

Whole Cluster and Dried Stem Additions' Effects on Chemical and Sensory Properties of Pinot noir Wines over Two Vintages

L. Federico Casassa,^{1*} Niclas P. Dermutz,¹ Paul F.W. Mawdsley,¹
Margaret Thompson,¹ Aníbal A. Catania,² Thomas S. Collins,³
P. Layton Ashmore,³ Fintan du Fresne,⁴ Gregory Gasic,⁵
and Jean C. Dodson Peterson¹

Abstract: The effect of additions of dried stems (DS) and whole clusters at 50% (50% WC) and 100% (100% WC) were investigated over two consecutive vintages of Pinot noir wines from the Edna Valley AVA of California (USA) at commercial scale. Addition of 100% WC led to significant increases in pH and volatile acidity. Anthocyanins, polymeric pigments, and color were more influenced by vintage than by the winemaking treatments, but they were unaffected or negatively affected in 100% WC wines. Conversely, the tannin content of the wines increased in accordance to the percentage of WC and stems added, with increases of 68% (50% WC), 100% (100% WC), and 90% (DS) in 2016 and 61% (50% WC), 123% (100% WC), and 137% (DS) in 2017 relative to control wines. Gas chromatography-mass spectrometry (GC-MS) data showed higher relative levels of ethyl cinnamate and benzaldehyde in 100% WC wines and relative abundance of esters in DS wines. Descriptive sensory analysis showed that 100% WC additions led to vegetal, cooked fruit flavors, and spicy (clove) notes, whereas DS additions produced wines that were more herbal, fruity, and astringent, with lifted fruity notes related to esters, and astringency sensations related to enhanced tannin extraction from the stems. Both WC and DS additions led to variable increases in perceived astringency, suggesting that they can be used to add mouthfeel and improve texture in Pinot noir wines.

Key words: phenolics, Pinot noir, sensory analysis, stems, whole cluster

Pinot noir accounted for only 7% of the total crush of the 2018 harvest in California (USA) (California Department of Food and Agriculture 2019), but the variety and their resulting wines have become increasingly fashionable in the California wine industry over the past 15 years. This trend has been anecdotally attributed to the “Sideways effect” (Cuellar et al.

2009), in which the eponymous movie cast aside Merlot and arguably managed to “put Pinot noir in the mind of the consumer.” In the Central Coast of California, Pinot noir claims ~7000 ha under vine. The Edna Valley AVA of San Luis Obispo County is especially suited for successful cultivation of Pinot noir due to an extended growing season that is often 50% longer than Burgundy and is marked by relatively cool temperatures during ripening (Robinson and Harding 2015).

Relative to other red cultivars, Pinot noir grapes and wines are relatively low in phenolic compounds (Harbertson et al. 2008) and have low concentrations of anthocyanin and tannin. Anthocyanins are phenolic compounds that provide color, whereas tannins are responsible for mouthfeel and textural properties in red wines (Casassa and Harbertson 2014). Low color saturation is a normal trait in the sensory make up of Pinot noir wines and is regarded as a major factor in its typicity and perceived quality (Valentin et al. 2016). Pinot noir wines are also often praised for their specific and desirable textural and mouthfeel features. Texture in liquids results from a combination of physical properties perceived through tactile, visual, and auditory receptors, whereas mouthfeel refers to tactile sensations felt once the wine has been placed in the mouth and is usually not affected by visual or auditory cues (Cheynier and Sarni-Manchado 2010). While elements other than phenolic compounds, including ethanol, polysaccharides, and sugars, may contribute to the mouthfeel of a wine, tactile sensations in red wines are primarily attributed to tannins and polymeric pigments (Gawel et al. 2007, Landon et al. 2008).

¹Wine & Viticulture Department, California Polytechnic State University, San Luis Obispo, CA 93407; ²Centro de Estudios de Enología, Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (INTA), San Martín 3853, Luján de Cuyo, Mendoza, Argentina; ³Viticulture and Enology Program, Washington State University, Richland, WA 99354; ⁴Chamisal Vineyards and Winery, 7525 Orcutt Rd, San Luis Obispo, CA 93401; and ⁵4035 NW Houston Place, Corvallis, OR 97330.

*Corresponding author (lcasassa@calpoly.edu)

Acknowledgments: This research was generously supported by Chamisal Vineyards (Crimson Wine Group) through their fruit contributions and donation of time and winemaking facilities. The authors are grateful for the opportunity to continue this work under their continued support. Funding was provided by the Agriculture Research Institute (ARI grant #17-03-007). Members of the trained sensory panel are thanked for outstanding commitment with this study over two years.

Supplemental data is freely available with the online version of this article at www.ajevonline.org.

Manuscript submitted May 2020, revised Aug 2020, accepted Aug 2020

Copyright © 2021 by the American Society for Enology and Viticulture. All rights reserved.

By downloading and/or receiving this article, you agree to the Disclaimer of Warranties and Liability. The full statement of the Disclaimers is available at <http://www.ajevonline.org/content/proprietary-rights-notice-ajev-online>. If you do not agree to the Disclaimers, do not download and/or accept this article. doi: 10.5344/ajev.2020.20037

Astringency is the main tactile sensation associated with red wine consumption and occurs due to the depletion of proteins from the tongue surface mediated by wine tannins, resulting in increased oral friction (Brossard et al. 2016). Astringency is a key determinant in consumer acceptance (Vidal et al. 2015), but tannin levels in wine vary widely as a function of climate, vineyard location, exposure, variety, and style, among other factors. A study including 1325 commercial wines reported higher tannin levels in Cabernet Sauvignon wines (672 mg/L, $n = 364$) than in Pinot noir wines, which had the lowest levels (348 mg/L, $n = 261$) (Harbertson et al. 2008). If tannin concentration alone is accepted as a primary driver of perceived astringency (and mouthfeel) in red wines, the later study (Harbertson et al. 2008) confirms the empirical observation of Pinot noir wines as light-bodied relative to full-bodied, tannic Cabernet Sauvignon wines. However, a recent study that extracted tannins from Pinot noir and Cabernet Sauvignon wines and added them back to model wines at iso-concentrations of 0.5 g/L found that Cabernet Sauvignon tannins were perceived as drier and longer lasting than Pinot noir tannins. This effect was attributed to larger (i.e., higher molecular weight) tannins in Cabernet Sauvignon than in Pinot noir wines (Watrelet et al. 2020). Thus, the inherently lower astringency of Pinot noir may be due to the extraction of low molecular weight tannins, which are less reactive toward salivary proteins during the development of oral astringency (Sun et al. 2013).

Because tannin content and extractability are generally low in Pinot noir grapes, several winemaking techniques have been specifically developed for its resulting wines. Moreover, some of these techniques also aim to enhance sensory characteristics of Pinot noir, such as wine color and aromas. Some of these winemaking techniques include prefermentative cold soak and whole cluster (WC) addition. Prefermentative cold soak involves allowing the contact of skins and seeds with the must held at low temperature (5 to 15°C), thereby preventing the onset of alcoholic fermentation and reportedly favoring anthocyanin extraction in the absence of ethanol. A study carried out in Mendoza (Argentina) reported that cold soak applied to Pinot noir wines decreased wine color and had no effect on sensory characteristics when compared to control wines (Casassa et al. 2015). However, another study reported that cold soak increased tannin extraction by 34% in Pinot noir wines from a cooler vintage, but not in those from a warmer vintage (Casassa et al. 2019b). Although widespread in Pinot noir winemaking, cold soak has produced mixed and sometimes conflicting results, likely related to variations in the original fruit chemistry.

WC addition entails keeping a portion of non-destemmed WC in the fermentor throughout alcoholic fermentation and maceration. The aim of WC appears to be two-fold. Keeping the clusters whole allows for tannin extraction from the stems, and it may also allow for a partial carbonic maceration inside the intact berries, thereby increasing aroma complexity and adding fruity esters such as isoamyl acetate and ethyl cinnamate (Tesniere and Flanzy 2011). The downsides of WC are that stems may not be lignified by harvest time, thereby

increasing the risk of adding vegetal or herbaceous notes to the wines, and that the sensory characteristics imparted by carbonic maceration that occurs during WC may mask varietal aromas. Indeed, some winemakers have empirically reported the benefits of adding stems while avoiding carbonic maceration for Pinot noir, which produces the aromatic lift of stems while avoiding the carbonic maceration character of WC addition.

Current research on Pinot noir wines from the Edna Valley AVA of California has investigated the effect of the timing of cluster thinning (Mawdsley et al. 2019) and the chemical outcomes of extended maceration (Casassa et al. 2019a). However, the effect of WC, one of the most popular winemaking techniques applied to Pinot noir in California, has yet to be reported. In this study, we conducted triplicate fermentations with different percentage additions of WC and dried stems (DS) to Pinot noir wines in a commercial winery from the Central Coast of California. The DS addition technique was developed with the goal of allowing the extraction into wine of stem-derived aromas and phenolics without the effect of partial carbonic maceration arising from WC additions.

Materials and Methods

Grapes. *Vitis vinifera* L. Pinot noir grapes (clone 777) grafted on 5C (Teleki) rootstock from a single vineyard block were grown in the Edna Valley AVA (35°11'N; 120°34'W), San Luis Obispo, California, during the 2016 and 2017 growing seasons. Grapes were harvested when a composite sample of ~500 berries reached 24 Brix and were analyzed for Brix, pH, titratable acidity (TA), and malic acid content (Supplemental Table 1).

