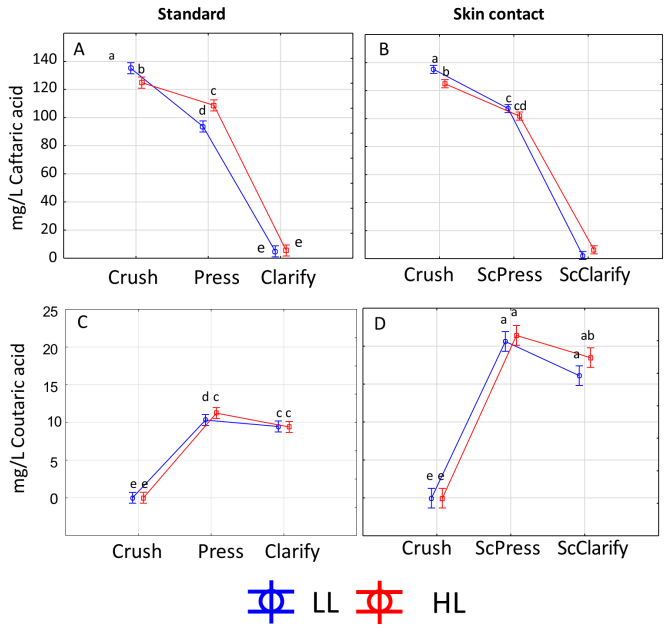


FIGURE 4. Juice. Fluctuations in the concentration of the polyphenolic compounds during juice processing steps (crushing, pressing and clarification) in Sauvignon blanc juices that originate from grapes from a High Light (HL), or Low Light (LL) microclimate in the bunch zones. Caftaric (A & B) and coutaric acid (C & D) are shown as typical examples of how some compound concentrations decreased (A) and others increased (C) and how skin contact can enhance (D) concentration or had no effect (B). The different letters indicate significance according to Fisher's LSD post hoc test with a 95 % confidence interval.

2.1. Skin contact

The juice processing steps caused bigger changes to the concentration of phenolic compounds, than the skin contact (Sc) treatment (Figure 3; Table S2) in the Sauvignon blanc matrices, corresponding with results seen by Honeth (2018). For both the HL and LL matrices, the skin contact treatment compensated, to a minor degree, for the general decrease in polyphenol concentrations during juice processing (Figure 3, and as can be seen for caffeic and coutaric acid [Figure 4D]). A few studies on the influence of skin contact on white cultivar juice have reported increases in polyphenolic compounds, especially caffeic acid and a decrease in glutathione (Gawel *et al*., 2014; Gómez-Míguez *et al*., 2007), a combination prone to juice browning. However, it should be noted that results were strongly influenced by the conditions (*e.g*., temperature, time) of the skin contact treatment.

3. Polyphenols in the waste matrices

3.1. The juice sediment could account for some of the metabolites "lost" during juice processing

The juice sediment that settled to the bottom of the tank during enzyme-assisted juice clarification was analysed to see whether it could explain the fate of some of the metabolites during juice processing (Table S3). The sediment had significant amounts of sugars and organic acids which corresponded with the decreasing trend for these compounds during juice processing (Table S2).

In terms of phenolics, all five compounds measured in juice (caftaric, coutaric- and caffeic acid, as well as catechin and epicatechin) were also found in the juice sediment. Quercetin-glucoside that was absent in the juice could be quantified in the sediment. Interestingly, skin contact increased the concentration in the sediment, suggesting that when this flavonol is extracted from the skins, it immediately settles in the sediment. The extraction of phenolic compounds during skin contact is accompanied by the extraction of other compounds such as polysaccharides and proteins which can polymerise and precipitate the phenolic compounds (Watrelot *et al*., 2017). When skin contact was applied, the increase in flavon-3-ols seen in clarified juice (*e.g*. LL_Clarified Juice has 29.0 mg/L vs. LL_Clarified Sc Juice has 41.9 mg/L flavon-3-ols; Table S2) is mirrored by a decrease in flavan-3-ols found in sediment after skin contact (672.4 µg/gDW in LL_Std Sediment vs. 494.0 µg/gDW in LL_Sc Sediment), suggesting that these compounds could

