AJEV | American Journal of Enology and Viticulture

Research Report
DOI: 10.5344/ajev.2022.22012

Freeze-Killed Leaf Material Causes Atypical Aromas and Astringency in Cabernet Sauvignon

Scott C. Frost,[2,1] Danielle J. Fox,[2] Markus Keller,[3] Thomas S. Collins,[2] and James F. Harbertson[2*]

Abstract

Background and goals

Washington State Cabernet Sauvignon wines made from fruit harvested after an autumn freeze have been shown to present potpourri, floral, and rose-like aromas. These aromas are described as atypical by Washington State winemakers and the affected wines are considered "rose-tainted" or "frost-tainted." Anecdotal evidence suggests that the inclusion of freeze-killed leaf material (FKLM) in the fermentation is the source of the taint.

Methods and key findings

Freeze-killed leaves were hand-collected from Cabernet Sauvignon vines in the Horse Heaven Hills, then crushed and added to Cabernet Sauvignon must at four addition rates: 0.0, 0.5, 2.0, and 8.0 g/kg. The Cabernet Sauvignon fruit was not exposed to freezing temperatures prior to harvest. Gas chromatography-mass spectrometry identified 60 volatile and semi-volatile organic compounds that correlated with FKLM addition. Additionally, the phenolic chemistry showed reduced concentrations of anthocyanin, tannin, and iron-reactive phenolics. Descriptive sensory analysis found that adding FKLM significantly increased the intensity of floral aroma, herbaceous/straw aroma, artificial fruit aroma, and floral aftertaste, but also decreased the intensity of dark fruit aroma and astringent mouthfeel.

Conclusions and significance

We estimate that approximately three freeze-killed leaves per vine will produce taint characteristics. These results clearly show the impact of freeze-killed grapevine leaves on Cabernet Sauvignon wine quality and provide convincing evidence of the taint's source.

Key words: Cabernet Sauvignon, frost taint, GC-MS, rose taint, sensory, wine taints

Introduction

The red table wine harvest often extends far into the fall season. During the late season, it is not uncommon for the vineyard to experience isolated overnight freezing temperatures. Although often brief, the freezing temperatures can kill grapevine leaves but leave the fruit unaltered. Anecdotal reports from winegrowers find that fruit harvested after a freeze event can produce wines with increased floral, rose, and potpourri-like aromas, and they characterize the affected wines as having "rose taint" or "frost taint." These anecdotal reports also suggest the inclusion of freeze-killed grapevine leaves into the wine ferment as the taint source.

In the arid winegrowing regions of the western United States, grapevine leaves killed during low temperatures are left desiccated, brittle, and easily broken into much smaller pieces. Here, we refer to the small, fractured leaves and petioles as freeze-killed leaf material (FKLM). FKLM is considered MOG or "material other than grapes." MOG, as the name implies, is all-inclusive and is comprised of grape rachis, petioles, leaves, and/or any other extraneous material not intended for the fermentation vessel. MOG is commonly associated with machine harvesting and ranges from 0 to 5% (w/w) in the harvested fruit. MOG composition can vary: one estimate finds that fresh leaf material comprises ~17 to 85% (Petrucci and Siegfried 1976, Huang et al. 1988, Parenti et al. 2015).

The addition of MOG to experimental fermentations has been shown to impact wine flavor and aroma. In Australian Shiraz, when compared to a berry fermentation, the inclusion of fresh grape leaves (1% w/w) produced wines with increased confectionary aromas, fruity flavors, and astringency; additionally, the inclusion of rachis (2.6% w/w) and peduncles (1.5% w/w) increased the "green" aroma and flavor (Capone et al. 2021). In Cabernet Sauvignon, petiole additions >5% by weight produced wine with stronger floral aromas than a control wine (Ward et al. 2015). A study using Sangiovese found the addition of 3% (w/w) of rachis altered wine color and astringency, which was attributed to increased flavonoid content

---

[1]Department of Biology, Tufts University, Medford MA, 02155; [2]Viticulture and Enology Program, Washington State University, Richland WA, 99354; [3]Viticulture and Enology Program, Washington State University, Prosser WA, 99350.

*Corresponding author (jfharbertson@wsu.edu; tel: 509-372-7506)

Manuscript submitted Feb 2022, accepted Sept 2022

(Guerrini et al. 2018). Lastly, the aroma chemistry of "green stemmy" was found to be driven by 2-methoxy-3-isopropoylpyrizine and 2-methoxy-3-isobutylpyrizine (Hashizume and Samuta 1997).

The literature reports few studies evaluating the effect of FKLM on wine quality. Increases in multiple monoterpenes and norisoprenoids were found with linear additions of frozen leaf blades and petioles to Cabernet franc (Lan et al. 2022a). Although the study showed strong evidence of the impact of frozen MOG on Cabernet franc, this experiment used fruit that experienced freezing temperatures, which may confound the MOG effect. In a second study, the authors report chemical changes across two vintages for Cabernet Sauvignon and Cabernet franc harvested before and after freezing temperatures (Lan et. al 2022b). For each cultivar, only four compounds varied consistently across the two vintages. In Cabernet Sauvignon, ethyl isobutyrate, ethyl heptanoate, ethyl decanoate, and citronellol concentrations changed consistently, while in Cabernet franc, ethyl hexanoate, ethyl octanoate, isoamyl acetate, and nerol changed. Of these compounds, only nerol and citronellol are likely not fermentation-derived. Although inconsistent across vintage and cultivar, these studies show that frozen MOG does impact the chemical composition of Cabernet franc and Cabernet Sauvignon wines (Lan et al. 2022a, 2022b).

Because frozen MOG can modify wine chemistry, it is reasonable to assume that FKLM can also modify wine aroma and flavor. Wine producers desire an understanding of how FKLM impacts wine aroma and flavor. This manuscript describes a replicated experiment using 220-L fermenters and known additions of FKLM. The flavor profile of the finished wines was assessed using descriptive analysis and the chemical profile was assessed using untargeted gas chromatography-mass spectrometry (GC-MS). The experiment demonstrates the effect of FKLM on Cabernet Sauvignon wine quality and the identification of potential frost taint marker compounds.