Winemaking. Winemaking occurred in Chamisal Vineyards and Winery (Edna Valley, California). Except when otherwise noted, processing was kept identical during both vintages. Approximately 6.1 t of grapes were harvested manually on 13 Sept 2016 and 12 Sept 2017 and processed immediately. A crusher-destemmer (Bucher Vaslin) was used to destem the grapes (if required) according to each treatment condition. After crushing, the musts were randomly allocated into twelve 0.5-t MacroBins, with 0.4 t of must distributed in each according to the experimental design detailed below. Sulfur dioxide (SO₂) was added at a rate of 30 mg/L in 2016 and 50 mg/L in 2017. An additional 50 mg/L addition was performed 24 hr post-crushing in 2017 because 20% of the clusters showed signs of powdery mildew (*Erysiphe* spp.). Four different treatments were established in triplicate ($n = 3$). The control treatment (C) received must containing crushed and completely destemmed berries. A 50% WC-added treatment (50% WC) combined 0.2 t of WC topped with ~0.2 t of crushed and destemmed berries. A 100% WC-added treatment (100% WC) placed 0.4 t of WC in the corresponding MacroBins. A treatment hereafter referred to as dried stems (DS) utilized an amount of previously air-dried stems (for five days) that corresponded to the total weight of stems present in 0.4 t of fruit and placed them in the MacroBins below the crushed berries. The percentage weight of fresh stems in the fruit was calculated based on weights recorded by an

analytical benchtop scale (Fisher Science Education ALF203 200 g scale, Thermo Fisher Scientific) of a random sample of 60 clusters taken three days prior to harvest. The percentage of stems was 5.8% in 2016 and 5.2% in 2017, corresponding to 23.3 kg and 20.8 kg of fresh stems, respectively, for each 0.4 t lot of fruit. The stems were harvested five days in advance from the same vineyard block and only for the purposes of this experiment, with the remaining fruit conveyed to rosé production. Upon destemming, the fresh stems were spread outdoors to allow drying for five days and flipped regularly once per day during the drying process, which led to weight losses of 74% and 70% in 2016 and 2017, respectively. Weights were recorded on site using an industrial scale (capacity 2268.0×0.5 kg), equipped with a digital display (Cardinal Detecto, Model 204). The fruit was transferred into the MacroBins, which were immediately moved to a room at 13°C where they underwent a two-day cold soak, during which they received additions of CO₂ gas twice per day (morning and afternoon). After cold soak and homogenization of the musts, all treatments received a tartaric acid addition (2 g/L) and were inoculated with a commercial yeast strain (RC-212, Lallemant). Tartaric acid additions are usually performed by the winemaking team if fruit pH is above 3.60, which was the case in both years studied (Supplemental Table 1). Contact time with the fermentation solids was set at 10 days in both vintages. Cap management regime consisted of two 10-min punch-downs a day (morning and afternoon) applied to all treatments. Alcoholic fermentation took place at average temperatures of 21.5°C and 22.2°C, with a temperature peak of 26°C and 26.5°C in 2016 and 2017, respectively. Brix, cap temperature, and wine temperature were recorded daily by use of Anton Paar Portable Density Meter DMA 35 (Anton Paar). Alcoholic fermentation was completed within seven to eight days, with temperature and sugar consumption curves showing good reproducibility within replicates of the same and different treatments (overall temperature variance of 0.54°C and 0.23°C in 2016 and 2017, respectively, and overall Brix variance of 2.05 Brix and 1.87 Brix in 2016 and 2017, respectively).

Upon completion of alcoholic fermentation, all treatments and their replicates were individually pressed in a horizontal press (Europress Scharfenberger Maschinenbau) with an upper pressure limit set at 0.6 bars. Wines were then transferred to neutral (four fills, four-years-old) medium-toasted 225-L French oak barrels that were previously conditioned with steam and ozonated water and deemed free of microbial spoilage, and placed into a temperature-controlled room ($18 \pm 1^\circ\text{C}$), where malolactic fermentation (MLF) took place. Wines were inoculated with malolactic bacteria (Viniflora CH16, Lallemant, in 2016; and Enoferm Alpha, Lallemant Inc., in 2017), and MLF was completed 45 to 55 days post-inoculation. After completion of MLF (malic acid <0.1 g/L), wines were racked off the lees, treated with 25 mg/L free SO₂, returned to the same barrels where they settled for one month, and racked off the fine lees again. Free SO₂ levels were kept at 20 mg/L during aging, which lasted approximately three months. Wines were bottled prior to adjustment to 30 mg/L

free SO₂ on 17 March 2017 and 26 Feb 2018, corked using a DIAM 5 micro-agglomerated cork closure (G3 Enterprises), and kept in cellar-like conditions until analysis.

Grape and wine analysis. Brix in the fruit at harvest was determined with a handheld refractometer (Vee Gee Scientific). TA (end-point at pH 8.2 titrating with 0.067 N NaOH) (Fisher Scientific) and pH (Thermo, Fisher Scientific) were determined manually. Ethanol in finished wines was measured by near-infrared spectroscopy with an Anton-Paar wine alcolyzer (model M/ME, Anton Paar). Glucose, fructose, and tartaric, malic, acetic, and lactic acid were measured with a semi-automatic enzymatic analyzer system (Admeo Inc., Biosystems group) using commercial enzymatic analysis kits (Biosystems). Free and total SO₂ levels were determined using the aspiration/oxidation method.

Spectrophotometric analysis. Spectrophotometric measurements were performed in a Cary 60 UV-vis spectrophotometer equipped with an 18-sample cell auto-sampler (Agilent Technologies). Analyses included phenolic compounds, color parameters (including CIE L*a*b* coordinates), and full visible spectrum absorption scans, and were systematically performed during winemaking and bottle aging to evaluate the effect of the different winemaking treatments on the evolution of phenolic compounds and color components. Samples were centrifuged for 8 min at 15,000 g in a microfuge (Eppendorf, model 5415D), and the supernatant was transferred into clean 1-mL Eppendorf tubes prior to analysis. Anthocyanins (mg/L malvidin-3-glucoside), total phenolics expressed as mg/L gallic acid equivalents, small polymeric pigments (SPP), large polymeric pigments (LPP), and total polymeric pigments (herein reported as SPP + LPP) were measured as previously described (Harbertson et al. 2003). Tannins in the wines were analyzed by protein precipitation and expressed as (+)-catechin equivalents (CE). Wine color parameters, including full-visible-spectrum absorbance spectrum scans and CIE L*a*b* color coordinates, were determined in 1-mm path-length quartz cuvettes. CIE L*a*b* coordinates were calculated using the Cary WinUV color software (version 6.0, Startek Technology) to extract CIE L*a*b* tri-stimulus colorimetry values (D65 illuminant). To explore overall chromatic differences between treatments, the CIE L*a*b* color difference (ΔE^*) between any given pair of wines was calculated as previously described (Pérez-Magariño and Gonzalez-Sanjosé 2003) after three and 15 months of bottle aging for both vintages (Supplemental Tables 2 and 3).

GC-MS analysis. Samples were analyzed following a previously published procedure (Pino 2007). Samples of each wine replicate were prepared with addition of 146 µg/L of an internal standard (99.0% 2-nonanone-1,1,1,3,3,3d₅, CDN Isotopes), and 5 mL was placed into 20-mL headspace vials (Restek), with all vials prepared and injected in triplicate. Extraction of volatiles for analysis consisted of headspace solid-phase microextraction using a Supelco Stableflex PDMS/DVB fiber (65 µm film thickness, Supelco). Samples were adsorbed at 60°C for 35 min with agitation using a Gerstel Robotic Pro MPS autosampler (Gerstel US Inc.). The fiber was desorbed in the inlet at 250°C for 5 min. Separations

were performed using a polar Agilent DB-WaxUI column (30 m × 0.250 mm, 0.25 µm film thickness, Agilent Technologies). The temperature regime consisted of an initial hold of 2 min at 60°C followed by a 4°C/min ramp to 250°C with a final 10-min hold. The transfer line to the MS was held at 250°C, and fragmentation was performed by electron impact (70 eV). The quadrupole and source were held at 150°C and 230°C, respectively. Deconvolution and peak alignment were performed using Masshunter Quantitative Analysis (version B.07.00, Agilent Technologies) with compound identification performed by NIST2014 library lookup. Extracted ion chromatogram (EIC) integration values were used to determine relative abundances, which were normalized by their respective internal standard EIC. Tentative compound identifications were supported by Kovats retention indices. A 4-µL aliquot of Kovats n-alkane solution (c8-c20 in methylene chloride, Sigma Aldrich) was placed in a headspace vial with internal standard and analyzed according to the previously stated method. Retention time correction was performed using the difference between internal standard retention times. Kovats indices were calculated and compared to NIST mass spectral library reported values (Supplemental Table 4).

Descriptive sensory analysis. The wines of both vintages and their replicates (n = 3) were evaluated by sensory descriptive analysis (Lawless and Heymann 2010), after approximately three months of bottle aging. Panelists were first screened for visual disorders and potential color perception deficiencies using pseudo-isochromatic color testing plates (Ishihara maps). Panelists were also screened for bitterness sensitivity to 6-n-propylthiouracil (PROP) (Fluka Chemical Company), a test that can assert a condition known as PROP status and breaks down screened individuals in non-, medium-, and super-tasters (Pickering et al. 2004). To reduce bias, no information about the nature of the study other than the variety was provided to the panelists. The Cal Poly Institutional Review Board for human subject participation approved the project.

For both sensory panels, panelists were chosen on a volunteer basis. All of the panelists had previous wine tasting experience (previous descriptive analysis panels, tasting room attendants, cellar workers, and students of the Wine and Viticulture department at Cal Poly, San Luis Obispo). For the 2016 wines (evaluated in 2017), the panel was composed of nine individuals (eight men and one woman) 20 to 35 years of age (mean age of 26 years). Seventy percent of the panelists were medium-tasters and 30% were super-tasters, and no deficiencies in color perception were found. For the 2017 wines (evaluated in 2018), the panel was composed of 10 individuals (five males and five females) 21 to 40 years of age (mean age of 29 years). Ten percent of the panelists were non-tasters, 60% were medium-tasters, and 30% were super-tasters, and no deficiencies in color perception were found. Panelists were trained over a total of six (2017) and eight training sessions (2018), and the wines were formally evaluated over five formal sessions during both panels. Training sessions were held at the same time twice per week and were 90 min long. During the training sessions, panelists discussed, agreed upon, and

were trained with reference standards (Supplemental Tables 5 and 6) based on the experimental wines. These standards included color, aroma, taste, and mouthfeel attributes, for a total of eight (2016 wines) and 10 (2017 wines) sensory descriptors to be assessed. For the 2017 wines, the panelists requested a low and high reference standard for color and astringency, which were prepared as reported in Supplemental Table 6. The standards were reviewed by all panelists at the beginning of each training session for calibration purposes and to ensure they were sound and true to the sensory attribute they were supposed to demonstrate. Standards were made the night before or three hours prior to each session to ensure they were fresh and true to the attribute they represented.

Training occurred as follows. Briefly, during the training sessions, wines were presented in clear ISO wine glasses covered with plastic lids (to trap volatiles) for aroma standards. The standards were reviewed by all panelists at the beginning of each training session for calibration purposes and to ensure they were sound and true to the sensory attribute they were supposed to demonstrate. Two flights of four to six wines each were assessed during each training session, but only one was scored for analysis of panel comprehension of the standardized attributes. Panelists assessed experimental wines as well as commercial wines to broaden understanding of characteristics such as acidity, astringency, and color. Panelists were exposed to all of the experimental wines while tasting blind. Scoring of the wines during the training sessions was performed on a 15-cm unstructured line scale anchored by the words “low” at 1 cm and “high” at 14 cm.