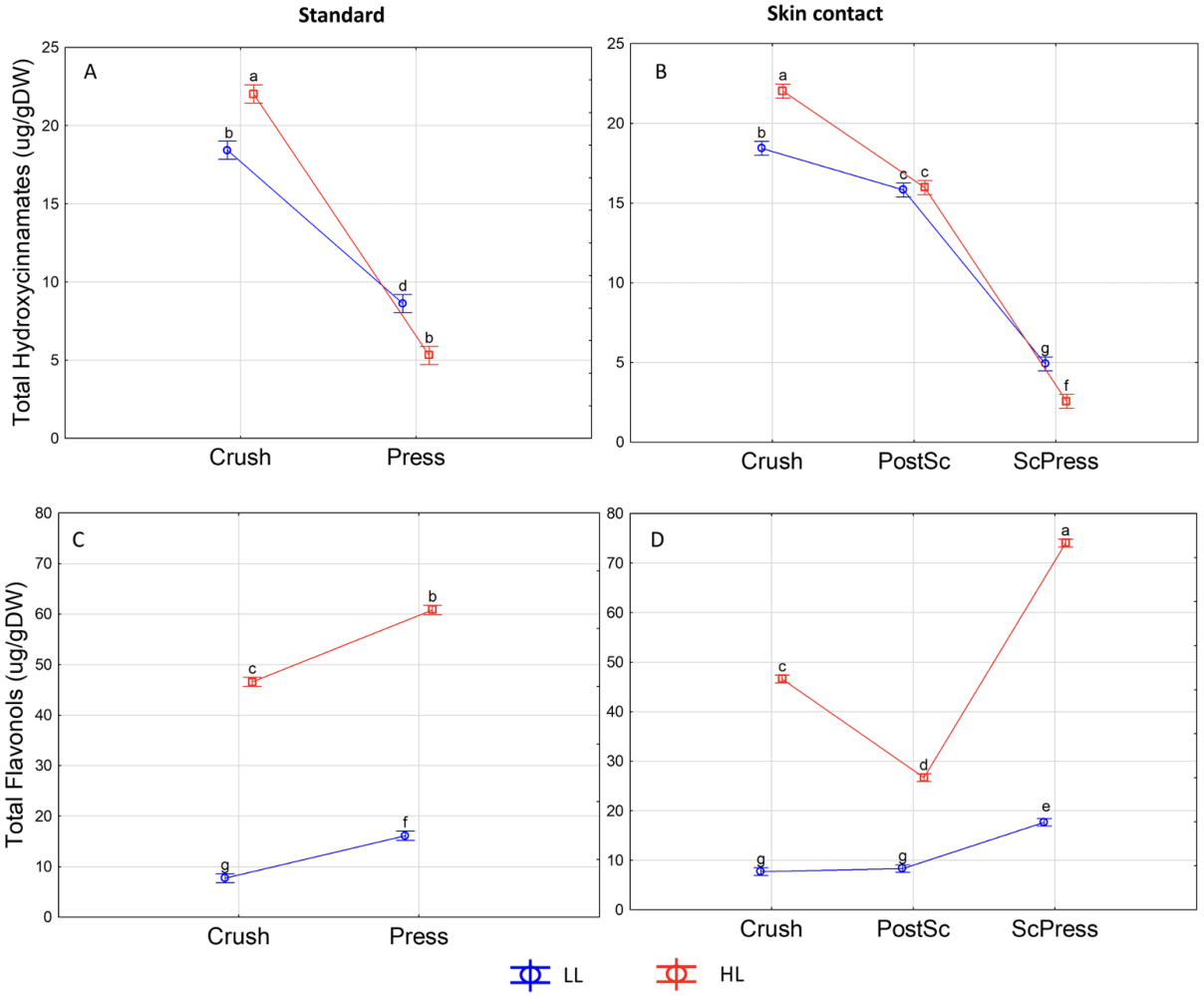


FIGURE 5. Pomace. The effect of juice processing and skin contact treatment on residual hydroxycinnamates (A & B) and flavonols (C & D) measured in pomace at crush, after skin contact (PostSc) and after press (Press and ScPress); the samples originated from Sauvignon blanc grapes from a High Light (HL), or Low light (LL) microclimate in the bunch zones. HCA, Hydroxycinnamic acids; PostSc, Samples taken after skin contact; ScPress, Samples taken after skin contact and pressing. The different letters indicate significance according to Fisher's LSD *post hoc* test with a 95% confidence interval.

have been extracted from both the skins and the sediment during juice processing.

3.2. The residual phenolic compounds in the pomace allude to secondary interactions with cell wall polymers

To investigate the phenolic compounds that remained in the grape skins, the pomace samples (pressed skins) were analysed for their phenolic content after the crush, after skin contact and after press, and compared to the samples that did not receive the skin contact treatment (Table S4). All the phenolic compounds measured in the grapes were present in the pomace except epicatechin. In pomace, the concentration of the hydroxycinnamates decreased during pressing (Figure 5A) and during skin contact (Figure 5B). This decrease corresponds with the increase in coutaric and caffeic acids in the juice during processing, but caftaric acid showed the opposite trend. In contrast, the residual flavonols (Figure 5C and 5D) in the pomace increased during juice processing. Although we see that skin contact treatment does seem to extract flavonols from the HL samples (decreasing values in pomace), the residual levels increase again during the subsequent pressing step, to result in an overall gain.