Materials and Methods

Wine production

Freeze-killed Cabernet Sauvignon leaves were hand-collected from vines in the Austin Sharp Vineyard in Alderdale, WA on 21 Oct 2019 (Supplemental Figure 1). The frozen leaves were transported back to the Ste. Michelle Wine Estates Washington State University Wine Science Center and stored at 15°C in plastic yard bags. Seven days later, on 28 Oct 2019, *Vitis vinifera* L. cv. Cabernet Sauvignon (clone 08) grapes were harvested from Cold Creek vineyard (Columbia Valley American Viticultural Area, Sunnyside, WA). Fruit (1877.9 kg) was machine-harvested into half-ton bins from vines planted in 2009 with north-south row orientation and 2.4 × 1.5 m vine spacing. Upon delivery to the Washington State University Winery in Richland, WA, the fruit was destemmed, then crushed into jacketed fermentors using an Armbruster Rotovib Destemmer-Crusher 10 with a single roller sorter (Armbruster Kelterei-Technologie). A total of 12 stainless steel-jacketed fermentors (Spokane Industries) were filled with 140 L of must. The average soluble solids across all 12 tanks was 28.6 Brix. All tanks were then adjusted to 25.0 Brix by watering-back with a 5g/L tartaric acid solution. Fifty mg/L sulfur dioxide (in the form of $K_2S_2O_5$) was added to each tank postcrush.

The 12 fermentors were split randomly into four lots of three tanks. The previously-harvested, freeze-killed Cabernet Sauvignon leaf matter (FKLM) was added to the four lots at a rate of 0.0, 0.5, 2.0, or 8.0 g/kg must. The addition rate was based on each full fermentor containing 136 kg of must. The leaves were hand-crushed, punched down, and incorporated into the must. Lalvin EC-1118 was rehydrated (0.3 g/L) with GO-Ferm (0.3 g/L) and added to all 12 tanks for inoculation. Diammonium phosphate (0.25 g/L) and Fermaid K (0.25 g/L) were added on day 1, postcrush, to reach a yeast assimilable nitrogen of 225 mg/L. Malolactic bacteria Lalvin VP41 (0.01g/L) was added to all 12 tanks 48 hrs after initial yeast inoculation. All fermentation support products were purchased through Scott Laboratories, Petaluma, CA.

Each tank was fitted with a variable capacity, stainless steel tank lid (Spokane Industries) and Cypress Integrated Fermentation Controller System 3.2 (IFCS) computer for temperature control, temperature monitoring, and reoccurring pump-overs (Cypress Semiconductor). The tanks were set to a maximum fermentation temperature of 30°C. Pump-overs were performed every four hrs for five min, six times daily, using a DDP 550 5 Chamber Diaphragm pump (Aquatec). Fermentation progress and temperature were monitored daily using a DMA 35N handheld densitometer (Anton-Paar), and by the IFCS computer. An Admeo Y15 Autoanalyzer (Admeo, Inc.) used enzymatic analysis to measure residual sugar of wines sampled at the end of fermentation.

A 10-day maceration period was applied to all ferments. Wines were pressed off the skins using a custom-built hydraulic tank press (Cypress Semiconductor) and transferred to sanitized 60-L stainless steel kegs. Kegs were stored at ambient temperature and topped with carbon dioxide (CO_2) daily until completion of the malolactic fermentation (MLF), which was monitored using an Admeo Y15 autoanalyzer to perform malic acid and lactic acid enzymatic assays. MLF was complete when <0.1 g/L malic acid was measured. Upon completion, wines were racked into 50-L stainless steel kegs and 60 mg/L sulfur dioxide (in the form of $K_2S_2O_5$) was added. Kegs were then stored in a temperature-controlled 12.8°C (±2°C) room to settle out for 71 days. An XpressFill XF4100 four-spout bottling machine (XpressFill Systems LLC) bottled wines at ambient temperature into 750 mL green glass bottles (M.A. Silva). Bottles were sparged with nitrogen gas (N_2) and left with 15 mL of headspace. A Technovin TVLV semiautomatic capper machine encapsulated bottles with Saranex liner 30 × 60 screwcaps (Scott Caps). Bottles were placed in cardboard cases (12 bottles/case) and moved to a 12.8°C (±2°C) room for storage. Approximately five cases were bottled per treatment. Final wine chemical parameters for each FKLM dose level are displayed in Table 1.

Chemical analysis

Total soluble solids (Brix), pH, and titratable acidity (TA) were measured on the harvested fruit and wine as described (Iland 2004). Wine ethanol concentration was measured with a near-infrared spectrophotometer (Anton Paar USA, Inc.). Tannins (mg/L catechin equivalents; CE), total iron-reactive phenolics (mg/L CE), anthocyanins (mg/L malvidin-3-glucoside equivalents; m3g eq) and total polymeric pigments (A_{520nm}) were measured following established methods (Harbertson et al. 2003, 2015).

Sensory analysis

The effect of FKLM on the flavor and aroma profile of the experimental wines was evaluated using descriptive analysis (Heymann and Lawless 2013). A panel of 13 judges (eight males, five female; aged 21 to 35) was recruited based on each member's interests and availability. All panelists had experience describing the aroma and flavor profile of Cabernet Sauvignon wine; additionally, 10 panelists had served on prior descriptive analysis panels. Panel training occurred over a two-week period, during which each member attended four sessions. Using black ISO glasses, six wines were evaluated at each training session; thus, after four sessions, each panelist had trained using each of the 12 experimental wines at least twice. Session one focused on attribute generation, reference standards for each attribute (Table 2) were presented and refined in session two, attributes and reference standards were finalized in session three, and panel alignment was evaluated in session four (data not shown). During formal data collection, each panelist evaluated all 12 experimental wines in triplicate over five evaluation sessions. During each session, two flights of four wines were presented and each wine was evaluated individually for all 20 attributes using a 15-cm unstructured lines scale. Service order was determined using a randomized block

Table 1 Attribute and reference standard preparation for descriptive analysis of Cabernet Sauvignon wines made with different dosages of freeze-killed leaf material.