The experimental wines were formally assessed over five evaluation sessions during both panels. The sessions were held in individual sensory analysis booths. Regular lighting (General Electric, Eco Luxe, 25 Watts) was used for color evaluations while red lighting (General Electric, Party Lights, 25 Watts) was used for taste, tactile, and aroma evaluations to decrease bias due to color. Color was assessed separately from aroma and taste/mouthfeel to ensure independent ratings. Panelists evaluated seven or eight wines during each of the evaluation sessions, with each wine and its replicates evaluated in triplicate.

The wines were presented monadically according to a Latin Square Design in clear ISO wine glasses labeled with three-digit random code numbers. Wines were served at room temperature in 30-mL aliquots per glass. Unsalted crackers (Nabisco), deionized water, and spit cups were provided for panelists during aroma and taste/mouthfeel sessions. Panelists were instructed to wait one minute and consume a cracker and water before moving to the next wine during the taste and mouthfeel assessment sessions. Panelists recorded their answers on ballots using a 15-cm unstructured line scale anchored by the words “low” at 1 cm and “high” at 14 cm. Results were collected on ballots, and responses (in cm) were decoded manually. In the 2017 panel, one panelist (code 117) missed Session 1, and the ratings of this panelist were computed as the arithmetic means of the other replicates for that session for each of the wines evaluated in Session 1 (Hopfer et al. 2012).

The performance of each panelist was evaluated by analyzing interaction plots generated by the Panel Check software (Tomic et al. 2010). Panel performance was also evaluated by assessing the correlation between each panelist and the panel mean, and by their contribution to the panelist \times wine interaction for each attribute.

Data analysis. The basic composition of the fruit at harvest during both vintages was analyzed by a Student's *t*-test with a 5% level for rejection of the null hypothesis. The phenolic, anthocyanin, and chromatic compositions of the wines were analyzed by two- and three-way analysis of variance (ANOVA) with interactions. Fisher's least significant difference (LSD) test was performed with a 5% level for rejection of the null hypothesis using XLSTAT v. 2019 (Addinsoft).

GC-MS data was standardized prior to statistical analysis due to variations in relative abundance over two orders of magnitude. Data were analyzed by a combination of heatmaps and hierarchical cluster analysis. A heatmap is a color-coded table that represents intensity or abundance where the rows (winemaking treatments) and columns (concentration of volatiles) are sorted by hierarchical clustering trees, thereby facilitating the identification of underlying patterns. GC-MS data was analyzed with R software, version 3.4.0 (R Foundation for Statistical Computing) using personally tailored "R" scripts.

The trained panel data from both years were analyzed by a three-way mixed-effects ANOVA with replication. Panelists were treated as random effects and wine treatments and replicates, including their interactions, were treated as fixed effects. Separation of the means was accomplished using Fisher's LSD with significance established as $p < 0.05$ using XLSTAT v. 2019 (Addinsoft). Principal component analysis

(PCA) using the correlation matrix with no rotation was applied to the significant ($p < 0.05$) sensory attributes, including the replicates, using R software version 3.4.0. Confidence ellipses indicating 95% confidence intervals were based on the multivariate distribution of Hotelling's test for $p < 0.05$ and were constructed using the *SensoMineR* panellipse function of R as described previously (Husson et al. 2005).

Results and Discussion

General chemistry of grapes and wines. WC and stem addition, which are in widespread use in Pinot noir winemaking, were studied over two consecutive vintages in the cool-climate Edna Valley AVA appellation of the Central Coast of California (USA). Grapes were harvested at full maturity during both vintages when they approached 24 Brix. Grapes of the 2017 vintage showed slightly higher acidity than in 2016 (Supplemental Table 1). The basic chemical composition of the wines was measured to characterize potential effects of the winemaking treatments and analyzed by a two-way ANOVA, which considered interactions between vintage and winemaking treatment (Table 1). With the exception of the 50% WC treatment in 2017, WC (at any percentage) and DS addition increased wine pH by 0.2 to 0.3 units relative to C wines in both vintages. The increase in pH is most likely attributed to the extraction of potassium and calcium ions from the stems into the wine, as these ions combine with tartaric acid and enhance the precipitation of tartrates (Hashizume et al. 1998). This process, however, did not affect the TA of the wines. Although all of the wines completed alcoholic and malolactic fermentation, none of the treatments significantly affected fermentation rate (data not shown), ethanol content, glucose, fructose, lactic, or malic acid (Table 1). However, DS

Table 1 Basic chemical analysis of Pinot noir wines produced with different maceration techniques over two consecutive vintages (2016 and 2017). A two-way analysis of variance (ANOVA) with the separate effects of vintage, maceration, and their interaction is also presented. Values represent the mean of three tank replicates.

Vintage/ maceration	Ethanol (% v/v)	pH	Titrateable acidity (g/L tartaric acid)	Lactic acid (g/L)	Malic acid (g/L)	Volatile acidity (g/L acetic acid)	Glucose + fructose (g/L)
2016							
C ^a	13.07 a ^b	3.36 b	6.4 b	0.86 ab	0.05 a	0.77 b	0.59 a
50% WC	13.24 a	3.55 a	6.8 a	0.81 ab	0.05 a	0.81 b	0.51 b
100% WC	13.02 a	3.53 a	6.8 a	0.79 b	0.06 a	1.11 a	0.51 b
DS	13.48 a	3.52 a	6.5 ab	0.87 a	0.06 a	0.85 b	0.59 a
<i>p</i> value	0.225	0.006	0.099	0.133	0.423	0.015	0.015
2017							
C	14.54 ab	3.31 c	7.1 a	0.58 b	0.04 a	0.79 b	1.17 a
50% WC	13.90 b	3.42 bc	7.2 a	0.66 ab	0.04 a	0.95 ab	1.12 a
100% WC	14.24 ab	3.60 a	7.2 a	0.76 a	0.04 a	1.12 a	1.13 a
DS	14.68 a	3.51 ab	7.1 a	0.72 a	0.06 a	0.91 ab	1.13 a
<i>p</i> value	0.164	0.003	0.243	0.025	0.274	0.044	0.238
ANOVA effects (<i>p</i> values)							
Vintage	<0.0001	0.802	<0.0001	<0.0001	0.068	0.219	<0.0001
Maceration	0.136	0.001	0.016	0.088	0.256	0.001	0.030
Vintage \times Maceration	0.354	0.018	0.727	0.007	0.387	0.699	0.628

^aC: Control; WC: whole cluster; DS: Dried stems.

^bDifferent letters within wines of the same vintage indicate significant differences for Fisher's least significant difference test and $p < 0.05$. Significant *p* values are shown in bold.

and WC addition increased the volatile acidity (VA) of the wines. VA was ~0.33 g/L higher in the 100% WC wines than in the C wines over the two vintages. Although no published scientific reports have studied the effect of WC additions (at or near 100%) on VA, winemakers have often empirically reported increases in VA when partial or 100% WC are used. This increase in VA is likely due to acetic acid bacteria that develop as a result of the “air pockets” that occur in a fermentor when a large proportion of the clusters remain intact during maceration and alcoholic fermentation.

Wine color and phenolics. The effect of WC and DS addition on wine color and several phenolic classes was evaluated throughout winemaking and bottle aging in both vintages. Results pertaining to selected phenolic classes are presented in Figure 1, and results pertaining to color are presented in Table 2 and Figure 2. Anthocyanins decreased over time from pressing onwards, with a 68% decrease observed after two years of bottle aging in 2016 and a 79% decrease observed after 15 months of bottle aging in 2017. Anthocyanin concentration did not differ significantly between any of the treatments and the C wines at similar time points (Figure 1A and 1B). However, anthocyanins were markedly higher in 2016 than in 2017. Grape and wine phenolic content and extractability usually show marked seasonal variations (Downey et al. 2006). In the present study, 2016 (1462 growing degree days) was markedly cooler than 2017 (1780 growing degree days) (Mawdsley et al. 2018). Cooler temperatures typically correlate with enhanced anthocyanin accumulation (Downey et al. 2006), which supports our observation of higher anthocyanin content in 2016. Levels of SO₂ added at and after crush were also higher in 2017 than in 2016, which could have bleached a portion of the anthocyanin pool. Although tannins showed less vintage variation than anthocyanins, the three treatments significantly increased tannins relative to C wines at pressing (Figure 1C and 1D). In 2016, levels of tannins at pressing were increased by 68% (50% WC), 100% (100% WC), and 90% (DS) relative to C wines. These differences were maintained throughout winemaking and bottle aging. In 2017, levels of tannins at pressing were increased by 61% (50% WC), 123% (100% WC), and 137% (DS) relative to C wines. These differences became more apparent after 15 months of bottle aging, especially for 100% WC and DS wines. In a previous study also in Pinot noir, additions of WC at a 20% rate had no effect on wine tannins, but additions of stems at 3% (by volume of crushed grapes, which was equivalent to approximately half of the stems of the batch) increased tannins by 60% (Casassa et al. 2019b). These and our results suggest that tannin extraction from stems in Pinot noir, either from WC or DS addition, seems to be more or less proportional to the amount of WC or stems added. Moreover, DS additions led to equivalent increases in wine tannins as 100% WC but without the additional effect on other chemical characteristics associated with the use of whole clusters (e.g., acetic acid production).

Reflecting seasonal vintage-related variations, total phenols were generally higher in 2016 than in 2017 (Figure 1) and followed a trend similar to that observed for wine tannins,

with a higher phenol content observed with all of the treatments than with C wines (Figure 1E and 1F). At the end of the study, 100% WC and DS wines had equivalent phenolic content in both vintages.

Polymeric pigments encompass a heterogeneous group of polymeric phenolics, including primarily covalent adducts between anthocyanins and tannins as well as other phenolic and non-phenolic materials. Polymeric pigment formation is relevant because these compounds provide stable color and positive mouthfeel modification (Casassa and Harbertson 2014). Polymeric pigment formation progressed faster initially in the 2016 wines, peaking after nine months of bottle aging and decreasing thereafter. After two years of bottle aging, polymeric pigment content increased by 15% and 18% in the 100% WC and DS wines, respectively, relative to C wines. Although polymeric pigment formation progressed more gradually in the 2017 wines, it increased steadily from pressing to 15 months of bottle aging. Unlike 2016, the winemaking treatments did not have an effect on polymeric pigment formation throughout winemaking and bottle aging in 2017. In a previous report on Pinot noir, a 20% WC addition did not affect polymeric pigment formation; however, addition of 3% stems by weight to the fermentors did affect polymeric pigment formation and was attributed to enhanced tannin extraction (60%) achieved by stem addition (Casassa et al. 2019b). Another study of Primitivo wines found that the proportion of pigments not bleachable by SO₂ was higher in wines made with additions of 25% and 50% WC than in fully destemmed control wine (Suriano et al. 2015). However, WC and DS additions had a relatively minor effect on polymeric pigment formation in our study, reaffirming that polymeric pigment formation is intrinsically regulated by anthocyanin content and type, tannin to anthocyanin ratio, and the predominance of skin- or seed-derived tannins, as well as by tannin content (Teng et al. 2019). For example, the comparatively simpler anthocyanin composition of Pinot noir, that is, lack of acylated anthocyanins, coupled with an intrinsically low concentration of anthocyanins, may cause a lower rate of polymeric pigment formation even in the presence of sufficient tannin. Under our experimental conditions, stem-derived tannins did not significantly favor the formation of polymeric pigment.