This was mirrored by the absence of flavonols in the juice, which may indicate that once extracted from the skins, these compounds bind to the pomace cell wall polysaccharides. Cell wall binding primarily affects flavonoids and not phenolic acids (Bindon *et al*., 2010a; Bindon *et al*., 2010b). The fate of the flavan-3-ols in the pomace samples is more obscure, since no epicatechin could be detected in the pomace and for catechin, no consistent trends were seen.

4. The wine phenolic compound profiles in response to the vineyard and winemaking treatments

4.1. Wine had a higher phenolic content than the clarified juice

The phenolic compounds were also measured in the wine samples (Table S5) that were prepared with pre-fermentative skin contact (Sc); or fermented in contact with the sediment

formed during juice clarification (Sed); a combination of these two treatments (ScSed); and a standard white winemaking procedure where no skin or sediment contact was conducted (Std).

Of the five different phenolic compounds measured in the juice, only four could be detected in the wine (no caffeic acid in wine) (Table S2 and S5). The total polyphenolic content of the wines was higher than their respective juices from which it was fermented *e.g.,* LL_Clarified juice had a total polyphenol concentration of 53.3 mg/L and LL_Std wine was 93.7 mg/L, and this was primarily due to high caftaric acid values in the wine compared to the juice. The higher caftaric acid content of the wine could originate from partial hydrolysis from hydroxycinnamoyltartaric acids (Garrido & Borges, 2013) during alcoholic fermentation. Other possible explanations for the increase in polyphenol concentration going from juice to wine, are hydrolysis from polymeric polyphenols (not measured by our analysis method), or the alcoholic extraction of flavan-3-ols from residual grape tissues (Su *et al*., 2014). Previous studies showed contradictory results: Patel *et al*. (2010) reported Sauvignon blanc wine with lower polyphenol concentrations than juice but Hernanz *et al*. (2007) showed the opposite in *Vitis vinifera* cv. Zalema wine. Overall, it seems that juice

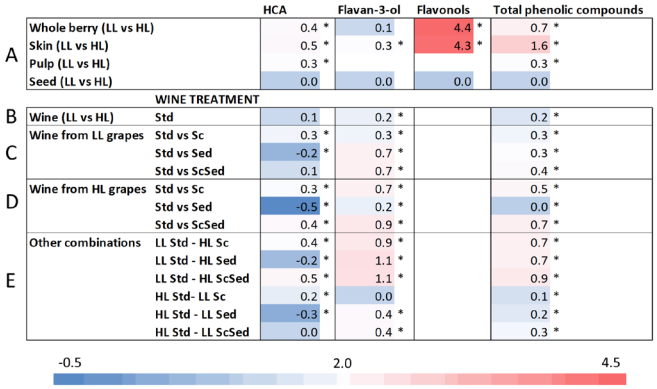


FIGURE 6. Grapes and Wine. Comparing the effect of different vineyard (A) and winemaking (B–E) treatments on the concentration of grape and wine phenolic compound groups in Sauvignon blanc. The juices and wines originated from grapes from a High Light (HL), or Low Light (LL) microclimate in the bunch zones. For grape samples, the log2 fold change was calculated between LL and HL samples from the different sample types (whole berry, skin, pulp and seed). Similar comparisons were made for the wine samples: LL_Std wine was compared to HL_Std wine (B) and LL_ or HL_Std were compared to skin contact (LL or HL_Sc) and sediment contact wine (LL or HL_Sed) or a combination of the latter two (LL or HL_ScSed) (C & D). In the last block (E) all other combinations of vineyard and winemaking treatments were compared to each other. Statistically significant differences, as determined with ANOVA and Fisher's LSD *post hoc* test ($p < 0.05$), are indicated with a *. HCA refers to hydroxycinnamates.

parameters cannot easily predict the phenolic content of the resulting wine. This is further illustrated by the juice before clarification which had a phenolic potential that was more than two times higher than the corresponding wines.