Attribute	Description	Reference standard preparation
Red fruit	Bright fruity, slightly floral aromas of raspberry jam, cherry jam, cranberry juice	0.5 Tbsp Bonne Maman Raspberry Preserves + 0.5 Tbsp Bonne Maman Cherry Preserves + 25 mL base red wine[a]
Dark fruit	Dark fruity, slight woody and herbal aromatics associated with blackberry jam, blueberry jam, cassis, plum	0.25 Tbsp O Organic Blackberry Preserves + 0.25 Tbsp Bonne Maman Blackcurrant jam + 15 mL crème de cassis + 10 mL base wine
Dried fruit	Aromas associated with dried raisins, prunes, and figs	1 Tbsp Sun-Maid Raisins, chopped + 1 Sunsweet Prunes, chopped + 25 mL base red
Stone fruit	The perfuming, somewhat cooked aromas associated with apricot jam and peach jam	0.25 Tbsp Bonne Maman Apricot Jam + 0.25 Tbsp Smuckers Peach Preserve
Artificial fruit	Lightly fruity aroma of cherry and grape Jolly Rancher candy	One cherry Jolly Rancher + one grape Jolly Rancher + 25 mL base wine
Tropical fruit	Fruity, pungent aroma of pineapple and passion fruit	10 mL Dole Pineapple Juice + 10 mL Welch's Passion Fruit
Citrus	Aromas associated with orange juice and orange peels	One fresh orange wedge, juiced with peel
Floral	Perfuming and floral aromas associated with roses	0.5 mL Rose water in base wine
Herbaceous/straw	Pungent and penetrating aromas of dried dill, sage, and dried grass	1/8 tsp dried dill + 1/8 tsp dried sage + 1/8 tsp dried grass + 20 mL base wine
Baking spices	Spicy, slightly woody, and pungent, aromas of cinnamon, clove, and allspice	3 whole cloves + 1/8 tsp cinnamon + 25 mL base red wine
Fresh green veg	Sharp, vegetative aromas of bell pepper, parsley, and grass	1 inch x 1 inch slice green bell pepper + 25 mL base red wine
Black pepper	Spicy, pungent, musty, aromas of black pepper	1/8 tsp whole black peppercorns + 25 mL base red wine
Sulfurous	Strongly pungent, piercing aromas of diced onion and dried onion	1 Tbsp diced onion + 0.25 Tbsp dried powdered onion + 25 mL base wine
Cooked veg	Pervasive aromas associated with cooked cabbage and canned asparagus	1 Tbsp liquid from Jolly Green Giant canned asparagus + 1 Tbsp cooked cabbage + 20 mL base wine
Oxidized	Aromas described as sherry, bruised apple, and expired fruit	25 mL Tío Pepe Fino Sherry
Sweet	Overall sweet intensity	1000 mg/L sugar solution
Sour	Overall sour intensity	400 mg/L tartartic acid
Bitter	Overall bitter intensity	200 mg/L caffeine solution
Astringent mouthfeel	Overall astringent intensity	200 mg/L alum solution
Hot mouthfeel	Pungent, chemical aroma of ethanol	15 mL vodka (Burnett's)

[a] Franzia chillable red was used as the base wine.

design to control for possible carryover effect. Additionally, a one-min rest between each wine and a five-min rest between flights was imposed. All evaluations were conducted using individual, temperature-controlled, tasting booths lit with red light, and each wine was presented in a black ISO glass filled with 25 mL of wine and labeled with a three-digit blinding code. Panelists were provided with unsalted crackers and distilled water to rinse their pallet between samples. Data was collected using Compusense Software.

Headspace solid-phase microextraction (HS SPME) GC-MS

The volatile profile of each wine was analyzed using an Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass selective detector (Agilent Technologies). The GC-MS was equipped with an MPS Robotic autosampler (Gerstel), a DB-WAX capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness [Agilent Technologies]). The method parameters and sampling protocols were adapted from Hjelmeland et al. (2013). Ten mL of wine was pipetted into a 20 mL round-bottom glass vial (Restek) with 3 g of NaCl. The vial was sealed with a screwcap and PTFE lined septum (Restek) and then placed on the sample tray. The sampling protocol began with the MPS Robotic autosampler spiking 2-undecanone, the internal standard, to an in-vial concentration of 50 ug/L. The vial was then warmed to 40°C with agitation at 500 rpm for five min; then a 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco), 23-gauge SPME fiber was inserted into the vial headspace. Agitation was slowed to 250 rpm and the fiber was exposed for 30 min. The SPME fiber was then desorbed at a 20:1 split with the inlet set to 240°C. The SPME fiber was retained in the inlet for three min. The GC oven temperature was programmed to remain at 40°C for five min, then increase at 3°C/min up to 180°C, followed by a ramp of 30°C/min to the final temperature of 240°C, which was held for 10 min. The helium carrier gas was set to a constant flow of 0.9 mL/min, such that the internal standard (2-undecanone) eluted at 30.00 min. A solvent delay was set for the initial 2.5 min and the mass spectrometry detector (MSD) was turned off from 3.40 to 3.80 min. The MSD was set to an electron energy of -70 eV, a source of 230°C, and quadrupole temperature of 150°C. Data was acquired in scan mode ranging from 40 m/z to 300 m/z. Triplicate samples of each wine were prepared and analyzed in randomized order for 36 total injections: three replicate injections, per fermentation replicate, per dose treatment.

Data processing

Deconvolution was completed using Agilent Qualitative Analysis software version 8.0. The following list of known contaminant m/z ions were excluded: 28, 44, 73, 147, 207, and 281. Deconvolution settings were as follows: RT window size factor: 75.00; spectrum peak threshold: 0.00%; SNR threshold: 1.50; extraction window: left m/z delta: 0.3% and right m/z delta: 0.3%. Use base peak shape was selected with Sharpness threshold: 50.00%. Peaks with area less than 500 counts were removed. All peak areas were standardized against the average (n = 36) area of the internal standard, such that after standardization, each internal standard peak area was equivalent across all 36 analyses. Lastly, a probable compound was included for a given treatment dose if it was found in seven of the nine replicates. Compound identity was determined by comparison of the mass spectral fragmentation patterns against the NIST 14 database and published retention index values. Compound identification was confirmed using authentic reference material: β-damascenone (Chem-Impex International), 6-methyl-3,5-heptadien-2-one, 1,1,6,-trimethyl-1,2-dihydronapthalene (Santa Cruz Biotechnology Inc.), 6-methyl-5-hepten-2-ol, ethyl isobutyrate, ethyl butyrate, ethyl isovalerate, ethyl-3-hexenoate, isobutanol, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl heptanoate, 1-hexanol, ethyl octanoate, 2-methyl-1-benzofuran, phenethyl acetate, isoamyl alcohol, phenethyl alcohol (Sigma Aldrich), propyl acetate, citronellol, ionone (Tokyo Chemical Industry Company). Unknown compounds were given a unique identifier.