Wine color (AU 420+520+620 nm) was followed throughout winemaking (Figure 1I and 1J), whereas selected CIE L*a*b* parameters (Table 2) and full absorbance spectrum scans (Figure 2) were determined after three and 15 months of bottle aging. Additionally, Supplemental Tables 2 and 3 show the CIE L*a*b* Color Difference (ΔE^*) calculated between each pair of wines after three and 15 months of bottle aging. Wine color and most chromatic parameters were generally higher in 2016, reflecting a cooler vintage, but there were relatively few major effects of the winemaking techniques on most wine chromatic parameters. In 2016, wine color in 100% WC wines decreased by 43% from pressing to two years of bottle aging, but these wines still showed slightly higher color than the wines from the other treatments (Figure 1I). CIE L*a*b* measurements indicated that saturation was initially higher in 100% WC wines but decreased through

aging. Likewise, DS wines were only higher in hue angle and yellow components (positive values of b^*) after 15 months of bottle aging (Table 2). The full absorption spectrum scans confirmed higher absorbances throughout the visible range in the 100% WC wines after three and 15 months of bottle

aging (Figure 2). However, these color differences in favor of 100% WC wines were only discernible by the human eye ($\Delta E^* > 5$) after three months of bottle aging and only when C wines were contrasted with 100% WC wines. No further color differences were discernible by the human eye between

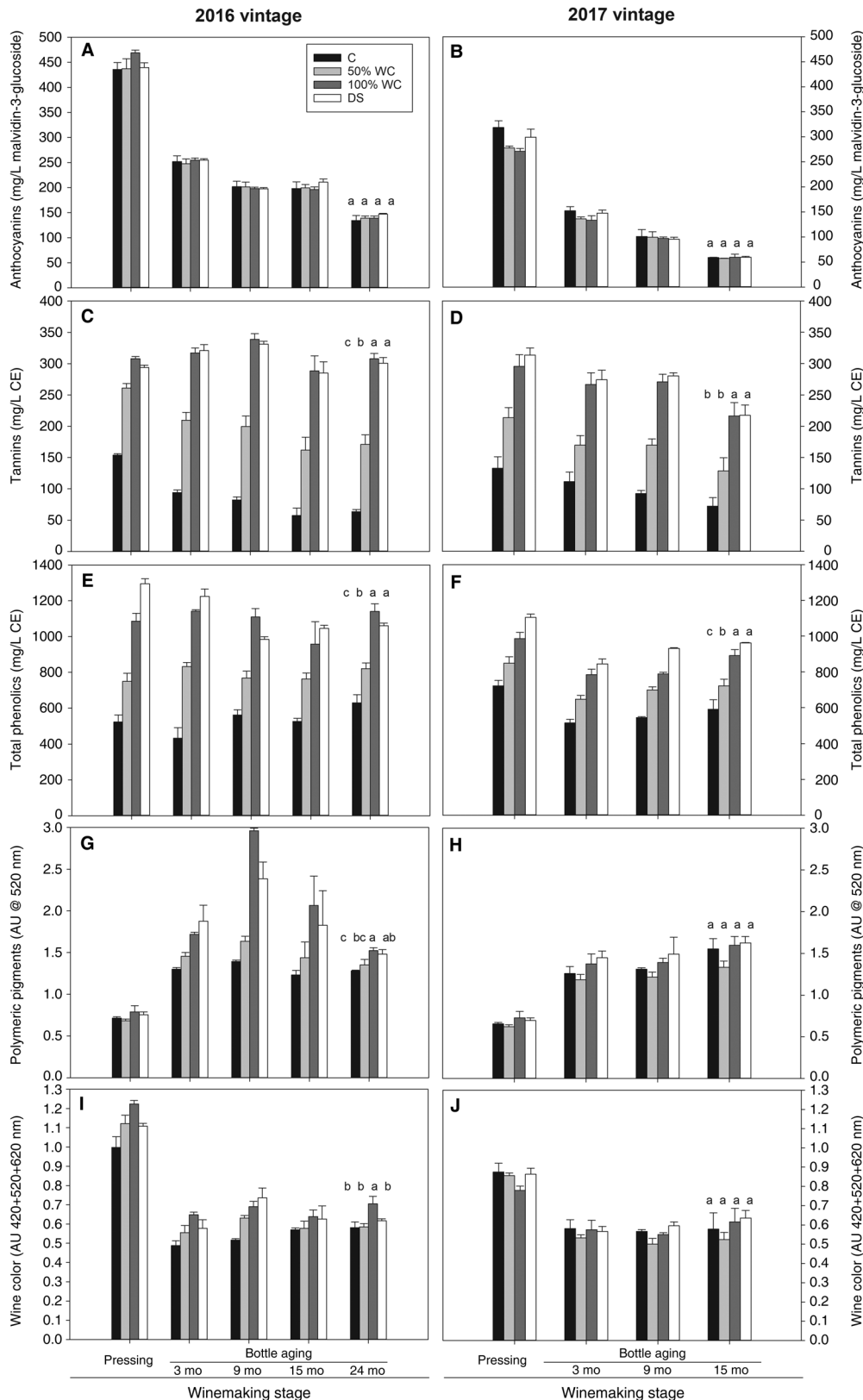


Figure 1 Evolution of phenolic compounds and wine color during winemaking and aging of Pinot noir wines over two consecutive vintages. **A** and **B**: anthocyanins; **C** and **D**: tannins; **E** and **F**: total phenolics; **G** and **H**: polymeric pigments; **I** and **J**: wine color. Different letters at the last sampling point indicate significant differences for Fisher's least significant difference test and $p < 0.05$. AU: absorbance units; CE: catechin equivalents; C: control; WC: whole cluster; DS: dried stems.

any other pair of wines after 15 months of bottle aging in 2016 (Supplemental Table 2). In 2017, color drop was only 30% throughout aging, and no differences between treatments were found (Figure 1J). Although the discrete absorbances included in the determination of wine color failed to capture an effect of the winemaking treatments on wine color, CIE $L^*a^*b^*$ measurements showed some discrimination among treatments, particularly after 15 months of bottle aging (Table 2). For example, 100% WC and DS wines had an increase in hue (more evolved or brownish color), a decrease in a^* (less red color), and an increase in b^* (more yellow color) relative to C wines. Full spectrum absorption scans clearly showed more color in the 2017 C wines after three months of bottle aging, although these differences subsided after 15 months of bottle aging (Figure 2). These early differences were confirmed by ΔE^* values of 5.30 and 5.46 when comparing C wines with 50% WC and 100% WC wines, respectively, after three months of bottle aging, indicating more perceivable color in C wines. However, as with full spectrum scans, the ΔE^* values in all cases were lower than 5 after 15 months of bottle aging, indicating no discernible differences in color between any pair of wines (Supplemental Table 3).

The addition of stems from WC has been shown to decrease anthocyanins attributed to the adsorption capacity of stems towards monomeric anthocyanins (Suriano et al. 2015). More generally, addition of extra solids to the fermentor, such as the added stems in the present study, results in lower color saturation and decrease in absorbance of the visible absorbance spectrum of wine color (Casassa et al. 2019a). However,

the major contributors to wine color, i.e., anthocyanins and polymeric pigments, were not significantly affected by the winemaking techniques herein applied. Yet in 2016, the 100% WC wines showed slightly higher color, whereas in 2017, C wines showed slightly higher color and better chromatic composition than the other wines from that year. Therefore, the inconsistency of WC and DS additions in achieving positive effects on wine color can be attributed to other phenolic and non-phenolic materials, which may vary in composition and content on a vintage by vintage basis.

GC-MS analysis. The wines of the 2016 and 2017 vintages were analyzed by GC-MS after two and one year of bottle aging, respectively. A total of 56 volatile compounds were identified by GC-MS, including alcohols, aldehydes, thiols, esters, organic acids, terpenes and terpenoids, volatile phenols, lactones, and oak aromatics, and were subjected to one-way ANOVA (Supplemental Tables 7 and 8). The GC-MS data set was separated by vintage and further analyzed by a combination of heatmap analysis and hierarchical cluster analysis (Figure 3). Heatmap analysis allows for vertical visualization of the full aroma profile of each wine, with red and orange hues indicating predominance, yellow hues indicating presence, and blue and light green hues indicating absence or very low presence. On the horizontal axis, the relative presence of the 56 aroma compounds can be visualized for each set of wines of the 2016 (Figure 3A) and 2017 vintages (Figure 3B).

The wines of the 2016 vintage were higher in alcohols and acids, in particular 1-butanol, 1-octen-3-ol, 3-octanol, 1-pentanol, benzyl alcohol, and caprylic, valeric, and caproic

Table 2 Detailed chromatic composition determined by tri-stimulus colorimetry (CieLab system) of Pinot noir wines at selected times during the aging over two consecutive vintages (2016 and 2017). A three-way analysis of variance (ANOVA) with the separate effects of vintage, maceration, aging time, and selected interactions is also presented. Values represent the mean of three tank replicates.