4.2. The increase in light exposure in the vineyard triggered the strongest metabolic change of all treatments

Log_2fold calculations were used to determine which of the treatments had the most prominent impact on the phenolic compound concentrations of the wine (Figure 6). Under both LL and HL conditions, the combination of skin contact and sediment contact (*e.g*. LL_Std vs LL_ScSed) had the strongest impact on the total phenolic compounds (Figure 6C and 6D). The flavan-3-ols (Figure 6E) increased with all treatments (both light exposure and winemaking treatments) with the strongest increase (log2 fold change of 1.1) seen when leaf removal was applied in combination with sediment contact and skin contact (LL_Std compared to HL_Sed and HL_ScSed). In contrast, hydroxycinnamates were reduced, specifically by sediment contact in the absence of skin contact. However, none of these changes were as pronounced as the change caused by the vineyard sun exposure to the concentration of flavonols in the grape skins (Figure 6A), caused by defoliation.

Interestingly when comparing HL_Std wine with all combinations of LL wines (Figure 6E), one sees that it was possible to obtain close to similar levels of total phenolic compounds in wines from shaded grapes by applying Sc or Sed, or the combination of the two.

4.3. Did the HL > LL profile persist throughout all the winemaking stages and in all the matrices?

The higher sugar concentration seen in the HL berries was still present in the HL_crush juice, but as juice processing progressed, the sugar difference between the HL and LL juices became statistically insignificant (Table S2). The LL clarified juice still had a higher total organic acid concentration than the HL equivalent, and skin contact caused a slight decrease in malic acid in the HL samples.

For the hydroxycinnamates, the HL > LL profile that was found in the berries was equalised during juice processing (Table 1). However, the HL > LL profile was preserved for the flavan-3-ols and total phenolic compounds in the clarified juice. This was also carried through to the final wines, and even for hydroxycinnamates the HL > LL profile was reinstated in some of the wines (Sc and SedSc). This HL > LL profile was also maintained in the pomace samples throughout juice processing, but it was not as well preserved in the sediment (Table S3 and S4).

It is interesting to note that the flavonols, that showed the strongest reaction towards the HL conditions in the vineyard, were not transferred to the juice and wine. This is in contrast to other studies that did report flavonols in juice (5–9 mg/L) and wine (0.5–7 mg/L) made from white grape cultivars (Makris *et al*., 2006).

TABLE 1. Persistence of the LL < HL trend. Comparing the levels of the phenolic compound groups in LL and HL samples in the different matrices (grapes, juice and wine) shows the persistence of the trend established in the vineyard, throughout juice processing up to the final product. Clarified Sc, clarified juice that received skin contact treatment; Std, standard winemaking procedures followed; Sc, skin contact; Sed, sediment contact; ScSed, skin and sediment contact applied.

	Grapes	Juice			Wine			
		Crush	Clarified	Clarified Sc	Std	Sc	Sed	SedSc
Total hydroxycinnamic acids	LL < HL	LL = HL	LL = HL	LL = HL	LL = HL	LL < HL	LL = HL	LL < HL
Total flavan-3-ols	LL=HL	LL < HL	LL = HL	LL < HL	LL < HL	LL < HL	LL < HL	LL < HL
Total flavonols	LL < HL							
Total phenolic compounds	LL < HL	LL < HL	LL = HL	LL < HL	LL < HL	LL < HL	LL < HL	LL < HL

5. Sensorial characteristics of the wine

Descriptive sensory analysis on taste, aroma and mouthfeel was performed on all wine samples after 6 months of bottle ageing (Table S6). Each of the eight different experimental wines had a unique sensory profile which included many typical Sauvignon blanc aromas e.g., pineapple, peach and asparagus. Sediment contact during fermentation was the strongest driver for variance amongst the aroma descriptors (PCA biplot, principal component 1 (PC1) = 69.2 %; Figure 7A), while PC2, explaining 21.5 % of the variance, was linked to light exposure.

Interpreting the 2-Way ANOVA LS means, the wines made from HL berries were reported to taste sweeter (Table S6 and Figure 7) and the HL_Std wine scored higher in body, floral and baked apple aromas, while the LL_Std wine scored higher in sourness (the aftertaste). The HL_Sc wine had a similar score for bitterness to the HL_Std wine, even though it had a significantly higher level of catechin (known to contribute to bitterness (Arnold *et al.*, 1980; Sokolowsky *et al.*, 2015). It is likely that the bitterness in HL_Sc wine might have been masked by the sweet taste. An interesting result is that the skin contact treatment was able to give the wine made from LL grapes some of the positive sensorial attributes seen in the HL_Std wines without any harsh or bitter attributes which is traditionally associated with a high phenolic profile. Sediment contact decreased the fruity and floral characteristics and increased the savoury and vegetable-associated (*e.g.*, gherkin, cooked-vegetable) aromas in the wines. Stabulation, the process where unracked juice is kept for a period at low temperatures to allow the extraction of esters and thiols from the solid juice particles (Philipp *et al.*, 2024; Seabrook & Van der Westhuizen, 2018) has enjoyed attention as a method to improve the aroma and mouthfeel properties of aromatic white cultivars such as Sauvignon blanc. This might be an indication that skin contact and harnessing the metabolite abundance of the sediment should be re-investigated for cultivars such as Sauvignon blanc.