Data analysis

Statistical analyses were performed using R version 4.1.0 “Camp Pontanezen ” (Team R 2021a) and the R Studio IDE version 1.4.1717 (Team R 2021b). Graphics were prepared using the ggplot2 package (Wickham 2016). A significance of α = 0.05 was set for all analyses. Descriptive analysis data was analyzed using fixed effects analysis of variance (ANOVA). Main effects of judge, dose, sensory replicate, and fermentation replicate nested within dose and all two- and three-way interactions were evaluated via the F-statistic. When the main effect of dose and the dose × judge interaction were both significant, the F-statistic for dose was recalculated using the interaction mean square. ANOVA tables were calculated using the Anova() function from the car package (Fox and Weisberg 2019). Post-hoc comparison of dose means ($p < 0.05$) were made using Tukey’s honest significant difference (HSD) and calculated using the agriocolae package (Mendiburu 2021). Canonical variate analysis (CVA) was

Table 2 Basic wine chemistry measures for Cabernet Sauvignon wines made with different dosages of freeze-killed leaf material (g/kg of must). TA, titratable acidity; VA, volatile acidity; RS, residual sugar.

Dose (g/kg)	pH	TA (g/L)	Malic (g/L)	Lactic (g/L)	VA (g/L)	RS (g/L)	Ethanol % (v/v)
0	3.7	6.23	0.04	1.13	0.14	0.13	14.01
0.5	3.6	7.19	0.04	1.11	0.13	0.13	13.77
2	3.6	6.95	0.05	1.02	0.14	0.13	14.11
8	3.6	7.07	0.04	1.02	0.16	0.13	14.21

applied to the raw scores of the significant sensory descriptors to show dose discrimination at the multivariate level. 95% confidence ellipses around mean points were also constructed (Owen and Chmielewski 1985). Correlation analysis was completed by calculating Pearson's correlation coefficient (r) between mean attribute by fermentation and the mean chemical measures by fermentation. The cor.test() function within base R was used with a two-side null alternative.

Results

Sensory analysis

Descriptive analysis found 10 attributes that differed significantly among the four dose levels (calculated mean square error presented in Supplemental Table 1). Figure 1 displays the mean intensity for each significant attribute at each dose level. Means separation was evaluated using Tukey's HSD and is displayed as letters above each bar. The addition of FKLM, at all dose levels, increased the intensity above the 0.0 g/kg dose for *artificial fruit*, *floral*, *floral aftertaste*, *herbaceous/straw*, *stone fruit*, and *tropical fruit*. While these six attributes did increase, only *floral*, *floral aftertaste*, and *herbaceous/straw* showed a significant effect at 2.0 g/kg freeze-killed leaf matter. Finally, no difference was found when comparing the 0.0 g/kg to the 0.5 g/kg, as indicated in Figure 1 by the bars sharing the same letter. In contrast to attributes which showed increased intensity, four attributes decreased with the addition of FKLM: *astringent mouthfeel*, *black pepper*, *dark fruit*, and *sulfurous*. Within each of the four attributes, no difference was shown between the 0.0 g/kg and 0.5 g/kg FKLM dose treatments. *Astringent mouthfeel* showed reduced intensity at 2.0 g/kg FKLM as compared to 0.0 g/kg FKLM, but *black pepper*, *dark fruit*, and *sulfurous* were only altered significantly at 8.0 g/kg FKLM.

CVA was applied to the four treatments using the 10 significant sensory attributes. The CVA results are displayed in Figure 2, allowing for the relationship between the sensory attributes and the four dose treatments to be visually evaluated. The first two canonicals described 98.7% of the treatment variation, with the first canonical accounting for 92.9%. Additionally, the four FKLM dose levels discriminate along the first canonical, moving from 0.0 g/L and 0.5 g/L, through 2.0 g/L, and ending at 8.0 g/L FKLM. Wines produced with two low dose levels (0.0 and 0.5 g/L FKLM) were described by increased intensities of *dark fruit*, *black pepper*, *astringency*, and *sulfurous aroma*, while wines produced with an addition of 8.0 g/L FKLM were described by *floral aftertaste*, *floral*, *herbaceous/straw*, *tropical fruit*, *stone fruit*, and *artificial fruit*. Wines fermented with the addition of 2.0 g/L FKLM were positioned near the biplot origin, and thus were not explicitly described by any one attribute or group of attributes.

Phenolics

Measures of tannins (mg/L CE), total iron reactive phenolics (IRP) (mg/L CE), anthocyanins (mg/L m3g), and total polymeric pigments (A_{520nm}) are found in Figure 3. Within each facet of the figure, four bars representing the mean concentration of each dose treatment are displayed (means by fermentation are displayed in supplemental material (Supplemental Table 2). Tannin (mg/L CE) and total IRPs (mg/L CE) both decreased with the addition of FKLM, but anthocyanins and total polymeric pigments showed an effect of treatment that did not correlate with the addition levels. Tannin concentration significantly decreased at 2.0 g/kg FKLM, but no significant difference was measured between 2.0 g/kg and 8.0 g/kg FKLM. Thus, tannin concentration grouped into "high" and "low," with the high group comprised of 0.0 and 0.5 g/kg FKLM, and the low group