Vintage/ maceration	L* (lightness)		C* (saturation)		H* (hue angle)		a*		b*	
	3 mo BA ^a	15 mo BA	3 mo BA	15 mo BA	3 mo BA	15 mo BA	3 mo BA	15 mo BA	3 mo BA	15 mo BA
2016										
C ^b	84.8 a ^c	82.2 a	18.4 b	16.4 a	2.2 ab	0.7 b	18.5 b	16.5 a	-0.7 c	-0.1 b
50% WC	83.1 a	82.7 a	18.5 b	17.5 a	0.9 b	1.3 b	18.5 b	17.5 a	0.1 b	0.4 b
100% WC	80.3 b	81.4 a	21.6 a	18.1 a	1.3 b	6.1 a	21.6 a	17.9 a	-0.5 bc	1.9 a
DS	82.8 a	81.9 a	18.1 b	15.9 a	4.1 a	8.1 a	18.1 b	15.7 a	1.3 a	2.2 a
<i>p</i> value	0.016	0.874	0.023	0.226	0.067	0.002	0.019	0.187	0.002	0.005
2017										
C ^b	81.9 b	83.4 a	18.7 a	14.5 ab	2.3 b	11.5 c	18.6 a	14.2 a	0.7 b	2.8 b
50% WC	84.9 a	85.2 a	14.5 b	12.6 b	7.8 ab	17.2 b	14.4 b	12.0 bc	1.9 ab	3.7 b
100% WC	82.8 ab	83.3 a	14.1 b	12.9 ab	12.2 a	23.7 a	13.7 b	11.8 c	2.9 a	5.2 a
DS	84.0 ab	85.5 a	15.2 b	14.9 a	11.1 a	21.8 a	14.9 b	13.9 ab	2.9 a	5.6 a
<i>p</i> value	0.070	0.681	0.009	0.072	0.029	0.000	0.007	0.047	0.038	0.001
ANOVA effects (<i>p</i> values)										
Vintage	0.047		<0.0001		<0.0001		<0.0001		<0.0001	
Maceration	0.087		0.089		<0.0001		0.029		<0.0001	
Aging time	0.636		<0.0001		<0.0001		<0.0001		<0.0001	
Vintage × Maceration	0.176		<0.0001		0.017		<0.0001		0.247	
Aging time × Maceration	0.654		0.306		0.349		0.301		0.153	

^amo: months; BA: bottle aging.

^bC: Control; WC: whole cluster; DS: dried stems.

^cDifferent letters within wines of the same vintage indicate significant differences for Fisher's least significant difference test and $p < 0.05$. Significant *p* values are shown in bold.

acid. In 2016, the dendrogram resulting from cluster analysis grouped closely the 50% WC wines with the 100% WC wines and the C wines with the DS wines. Generally, 100% WC wines showed higher predominance of alcohols such as 1-butanol, 1-octen-3-ol, 3-octanol, and 1-hexanol, as well as ethyl lactate, ethyl cinnamate, ethyl caprate, and phenethyl acetate. 1-octen-3-ol, also known as mushroom alcohol, has a strong mushroom aroma, and it is usually considered a negative, off-odor wine aroma (Boutou and Chatonnet 2007) associated also with cork taint and/or the perception of oxidation (Escudero et al. 2000). The same is true for 1-butanol and 3-octanol, which are higher alcohols bearing a strong spirituous aroma and a strong mushroom-like aroma, respectively, and were both previously detected in Pinot noir wines (Brander et al. 1980). The 100% WC wines also showed a relative abundance of ethyl cinnamate, which was almost nine times more abundant than in C wines (Supplemental Table 7). Ethyl cinnamate has been identified as a key odorant in Burgundy Pinot noir wines, where it is responsible for cinnamon-like, fruity, and plum- and cherry-like aromas (Moio and Etievant 1995). All of these compounds were particularly associated with 100% WC wines. The 50% WC wines showed predominance of *cis*-lactone, β -damascenone, linalool, and 1-octanol and were also characterized by the esters butyl ethyl suc-

cinatate and diethyl succinate, the latter of which bears fruity, melon-like notes (Pérez-Coello et al. 2003). C wines of the 2016 vintage showed low presence of most compounds except for 1-pentanol, which did not show significant differences between treatments, and methionol, which was different between treatments (Supplemental Table 7). Conversely, DS wines were characterized by high abundance of benzyl alcohol and γ -nonalactone and showed an abundance of esters, namely ethyl 9-hexadecenoate and 9-octadecanoate, ethyl stearate, ethyl myristate, ethyl palmitate, ethyl pentadecanoate, and linolenic and linoleic acid ethyl esters. The ester content in DS wines bears potential positive effects, including fruity and floral notes. Ethyl 9-hexadecenoate has been previously identified in Cabernet Sauvignon wines (Liang et al. 2013) and is related to the perception of fruity aromas, whereas γ -nonalactone imparts a coconut-like, waxy, and buttery aroma. The predominance of benzyl alcohol in DS wines, on the other hand, suggests that this alcohol can be eventually oxidized to benzaldehyde, imparting a bitter almond aroma (Delfini et al. 1991).

The 2017 wines were generally higher in esters, including ethyl esters of fatty acids, isoamyl acetate, and ethyl phenylacetate, and nor-isoprenoids such as β -damascenone and trimethyl-1,2-dihydronaphthalene (TDN) (Supplemental

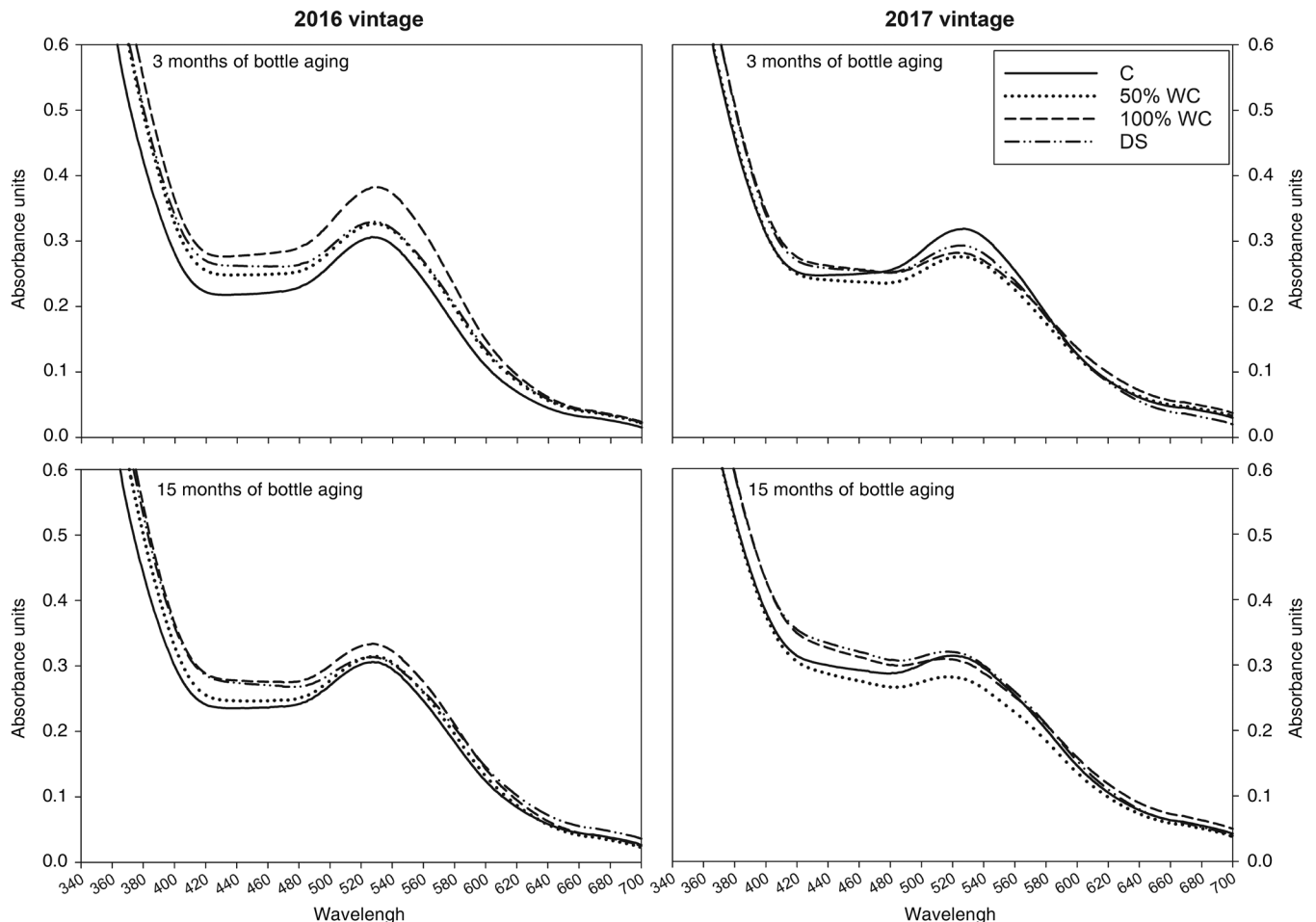


Figure 2 Full visible absorption spectrum scans of Pinot noir wines after three and 15 months of bottle aging over two consecutive vintages (2016 and 2017). C: control; WC: whole cluster; DS: dried stems.

Table 8). These compositional differences between vintages are expected because the 2016 wines had 28 months of bottle aging at the time of this analysis, whereas the 2017 wines had 16 months. Indeed, esters and certain nor-isoprenoids such as β -damascenone degrade over time during wine aging mostly due to the effect of accumulated temperature (Bordiga et al. 2013) and the progressive loss of free SO₂ during bottle aging (Garde-Cerdán and Ancín-Azpilicueta 2007). Moreover, the acid hydrolysis of acetate esters during aging, which yields their corresponding acids and higher alcohols, has been linked with the rapid loss of varietal character in Sauvignon blanc wines (Herbst-Johnstone et al. 2011). Similarly, we observed a decrease in esters and a concomitant increase in alcohols and acids in the more aged 2016 wines.

Cluster analysis also grouped the 2017 wines as a function of winemaking technique. C and 50% WC wines were

grouped together, with a subgroup of these grouped with DS wines, and 100% WC grouped in a separate cluster (Figure 3B). C wines were characterized by relatively high predominance of β -damascenone, 1-octen-3-ol, 1-butanol, 1-octanol, ethyl heptadecanoate, and acetoin. The presence of esters in C wines suggest fruity and floral notes in these wines. Similarly, C wines had the highest concentration of β -damascenone, which also bears fruity and floral notes (Supplemental Table 8). This nor-isoprenoid was recently identified as a key odorant with high odor activity value in Pinot noir wines (Casassa et al. 2019b), in agreement with the results presented here. The aroma profile of 50% WC wines was generally similar to that of C wines.