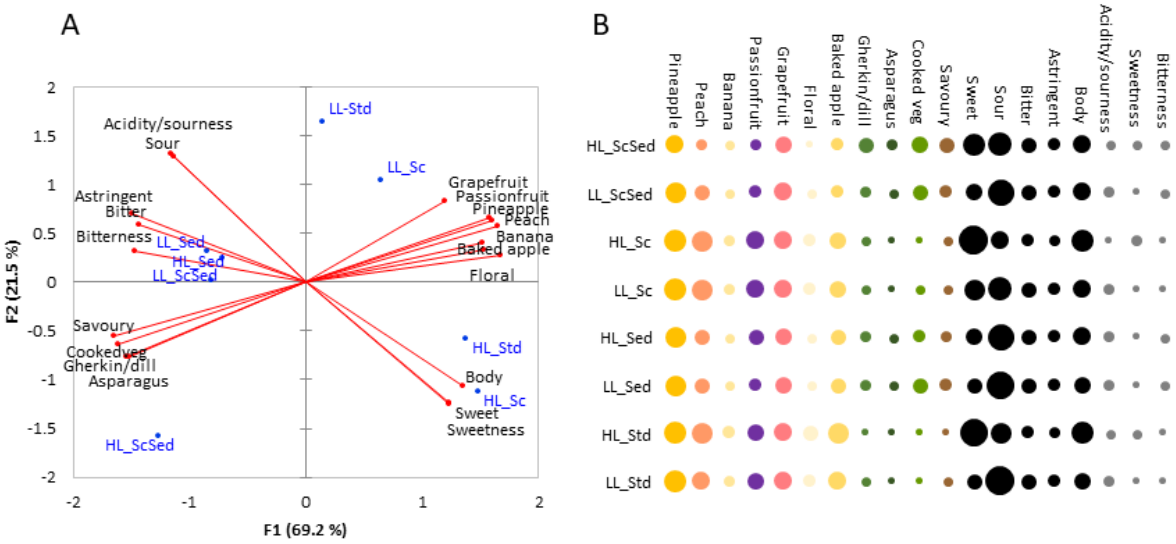


FIGURE 7. Wine sensory analysis on eight Sauvignon blanc wines that originated from a single vineyard where light exposure to bunches were modulated, in combination with juice and winemaking steps to study the polyphenolic compounds from grapes to wine. PCA biplot (A) of the aroma characteristics where the proximity between a wine sample and an aroma descriptor indicates a positive correlation. (B) Two-way ANOVA LS means representing the average intensity scores of the significant sensory descriptors ($p < 0.05$) are represented by the size of the circle. The last three aroma descriptors *i.e.* acidity/sourness, sweetness and bitterness, refers to the aftertaste. Abbreviations: LL, low light; HL, high light; Std, standard; Sc, skin contact; Sed, sediment contact; ScSed, skin and sediment contact.

CONCLUSION

This study provides a comprehensive grape-to-juice-to-wine-to-glass analysis of Sauvignon blanc polyphenolic compounds to help us understand how environmental conditions/perturbations in the vineyard influence the metabolic and sensorial potential of wines. By including waste matrices in the analyses, the metabolic loss to these matrices could be followed.

Sun exposed grapes had elevated levels of flavan-3-ols, hydroxycinnamates and flavonols and we could account for all these phenolic compounds of the grapes as they were either transferred to the juice and/or ended up in the juice sediment and/or remained in the pomace. According to our knowledge, this is the first attempt to define the phenolic composition of the sediment formed during enzyme-assisted juice clarification. The sediment was revealed to be an extremely rich source of metabolites that can account for some, but not all the "lost" juice metabolites Similarly, this is the first report showing the influence of the pomace during juice processing, with the results suggesting further entrapment of flavonols.

Despite the entrapment of flavonols by both the sediment and the pomace the wines made from the sun exposed grapes (HL) still had higher levels of phenolic compounds than the LL wines. The treatment in the vineyard had a secondary impact on the sensorial characteristics of the wine, being overshadowed by sediment contact during fermentation. The sediment however did not promote the typical Sauvignon blanc wine characteristics. The approach that was followed in this study highlights the importance of considering the juice processing and wine-making steps carefully to optimally make use of the phenolic potential in white grape cultivars.

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