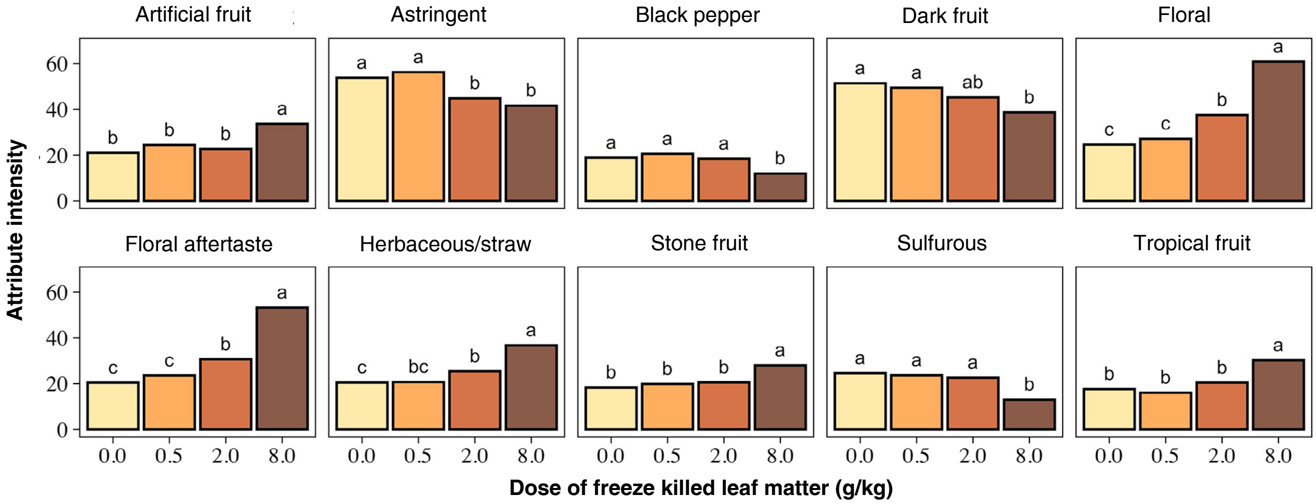

Figure 1 Mean attribute intensity for each significant attribute showing an effect for freeze-killed leaf material dosage (0, 0.5, 2.0, 8.0 g/kg must) on Cabernet Sauvignon wines. Within each attribute, treatment additions are not significantly different by Tukey's honest significant difference test if they are labeled with the same letter.

comprised of 2.0 and 8.0 g/kg FKLM. Total IRP measures decreased with treatment and correlated strongly with the FKLM dose ($\rho = -0.78$, df = 34, $p < 0.001$).

Volatile analysis

HS SPME GC-MS returned 126 tentative compounds across the 36 injections. From the 126, 60 unique compounds were carried forward. Forty-six were identified and 14 were assigned a numeric label. Table 3 displays the retention time, calculated retention index, mean peak area, fragmentation ions, and a compound name or identifier for all 60 relevant compounds. ANOVA found 45 compounds that showed a significant change in concentration among the dose levels, and 31 of the 45 compounds increased in concentration with increased FKLM addition. Mean peak areas and Tukey's HSD results are displayed in Table 3.

Correlation analysis

Correlation analysis was used to explore the relationship between the chemistry and sensory measures of the experimental wines. Additionally, hierarchal clustering was applied by row and by column to explore the similarities among the sensory and chemical measures. The dendrogram positioned above the columns describes the relationship among the sensory attributes, and the dendrogram to the right describes the relationship among the chemical measures. After organizing the rows and columns via the clustering relationship, a "correlation heat map" is created, and relationships among the sensory and chemical measures can be visually assessed.

The sensory dendrogram, shown at the top of the correlation heat map, clusters the 10 sensory attributes into two groups that split the heat map down the middle (Figure 4). The first cluster is comprised of six attributes: *tropical fruit aroma*, *herbaceous/straw aroma*, *floral aroma*, *floral aftertaste*, *stone fruit aroma*, and *artificial fruit aroma*. The second cluster is comprised of four attributes: *dark fruit aroma*, *astringent mouthfeel*, *black pepper aroma*, and *sulfurous aroma*. The delineation between the two sensory clusters is clearly captured for each chemical measure. For example, total phenolics correlates negatively with all attributes in cluster 1 and positively with all attributes in cluster 2. p-Menth-1-en-9-al shows a similar but opposite relationship, correlating positively with cluster 1 attributes and negatively with cluster 2 attributes. For most of the measured chemistry, there was a similar diametric correlation between cluster 1 and cluster 2.

The chemistry dendrogram, shown to the right of the correlation heat map, clusters the 64 chemical measures into two groups (Figure 4). The hierarchical clus-