The 100% WC wines, which formed a separate cluster in 2017, showed higher predominance of benzaldehyde, 2,3-butanediol, 1-butanol, glycerol, acetic acid, TDN, ethyl caprate,

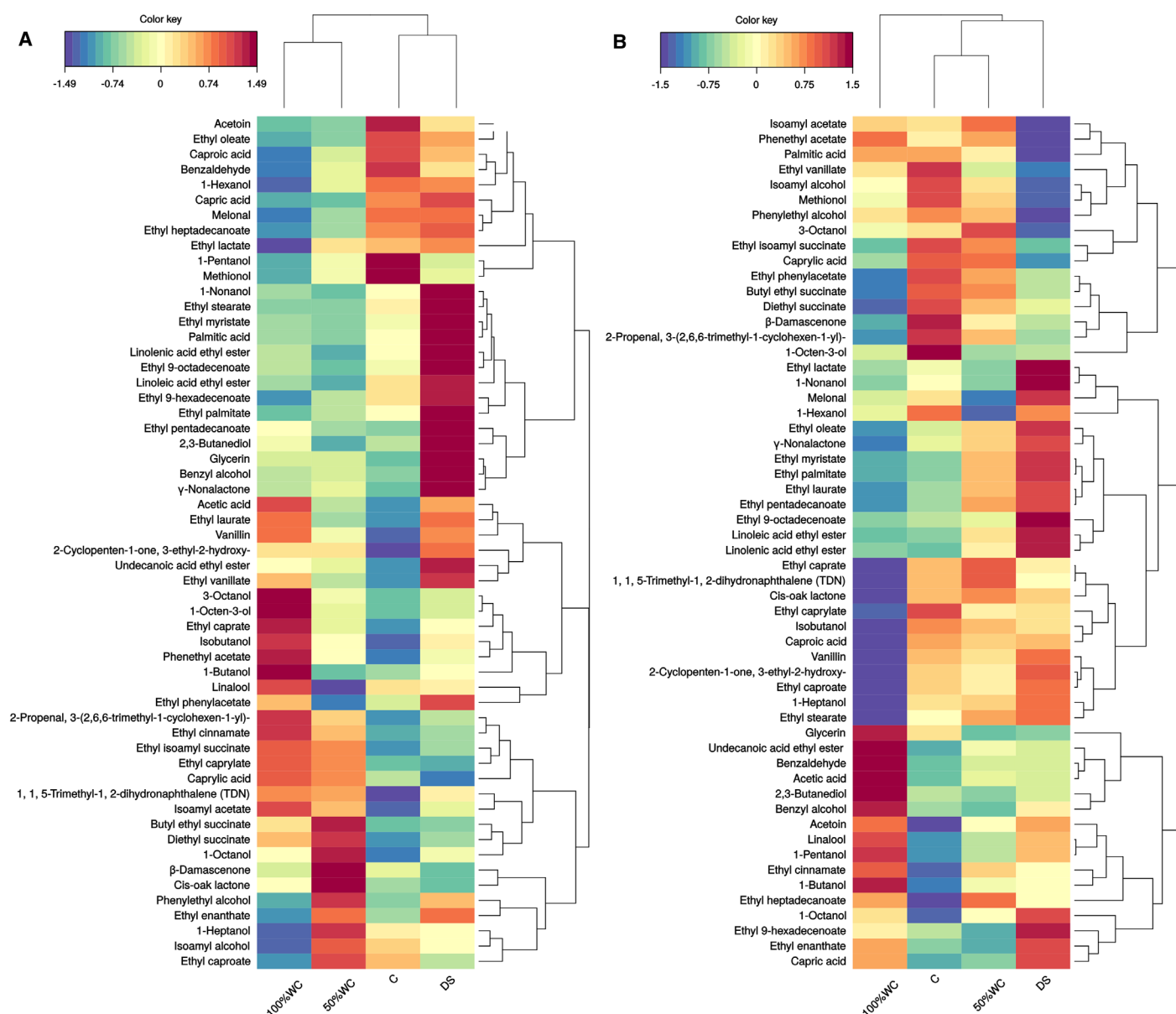


Figure 3 Heat map combined with hierarchical cluster analysis of standardized gas chromatography-mass spectrometry data of Pinot noir wines of the different winemaking treatments of the 2016 (A) and 2017 vintage (B). C: control; WC: whole cluster; DS: dried stems.

ethyl cinnamate, and caproic acid, than the other wines. Benzaldehyde is often considered an important aroma compound in Pinot noir wines. A study reported extract dilution and flavor dilution values above 64 for benzaldehyde in Pinot noir wines, which were described as nutty and cherry-like (Fang and Qian 2005). Benzaldehyde has also been linked with infection with *Botrytis cinerea* and other fungal diseases but can also be formed by action of the microorganisms on the aromatic amino acid phenylalanine (Genovese et al. 2007). Thus, the predominance of benzaldehyde in 100% WC wines may be related to the incidence of powdery mildew in 2017. It is also possible that the addition of WC may have led to oxidative conditions during the prefermentative phase that contributed to the formation of this volatile compound. DS wines from the 2017 vintage showed more similarities with C and 50% WC wines than with 100% WC wines, with a particular predominance of 1-nonanol and the ethyl esters of fatty acids including ethyl 9-octadecenoate, ethyl-9-hexadecenoate, ethyl oleate, ethyl myristate, ethyl palmitate, and ethyl pentadecanoate, as well as ethyl lactate and the ethyl esters of

linoleic and linolenic acids. Ethyl esters of fatty acids bearing an even number of carbon atoms, such as ethyl hexanoate, ethyl octanoate, or ethyl decanoate, are considered important contributors to the aroma of young wines and bear floral and fruity odors (van der Merwe and van Wyk 1981), whereas 1-nonanol, which also appeared in the DS wines of the 2016 vintage, imparts a citrus, citronella-like aroma.

Sensory descriptive analysis. The wines of the 2016 and 2017 vintages were evaluated by two trained sensory panels after approximately three months of bottle aging, with sensory descriptors and their respective standards established by consensus (Supplemental Tables 5 and 6). Results were analyzed by a combination of univariate statistical analysis, including three-way ANOVA (Tables 3 and 4) and PCA with confidence ellipses (Figure 4). ANOVA results indicated that in 2016, addition of WC at 50% and 100% and DS increased brown hue and herbal aroma, whereas a more prominent effect of oak-derived aromas, less brown hue, and less cooked vegetal aromas were observed in C wines (Table 3). Relative to C wines, 100% WC wines had enhanced vegetal aroma

Table 3 Three-way analysis of variance (ANOVA) with interaction showing mean separation and *p* values of descriptive sensory attributes of Pinot noir wines from the 2016 vintage assessed by a trained panel (*n* = 9). Main effects and interactions between selected ANOVA factors are also presented.

Treatment	Brown hue	Red hue	Oak aroma	Herbal aroma	Vegetal aroma	Cooked vegetal aroma	Red berry aroma	Astringency
C ^a	3.3 b ^b	8.9 a	8.0 a	6.8 b	5.1 b	4.2 c	6.2 b	5.4 b
50% WC	4.2 a	8.0 ab	6.6 b	8.0 a	5.6 ab	5.6 ab	5.9 b	6.5 ab
100% WC	4.7 a	8.6 a	6.2 b	8.1 a	6.5 a	6.5 a	6.3 ab	6.6 a
DS	5.1 a	7.4 b	6.9 ab	8.7 a	5.6 ab	5.1 bc	7.4 a	5.9 ab
ANOVA factors and interactions (<i>p</i> values)								
Wine (W)	0.005^c	0.008	0.025	0.006	0.126	0.015	0.094	0.119
Panelist (P)	<0.0001	<0.0001	<0.0001	0.003	<0.0001	0.001	0.089	0.001
Replicate	0.919	0.407	0.807	0.275	0.209	0.013	0.539	0.531
P × W interaction	0.306	0.974	0.153	0.253	0.085	0.436	0.474	0.566

^aC: Control; WC: whole cluster; DS: dried stems.

^bANOVA to compare data: different letters within a column indicate significant differences for Fisher's least significant difference at *p* < 0.05.

^cSignificant *p* values are shown in bold fonts.

Table 4 Three-way analysis of variance (ANOVA) with interaction showing mean separation and *p* values of descriptive sensory attributes of Pinot noir wines from the 2017 vintage assessed by a trained panel (*n* = 10). Main effects and interactions between selected ANOVA factors are presented.

Treatment	Saturation	Brown hue	Red hue	Purple hue	Red fruit aroma	Dark fruit aroma	Dried fruit aroma	Vegetal aroma	Clove aroma	Astringency
C ^a	12.6 a ^b	0.3 b	12.6 a	2.9 a	8.3 ab	7.0 b	4.0 b	2.5 b	1.0 b	6.4 c
50% WC	11.8 b	0.3 b	12.1 b	2.1 bc	7.7 b	7.2 ab	4.2 b	2.5 b	1.9 a	7.5 b
100% WC	11.9 b	0.8 a	11.2 c	1.8 c	8.6 a	7.8 a	4.1 b	3.1 a	1.8 a	8.0 ab
DS	12.7 a	0.8 a	11.5 c	2.2 b	8.5 ab	6.7 b	5.4 a	3.2 a	1.9 a	8.3 a
ANOVA factors and interactions (<i>p</i> values)										
Wine (W)	<0.0001^c	<0.0001	<0.0001	<0.0001	0.113	0.024	0.001	0.020	<0.0001	<0.0001
Panelist (P)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Replicate	0.132	0.218	0.028	0.237	0.998	0.041	0.041	0.706	0.229	0.012
P × W interaction	0.007	0.013	0.005	0.082	0.072	0.110	0.103	0.405	0.302	0.338

^aC: Control; WC: whole cluster; DS: dried stems.

^bANOVA to compare data: different letters within a column indicate significant differences for Fisher's least significant difference at *p* < 0.05.

^cSignificant *p* values are shown in bold fonts.

(reminiscent of grape tendrils, Supplemental Table 5) and astringency, whereas DS wines had less red hue and enhanced berry aromas. No panelist \times wine interactions were observed, indicating that the sensory panelists were consistent in their evaluation of the wines.

Figure 4 shows a PCA with confidence ellipses. The PCA biplot and confidence ellipses were constructed with 95% certainty according to the Hotelling's test, which provides significance testing. The ellipses represent empirical descriptions of the variability of the sensory evaluations (Husson et al. 2005), and superimposition of the ellipses indicated that the wines were not significantly different from a sensory standpoint. In 2016, the ellipses corresponding to 50% WC wines overlapped with those of 100% WC wines, suggesting sensory similarities between these two treatments. Conversely, the relative position on the PCA plot as well as the lack of overlap between the ellipses of the DS wines and the ellipses of the remaining treatments suggests that DS wines were the most distinctive from a sensory viewpoint (Figure 4A and 4B). The PCA solution, which accounted for 94% of the variability, confirmed the results of the ANOVA. The 50% WC and 100% WC wines were higher in vegetal aromas, cooked vegetal aromas, and astringency; DS wines were higher in brown hue, red berry aroma, and herbal aroma; and C wines demonstrated an effect of oak-derived aromatics in their sensory profile (unlike the wines from the other treatments). The wines were aged in neutral barrels because this experiment

was conducted at a commercial winery and treatment replicates needed to be kept separated throughout winemaking. Although we intended to avoid adding oak-derived flavors to the wines, C wines showed a more prominent impact of oak-derived aromatics, which could be due to the relatively lower intrinsic complexity of aromas in these wines. Alternatively, antagonistic effects between oak-derived volatiles, such as oak lactones and furanic compounds, and fruity aromas (Prida and Chatonnet 2010), may explain the prevalence of oak-derived aromatics in the aromatic profile of said wines.

In 2017, red and purple hues were decreased and clove aroma and astringency were increased in wines with DS or WC additions (50% and 100%) relative to C wines. Specifically, 50% WC and 100% WC wines were lower in color saturation, 100% WC and DS wines were higher in vegetal aroma (reminiscent to red and green bell pepper, Supplemental Table 6), and DS wines were higher in dried fruit aroma (Table 4). Significant panelist \times wine interactions were also observed for some of the sensory descriptors pertaining to color. PCA of the 2017 sensory data explained ~85% of the variability and generally confirmed the ANOVA results (Figure 4C and 4D). No overlap between any of the winemaking treatments was observed. Moreover, confidence ellipses for C, 50% WC, and 100% WC wines were considerably narrower, suggesting lower variability in the sensory profile of the replicates within the treatments (Figure 4C). C wines were differentiated on the basis of more purple and red hues as well as color

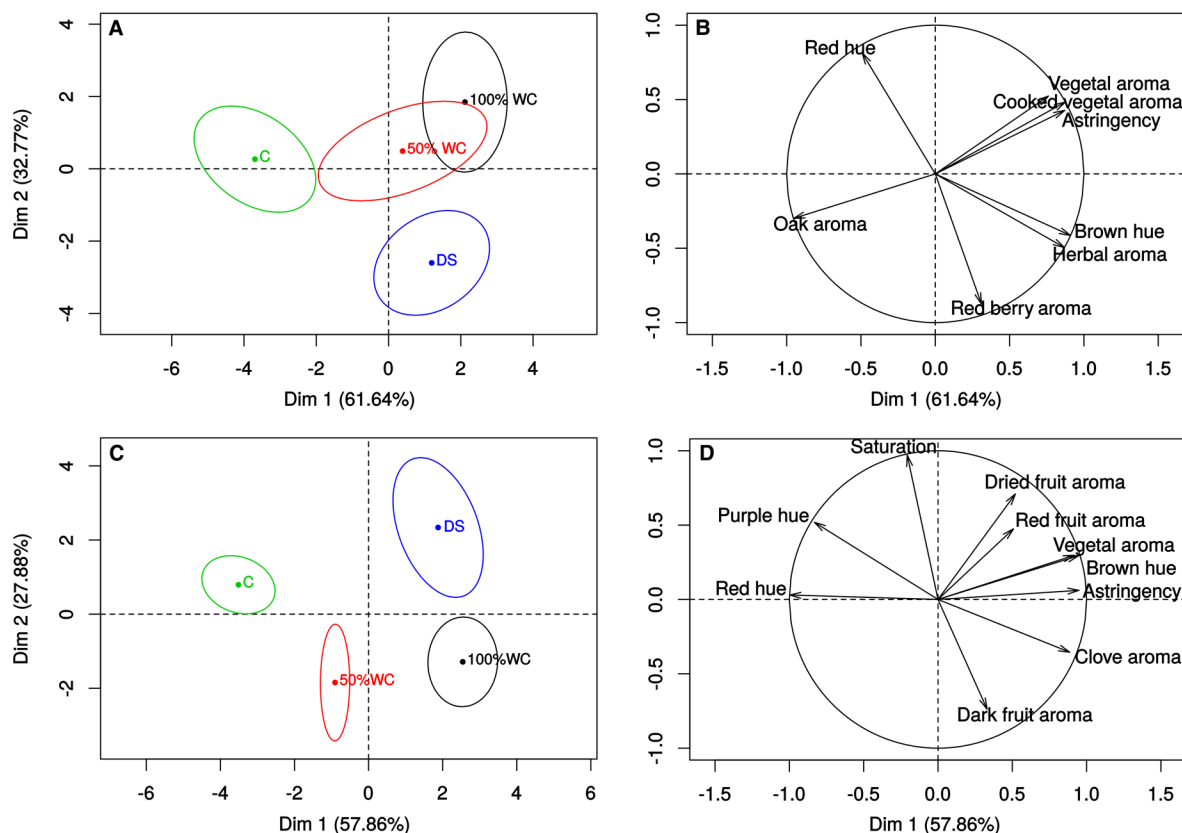


Figure 4 Principal component analysis of descriptive sensory data of Pinot noir wines from the 2016 (A and B) and 2017 vintages (C and D) evaluated by a trained sensory panel (2016, $n = 9$; 2017, $n = 10$). Confidence ellipses indicate 95% confidence intervals. C: control; WC: whole cluster; DS: dried stems.

saturation, and placed, along with the 50% WC wines, in the negative dimension of the PCA plot. DS and 100% WC wines were located in the positive dimension of the PCA plot, indicating shared similarities in their respective sensory profile but fairly different sensory profiles than the C and 50% WC wines. The 100% WC wines were confirmed to be higher in astringency and clove and dark fruit aroma, whereas the DS wines were higher in dried fruit, red fruit, and vegetal aroma and brown hue, with higher relative astringency than 100% WC wines.

Expectedly, the most salient sensory features of each treatment were generally consistent over the two vintages, as the fruit, vineyard source, and winemaking techniques applied here were kept constant over the two years. For example, the sensory panel consistently indicated an increase in perceived astringency in the 100% WC and DS wines, and although this increase was not proportional to the increase in tannins caused by WC and DS addition (Figure 1C and 1D), astringency was one of the most discriminant sensory attributes. Previous research has shown that stem-only or WC additions lead to a significant increase in perceived astringency in Cabernet Sauvignon wines (Pascual et al. 2016) and Primitivo wines (Suriano et al. 2016), but a 20% WC addition in Pinot noir wines did not affect astringency (Casassa et al. 2019b). Herein, we showed that either additions of DS or 50% or 100% WC led to significant increases in astringency. Therefore, the addition of WC or DS could be used a tool to improve texture in an otherwise light-bodied wine such as Pinot noir.

The most distinctive sensory features were achieved by the DS treatment. These wines were more aromatic and showed enhanced or lifted red berry, herbal, red fruit, and dried fruit aromas, albeit at the expense of wine color. This sensory outcome is consistent with the volatile composition of these wines discussed above; i.e., DS wines showed a relatively higher abundance of esters, whereas C wines were more affected by oak-derived aromas (particularly in 2016) and retained more color (particularly in 2017) (Figure 3). Studies reporting on the sensory effects of stem additions under actual winemaking conditions are scarce, but some chemical reports exist. For example, addition of stems increased the content of methoxypyrazines in Cabernet Sauvignon (Hashizume and Samuta 1997), and treatment of the stems with steam prior to fermentation decreased pyrazines by 95% while increasing the levels of extractable flavonoids in Cabernet Sauvignon, Merlot, Pinot noir, and Muscat Bailey wines (Hashizume et al. 1998). In the present study, addition of WC, but not DS, led to increased vegetal and cooked notes, suggesting that the process of drying the stems prior to their addition decreased undesirable sensory notes while allowing for the development of more pleasant ones. Alternatively, enhanced fruity notes in the DS wines may have effectively masked vegetal notes (Hein et al. 2009). Therefore, the practice of drying the stems prior to addition seems preferable from a chemical (lower VA) and sensory standpoint.

By contrast, addition of WC at 100% increased spicy (clove) and cooked vegetal notes, which may be related to the higher levels of ethyl cinnamate and benzaldehyde in these

wines (Figure 3). Although these sensory notes may not be fully desirable as standalone sensory features, they may be useful as blending options. Therefore, use of WC and DS additions appear to be effective tools to increase the aromatic and textural complexity of Pinot noir wines.

Conclusions

The present experiment was conducted at industrial scale in a commercial winery in the Central Coast of California, providing winemakers with a practical baseline for comparison when implementing WC and stem additions to Pinot noir wines. Furthermore, this study spanned a relatively cool (2016) and a relatively warm growing season (2017), which allowed us to characterize potential effects of vintage on the practice of WC addition.

Additions of 100% WC consistently led to significant increases in pH and VA. The former is a negative outcome in warm climates in which lower acidity and high pH may lower free SO₂ levels, thereby affecting the aging potential and bottle shelf life of the resulting wines. We speculated that VA was caused by the development of acetic acid bacteria within air pockets in WC fermentations. Therefore, the diligent use of inert gases such as carbon dioxide should be instituted if these techniques are implemented. Alternatively, partial crushing of a portion of the fruit as opposed to 100% WC allows alcoholic fermentation to start, thereby allowing the released CO₂ to displace oxygen and potentially minimize VA.

Anthocyanins, polymeric pigments, and color were influenced more strongly by vintage than by the winemaking treatments, with either null or negative results in 100% WC additions. Conversely, the tannin content of the wines was clearly affected by the winemaking techniques. Overall, tannin increases were generally proportional to the percentage of WC and stems added. Concurrently, increases in perceived astringency were also noted by the trained panels during sensory evaluation, suggesting that WC and DS addition can be used to add mouthfeel and improve texture to an otherwise light-bodied wine such as Pinot noir.

Notwithstanding the fact that the 2016 and 2017 wines were at different aging stages at the time of volatile analysis, some common aroma compounds were present in the wines of the different treatments. For example, 100% WC wines were higher in ethyl cinnamate and benzaldehyde, suggesting the presence of spicy and almond-like notes. DS wines showed relative abundance of esters, denoting potential fruity and floral notes.

Sensory results showed good alignment with the phenolic and volatile composition of the wines. For example, 100% WC addition led to vegetal, cooked fruit flavors and spicy (clove) notes, whereas DS additions produced wines that were herbal, fruity, and astringent, with fruity notes related to esters and astringency sensations related to enhanced tannin extraction from the stems. An empirical observation often quoted by winemakers is that Pinot noir wines produced with WC and/or stem addition are often perceived as “fresher.” Analysis of the composition of these wines suggested absence of a clear chemical effect explaining enhanced perception of freshness

due to stem addition (e.g., higher acidity), yet these wines were indeed perceived as “fresher.” Thus, we hypothesize that herbal and fruitier notes in wines made with DS additions may enhance perceived freshness through cross-modal association processes. This hypothesis also supports the rationale followed by some Pinot noir producers, who typically increase the proportion of WC added in especially warm or hot vintages to add “freshness” to the resulting wines. While these specific sensory features can be considered “markers” of WC or stem additions, they can also be desirable as blending options, thereby adding complexity and aromatic lift to other wines. Overall, chemical and sensory results concur that 100% WC and DS wines are generally more complex and have a more diverse aromatic palette than 50% WC and C wines.