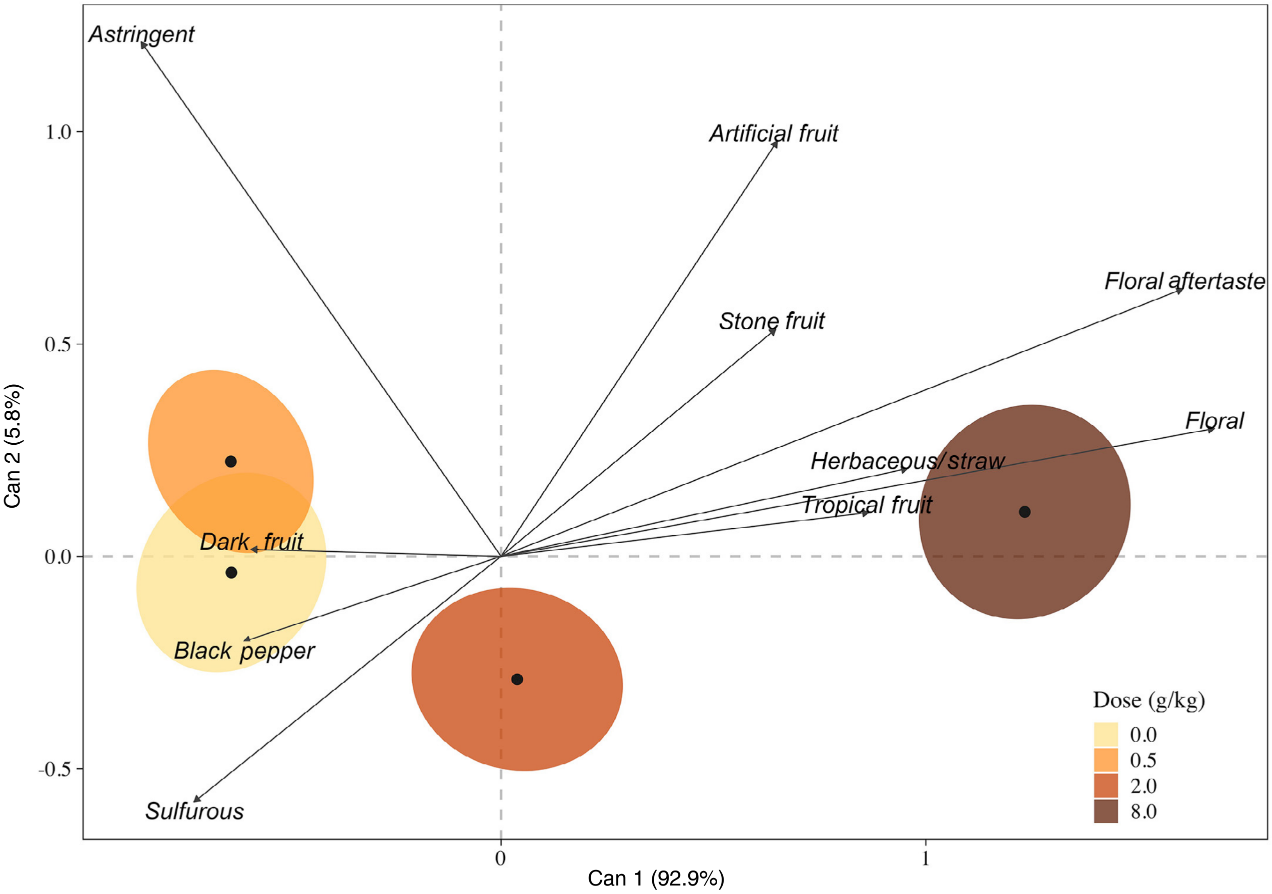

Figure 2 Canonical variate analysis of Cabernet Sauvignon wines made from four different dosages of freeze-killed leaf material (0, 0.5, 2.0, 8.0 g/kg must). The canonical variate scores for the four treatments are color-coded and enclosed by a 95% confidence ellipse.

tering of the chemical measures produced two primary clusters, the upper cluster which shows mostly weak correlations, and the lower cluster which shows mostly strong correlations. When evaluated in conjunction with the two sensory clusters, four heat map zones are formed. The first zone is positioned in the top left of the heat map and is comprised of 33 chemical measures showing a general negative correlation with the six sensory attributes from sensory cluster 1 (*tropical fruit aroma*, *herbaceous/straw aroma*, *floral aroma*, *floral aftertaste*, *stone fruit aroma*, and *artificial fruit aroma*). The second heat map zone is positioned in the top right. This zone is comprised of positive correlations between the same 33 chemical measures as the first group and the four sensory attributes from cluster 2 of the sensory dendrogram (*black pepper aroma*, *sulfurous aroma*, *dark fruit aroma*, *and astringent mouthfeel*). The third and fourth heat map zones are positioned in the lower half of the correlation heat map. The two zones show relationships between 31 chemical measures and the 10 sensory attributes, where zone 3 aligns with sensory cluster 1, and zone 4 aligns with sensory cluster 2.

Heat map zones 1 and 2 have a contrasting, but analogous, relationship to each other. The contrasting relationship between the two zones can be visualized by the colors: zone 1 shows a negative correlation (blue) and zone 2, a positive correlation (red), but the two zones are analogous in that the magnitude of the correlation is similar for each compound. Additionally, the correlations generally become less significant as you move down the two groups. Overall, except for methionol, 5-nitro-1-benzofuran, total phenolics, and tannins, the correlations found in groups 1 and 2 were not significant ($-0.53 < \rho < 0.53$); thus, few measures within groups 1 and 2 provided meaningful evidence on how FKLM additions alter specific sensory attributes.

In contrast, heat map zones 3 and 4 contain most of the strong, significant correlations found between chemical measures and the sensory attribute intensities. The chemistry dendrogram can be used to further subdivide heat map groups 3 and 4 to highlight the most meaningful relationships. Four subclusters were evaluated, of which one cluster contains most of the relevant chemical measures showing a strong positive or negative correlation with the 10 sensory attributes. The cluster comprises the 26 compounds between unk-107 and ethyl hexanoate and the correlation strength is visible as dark red or blue coloring. Overall, this subcluster contains the chemical measures which show the strongest correlation with each sensory attribute. Additionally, the 13 compounds with the greatest change are included (Table 3).

Discussion

To our knowledge, this is the first peer-reviewed study evaluating how FKLM impacts wine aroma and flavor. The results clearly show that the addition of FKLM prior to fermentation profoundly changed the flavor profile and mouthfeel of the experimental Cabernet Sauvignon wines. Additions >2.0 g/kg FKLM showed intense *floral aroma* and *floral aftertaste*, which was coupled with reduced Cabernet Sauvignon varietal characters such as *dark fruit aroma* and *astringent mouthfeel* (Figure 1). The *floral aroma* and *floral aftertaste* correlated with increased concentrations of

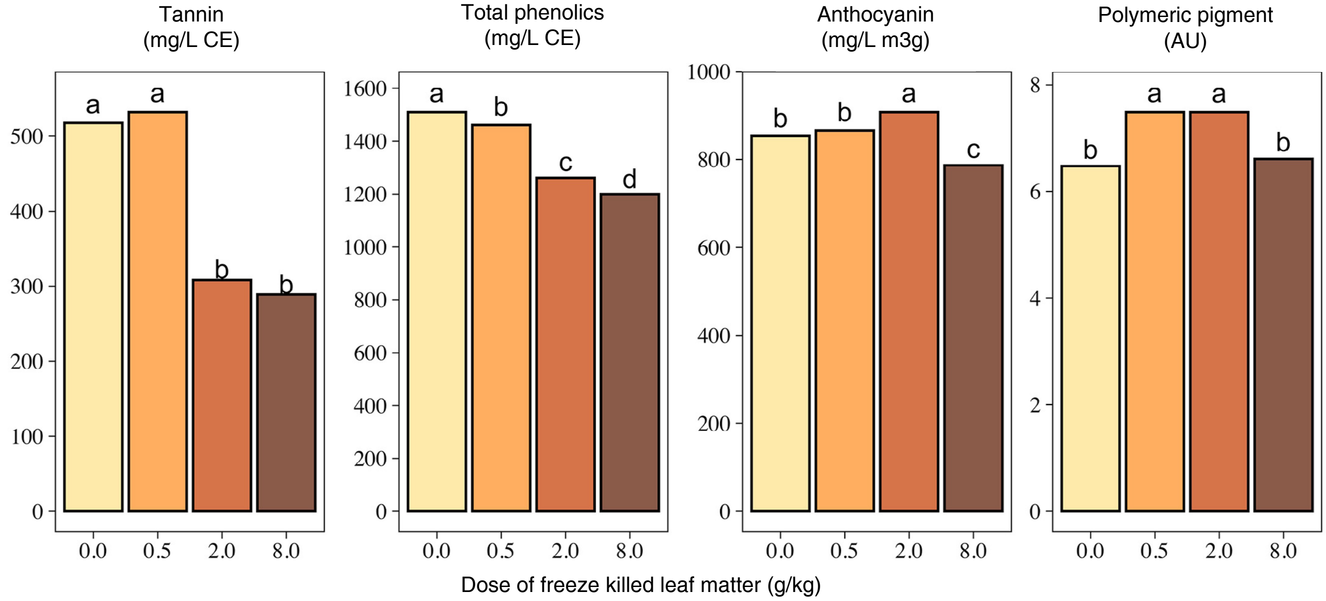

Figure 3 Mean concentrations of four phenolic measures of Cabernet Sauvignon wines made with four different dosages of freeze-killed leaf material (0, 0.5, 2.0, 8.0 g/kg must). Within each attribute, treatment additions are not significantly different by Tukey's honest significant difference test if they are labeled with the same letter. Tannins and total iron-reactive phenolics (listed as total phenolics) are measured in catechin equivalents (CE), anthocyanins are expressed as malvidin-3-glucoside equivalent (m3g), and polymeric pigments, as A520nm absorbance units (AU).

6-methyl-5-hepten-2-ol, *p*-menth-1-en-9-al, 6-methyl-3,5-heptadien-2-one, unknown 40, and unknown 109.

The three identified compounds have been found in other natural products. 6-Methyl-5-hepten-2-ol, also known as sulcatol or "coriander heptanol", is described as sweet, oily, green, and coriander-like. It has been identified in leaves of Muscat of Alexandria grapes (Wirth et al. 2001), fresh distilled Calvados (Ledauphin 2003), and yuzu citrus fruit (Song et al. 2000). *p*-Menth-1-en-9-al is described as herbal and has been identified in honey (Soria et al. 2009) and yuzu citrus fruit (Song et al. 2000). The referenced study on yuzu fruit (Song et al. 2000) used gas chromatographic olfactometry flavor dilution and showed that 6-methyl-5-hepten-2-ol and *p*-menth-1-en-9-al had the greatest flavor dilution values. Thus, these two compounds had an impact in the yuzu citrus fruit at very low concentrations. Lastly, 6-methyl-3,5-heptadien-2-one is described as herbal, spicy, and wood and is a component of basil (Lee et al. 2005) and green tea (Shimoda et al. 1995, 1996).

Previous work evaluating the effect of frozen MOG on wine chemistry reported multiple monoterpenes showing correlative relationships; specifically, geraniol, linalool, nerol, *cis*- and *trans*-rose oxide (Lan et al. 2022a, 2022b). Our GC-MS method did not detect these compounds, but it is possible they were present. These differences could also be attributed to microclimate and winegrowing conditions. The low

Table 3 Mean peak areas, confirmatory information of identified compounds (compound name; RT, retention time; RI, retention index), and statistical significance (*p* value) from gas chromatography-mass spectrometry analysis of Cabernet Sauvignon wines made with different dosages of freeze-killed leaf material (0 to 8 g/kg of must).

Compound	0.00 g/kg	0.50 g/kg	2.00 g/kg	8.00 g/kg	*p* value	RT (min)	RI (Meas.)	RI (Ref.)[a]	Ions
Methylfuran	931c[b]	645 c	4256 b	18572 a	<0.001	2.81	-	877[d]	44; 53; 82
Ethyl acetate	457954	501036	444165	500258	0.413	3.00	-	898[e]	61; 88
Ethyl propanoate	8489 b	10318 a	9669 ab	10351 a	0.004	4.15	949	950[e]	57; 102
Ethyl isobutyrate*[c]	5899	10148	5950	6981	0.075	4.35	958	965[e]	71; 88; 116
Diacetyl	30901	32477	28905	31294	0.8	4.53	967	977[f]	43; 86
Ethyl butyrate*	46638 ab	44028 ab	39681 b	47463 a	0.03	6.33	1031	1036[f]	71; 88; 101; 116
1-Propanol	15613	15352	17586	17061	0.705	6.51	1036	1038[e]	31, 59
Ethyl isovalerate*	10547	10704	10858	9247	0.849	7.45	1062	1070[f]	59; 73; 88
Isobutanol*	78633	86514	76434	77459	0.092	8.53	1092	1108[f]	43; 55; 74
Isoamyl acetate*	973975 ab	1032325 a	887889 b	979752 ab	0.034	9.61	1118	1132[f]	55; 70; 87
1-Butanol	4363 b	4210 b	5716 ab	7304 a	0.016	10.72	1143	1138[e]	56; 69
unk-28	7741 a	9058 a	7499 a	8428 a	0.668	12.73	1189	-	59; 74
Isoamyl alcohol*	3821739 b	4478990 a	4006295 ab	3887873 b	0.003	13.59	1208	1206[e]	55; 70; 87
Ethyl hexanoate*	1146727	1109948	1088969	1243926	0.118	14.57	1230	1229[e]	88; 99; 115; 144
Hexyl acetate*	44786 c	57976 b	54043 bc	71530 a	<0.001	16.30	1268	1264[g]	56; 69; 84; 101
unk-36	16788 a	18030 a	15830 a	16149 a	0.07	16.88	1281	-	71; 77; 105
Ethyl-3-hexenoate*	ND b	ND b	ND b	1920 a	<0.001	17.58	1296	1290[h]	69; 88; 142
unk-39	ND c	ND c	4189 b	35637 a	<0.001	17.70	1299	1297[h]	69;125;140
unk-40	ND c	2411 c	7677 b	53203 a	<0.001	17.80	1301	1297[h]	69;125;140
Isohexyl alcohol	1427	2336	1618	2323	0.406	18.37	1314	1301[h]	56; 69; 73; 99
3-Methylpentan-1-ol	9226 b	10587 a	9403 ab	9773 ab	0.044	18.91	1326	1325[i]	56; 69; 84
Ethyl heptanoate*	1478 c	1734 c	2725 b	4636 a	<0.001	19.07	1330	1332[j]	88; 101; 115
Ethyl lactate	63719 b	75267 a	67521 ab	72899 a	0.006	19.33	1336	1331[h]	45; 75
1-Hexanol*	105508 d	126685 c	145225 b	162126 a	<0.001	20.08	1353	1354[e]	56; 69; 84
unk-49	1090 a	1293 a	1199 a	1112 a	0.962	20.73	1368	-	59; 70; 74
unk-53	1958 a	1147 ab	ND b	718 b	0.004	21.47	1384	-	69; 74; 87
5-Nitro-1-benzofuran	3065 a	2858 ab	2337 ab	1366 b	0.028	22.21	1401	-	135; 151; 179
unk-56	ND c	ND c	5760 b	37077 a	<0.001	22.47	1408	-	67; 85; 119; 137; 151; 166
unk-57	ND b	ND b	973 b	16197 a	<0.001	23.22	1425	-	67; 85; 119; 137; 151; 166
Ethyl octanoate*	5738745	5444089	4952660	5354800	0.065	23.53	1433	1427[e]	88; 101; 127; 172
Acetic acid	88175	81108	81158	88763	0.545	23.86	1441	1434[e]	43; 45; 60
Isoamyl hexanoate	21522 b	21197 b	22263 b	28895 a	<0.001	24.50	1456	1450[e]	70; 99; 117
6-Methyl-5-hepten-2-ol*	NDc	ND c	7927 b	49266 a	<0.001	24.82	1463	1473[k]	69; 95; 110; 128
unk-70	ND b	ND b	ND b	2447 a	<0.001	26.38	1501	-	55; 67; 81; 93; 121; 136