From a practical standpoint, the results herein presented argue in favor of drying stems prior to their addition to the fermentor if logistics permit. Regular spraying of drying stems with a solution of SO₂ is recommended to combat possible mold development during the drying process. Tannin extractability and wine mouthfeel do not appear to be different between DS and 100% WC, with the added benefits that the DS treatment may result in lower production of acetic acid and facilitates cap management.

Literature Cited

- Bordiga M, Rinaldi M, Locatelli G, Travaglia F, Coisson JD and Arlorio M. 2013. Characterization of Muscat wines aroma evolution using comprehensive gas chromatography followed by a post-analytic approach to 2D contour plots comparison. *Food Chem* 140:57-67.
- Boutou S and Chatonnet P. 2007. Rapid headspace solid-phase microextraction/gas chromatographic/mass spectrometric assay for the quantitative determination of some of the main odorants causing off-flavours in wine. *J Chromatogr A* 1141:1-9.
- Brander CF, Kepner RE and Webb AD. 1980. Identification of some volatile compounds of wine of *Vitis vinifera* cultivar Pinot noir. *Am J Enol Vitic* 31:69-75.
- Brossard N, Cai H, Osorio F, Bordeu E and Chen J. 2016. “Oral” tribiological study on the astringency sensation of red wines. *J Texture Stud* 47:392-402.
- California Department of Food and Agriculture. 2019. California Grape Crush Final Report 2018. California Department of Food and Agriculture, Sacramento, CA.
- Casassa LF and Harbertson JF. 2014. Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. *Annu Rev Food Sci Technol* 5:83-109.
- Casassa LF, Bolcato EA and Sari SE. 2015. Chemical, chromatic, and sensory attributes of 6 red wines produced with prefermentative cold soak. *Food Chem* 174:110-118.
- Casassa LF, Huff R and Steele NB. 2019a. Chemical consequences of extended maceration and post-fermentation additions of grape pomace in Pinot noir and Zinfandel wines from the Central Coast of California (USA). *Food Chem* 300:125-147.
- Casassa LF, Sari SE, Bolcato EA, Diaz-Sambueza MA, Catania AA, Fanzone ML, Raco F and Barda N. 2019b. Chemical and sensory effects of cold soak, whole cluster fermentation, and stem additions in Pinot noir wines. *Am J Enol Vitic* 70:19-33.
- Cheyrier V and Sarni-Manchado P. 2010. 2 - Wine taste and mouthfeel. In *Managing Wine Quality*. Reynolds AG (ed.), pp. 29-72. Woodhead Publishing, Oxford, UK.
- Cuellar SS, Karnowsky D and Acosta F. 2009. The sideways effect: A test for changes in the demand for Merlot and Pinot noir wines. *J Wine Econ* 4:219-232.
- Delfini C, Gaia P, Bardi L, Mariscalco G, Contiero M and Pagliara A. 1991. Production of benzaldehyde, benzyl alcohol and benzoic acid by yeasts and *Botrytis cinerea* isolated from grape musts and wines. *Vitis* 30:253-263.
- Downey MO, Dokoozlian NK and Krstic MP. 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. *Am J Enol Vitic* 57:257-268.
- Escudero A, Cacho J and Ferreira V. 2000. Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography–olfactometric study. *Eur Food Res Technol* 211:105-110.
- Fang Y and Qian MC. 2005. Aroma compounds in Oregon Pinot noir wine determined by aroma extract dilution analysis (AEDA). *Flavour Frag J* 20:22-29.
- Garde-Cerdán T and Ancín-Azpilicueta C. 2007. Effect of SO₂ on the formation and evolution of volatile compounds in wines. *Food Control* 18:1501-1506.
- Gawel R, Francis L and Waters EJ. 2007. Statistical correlations between the in-mouth textural characteristics and the chemical composition of Shiraz wines. *J Agric Food Chem* 55:2683-2687.
- Genovese A, Gambuti A, Piombino P, Moio L. 2007. Sensory properties and aroma compounds of sweet Fiano wine. *Food Chem* 103:1228-1236.
- Harbertson JF, Picciotto EA and Adams DO. 2003. Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. *Am J Enol Vitic* 54:301-306.
- Harbertson JF, Hodgins RE, Thurston LN, Schaffer LJ, Reid MS, Landon JL, Ross CF and Adams DO. 2008. Variability of tannin concentration in red wines. *Am J Enol Vitic* 59:210-214.
- Hashizume K and Samuta T. 1997. Green odorants of grape cluster stem and their ability to cause a wine stemmy flavor. *J Agric Food Chem* 45:1333-1337.
- Hashizume K, Kida S and Samuta T. 1998. Effect of steam treatment of grape cluster stems on the methoxypyrazine, phenolic, acid, and mineral content of red wines fermented with stems. *J Agric Food Chem* 46:4382-4386.
- Hein K, Ebeler SE and Heymann H. 2009. Perception of fruitiness and vegetative aromas in red wine. *J Sens Stud* 24:441-455.
- Herbst-Johnstone M, Nicolau L and Kilmartin PA. 2011. Stability of varietal thiols in commercial Sauvignon blanc wines. *Am J Enol Vitic* 62:495-502.
- Hopfer H, Ebeler SE and Heymann H. 2012. How blending affects the sensory and chemical properties of red wine. *Am J Enol Vitic* 63: 313-324.
- Husson F, Lê S and Pagès J. 2005. Confidence ellipse for the sensory profiles obtained by principal component analysis. *Food Qual Prefer* 16:245-250.
- Landon JL, Weller K, Harbertson JF and Ross CF. 2008. Chemical and sensory evaluation of astringency in Washington State red wines. *Am J Enol Vitic* 59:153-158.
- Lawless HT and Heymann H. 2010. *Sensory Evaluation of Food – Principles and Practices*. Springer, New York.
- Liang HY, Chen JY, Reeves M and Han BZ. 2013. Aromatic and sensorial profiles of young Cabernet Sauvignon wines fermented by different Chinese autochthonous *Saccharomyces cerevisiae* strains. *Food Res Int* 51:855-865.
- Mawdsley PFW, Dodson Peterson JC and Casassa LF. 2018. Agronomical and chemical effects of the timing of cluster thinning on Pinot noir (clone 115) grapes and wines. *Fermentation* 4:60.

- Mawdsley PFW, Dodson Peterson JC and Casassa LF. 2019. Multi-year study of the effects of cluster thinning on vine performance, fruit and wine composition of Pinot noir (clone 115) in California's Edna Valley AVA (USA). *Sci Hortic* 256:108631.
- Moio L and Etievant PX. 1995. Ethyl anthranilate, ethyl cinnamate, 2,3-dihydrocinnamate, and methyl anthranilate: Four important odorants identified in Pinot noir wines of Burgundy. *Am J Enol Vitic* 46:392-398.
- Pascual O, González-Royo E, Gil M, Gómez-Alonso S, García-Romero E, Canals JM, Hermosín-Gutiérrez I and Zamora F. 2016. Influence of grape seeds and stems on wine composition and astringency. *J Agric Food Chem* 64:6555-6566.
- Pérez-Coello MS, González-Viñas MA, García-Romero E, Díaz-Maroto MC and Cabezudo MD. 2003. Influence of storage temperature on the volatile compounds of young white wines. *Food Control* 14:301-306.
- Pérez-Magariño S and González-Sanjosé ML. 2003. Application of absorbance values used in wineries for estimating CIELAB parameters in red wines. *Food Chem* 81: 301-306.
- Pickering GJ, Simunkova K and DiBattista D. 2004. Intensity of taste and astringency sensations elicited by red wines is associated with sensitivity to PROP (6-n-propylthiouracil). *Food Qual Prefer* 15:147-154.
- Pino JA. 2007. Characterization of rum using solid-phase microextraction with gas chromatography–mass spectrometry. *Food Chem* 104:421-428.
- Prida A and Chatonnet P. 2010. Impact of oak-derived compounds on the olfactory perception of barrel-aged wines. *Am J Enol Vitic* 61:408-413.
- Robinson J and Harding J. 2015. *The Oxford Companion to Wine*. Oxford University Press.
- Sun B, de Sá M, Leandro C, Caldeira I, Duarte FL, and Spranger I. 2013. Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *J Agric Food Chem* 61:939-946.
- Suriano S, Alba V, Tarricone L and Di Gennaro D. 2015. Maceration with stems contact fermentation: Effect on proanthocyanidins compounds and color in Primitivo red wines. *Food Chem* 177:382-389.
- Suriano S, Alba V, Di Gennaro D, Basile T, Tamborra M and Tarricone L. 2016. Major phenolic and volatile compounds and their influence on sensorial aspects in stem-contact fermentation winemaking of Primitivo red wines. *J Food Sci Technol* 53:3329-3339.
- Teng B, Hayasaka Y, Smith PA and Bindon KA. 2019. Effect of grape seed and skin tannin molecular mass and composition on the rate of reaction with anthocyanin and subsequent formation of polymeric pigments in the presence of acetaldehyde. *J Agric Food Chem* 67:8938-8949.
- Tesniere C and Flanzky C. 2011. Chapter 1 – Carbonic maceration wines: Characteristics and winemaking process. *In* *Advances in Food and Nutrition Research*. Ronald SJ (ed.), pp. 1-15. Academic Press.
- Tomic O, Luciano G, Nilsen A, Hyldig G, Lorensen K and Næs T. 2010. Analysing sensory panel performance in a proficiency test using the PanelCheck software. *Eur Food Res Technol* 230:497-511.
- Valentin D, Parr WV, Peyron D, Grose C and Ballester J. 2016. Colour as a driver of Pinot noir wine quality judgments: An investigation involving French and New Zealand wine professionals. *Food Qual Prefer* 48:251-261.
- van der Merwe CA and van Wyk CJ. 1981. The contribution of some fermentation products to the odor of dry white wines. *Am J Enol Vitic* 32:41-46.
- Vidal L, Giménez A, Medina K, Boido E and Ares G. 2015. How do consumers describe wine astringency? *Food Res Int* 78:321-326.
- Watrelet AA, Heymann H and Waterhouse AL. 2020. Red wine dryness perception related to physicochemistry. *J Agric Food Chem* 68:2964-2972.