relative humidly of eastern Washington compared to Ontario, Canada would produce MOG with different characteristics (Supplemental Figure 1).

Changes in the perceived *astringent mouthfeel* correlated with a reduction in measured tannins (Figure 4). A difference of 228 mg/L CE was measured between the 0.0 g/kg FKLM (517 mg/L CE) and the 8.0 g/kg FKLM (289 mg/L CE) treatment. This difference aligns with previously reported sensory differences (Hopfer et al. 2012, Frost et al. 2018). It is also notable that the reduction in tannin concentration was not linear, but occurred with additions of >0.5 g/kg FKLM (Figure 3). It is probable that FKLM removed tannin in a similar fashion as fining agents such as egg whites or gelatin, which are described by the Langmuir adsorption isotherm (Boulton et al. 1999). Overall, the removal of tannin and the reduction in *astringent mouthfeel* as a function of added FKLM was a key marker of frost taint.

In addition to reducing *astringent mouthfeel*, *dark fruit aroma* also decreased with each increasing FKLM treatment. *Dark fruit aroma* is often associated with the presence of esters; for example, ethyl propanoate, ethyl-2-methylpropanoate, and ethyl-2-methylbutanoate were associated with dark berry aroma in red Bordeaux wine (Pineau et al. 2009). We found little change in esters with increased addition of FKLM. This key result suggests how leaf material interacts with aroma chemistry during

Table 3 *continued* Mean peak areas, confirmatory information of identified compounds (compound name; RT, retention time; RI, retention index), and statistical significance (*p* value) from gas chromatography-mass spectrometry analysis of Cabernet Sauvignon wines made with different dosages of freeze-killed leaf material (0 to 8 g/kg of must).

Compound	0.00 g/kg	0.50 g/kg	2.00 g/kg	8.00 g/kg	*p* value	RT (min)	RI (Meas.)	RI (Ref.)[a]	Ions
Ethyl nonanoate	4120 b	4149 b	5225 a	5274 a	0.001	27.68	1533	1526[h]	88; 101; 115; 141
2,3-butanediol	26927	37533	22247	33035	0.141	27.77	1535	1523[m]	45; 57
unk-82	ND b	ND b	548 b	13414 a	<0.001	28.41	1551	-	68; 81; 97
1-Octanol	4525 b	4731 b	6239 b	9447 a	<0.001	28.66	1558	1547[h]	56; 70; 84
unk-87	10928 a	11539 a	1549 b	1974 b	0.001	29.27	1573	-	45; 57
2-Methyl-1-benzofuran*	ND b	ND b	ND b	5539 a	<0.001	29.35	1575	-	55; 77; 103; 131
6-Methyl-3,5-heptadien-2-one*	ND b	ND b	ND b	9067 a	<0.001	29.46	1578	1587[l]	55; 81; 109; 124
unk-92	2503 a	1200 a	1990 a	2690 a	0.327	30.33	1600	-	42; 56; 86
p-Menth-1-en-9-al	ND b	ND b	565 b	12099 a	<0.001	31.20	1622	1620[m]	60; 79; 94; 152
Ethyl decanoate	3115933 a	2784211 ab	2558398 b	2703806 b	0.002	31.73	1636	1630[f]	70; 88; 101. 115; 155; 200
Isoamyl octanoate	39241 a	31711 b	29682 b	32912 b	0.001	32.47	1656	1651[h]	70; 127; 144
2-Methylhexanoic acid	11043	14838	12436	11975	0.367	32.68	1661	-	60; 74; 87
Diethyl succinate	43343 c	59009 a	51065 b	52114 b	<0.001	32.97	1669	1690[j]	74; 101; 129
Ethyl 9-decenoate	13824 b	33727 a	19318 b	10116 b	<0.001	33.62	1686	1685[h]	69; 88; 101; 110; 135;152
Methionol	5242 b	7374 a	5094 b	2404 c	<0.001	34.32	1705	1738[f]	57; 61; 75; 106
1,1,6,trimethyl-2H-naphthalene (TDN)*	ND b	ND b	ND b	2966 a	<0.001	34.99	1723	1722[h]	142; 157; 172
unk-107	ND b	ND b	ND b	19326 a	<0.001	35.12	1727	-	43; 67; 81; 96
unk-109	ND b	525 b	838 b	3415 a	<0.001	36.39	1762	-	41; 55; 70; 82
Phenethyl acetate*	56703 b	70654 a	51774 b	52336 b	<0.001	37.72	1799	1803[e]	43; 65; 77; 91; 104
ß-Damascenone*	5169 b	5150 b	6707 a	7195 a	<0.001	37.94	1805	1832[f]	69; 77; 91; 105; 121; 190
Hexanoic acid	56967 ab	50521 b	53309 b	65971 a	0.001	39.04	1837	1840[e]	60; 73; 87
Ethyl dodecanoate	385821 a	317100 b	288677 b	317128 b	<0.001	39.17	1841	1835[h]	88; 101; 115; 157; 183; 228
Isopentyl decanoate	16411 a	12273 bc	9669 c	12500 b	<0.001	39.83	1860	1863[h]	70; 155; 173
Phenethyl alcohol*	1724285 b	2061510 a	1799951 b	1692450 b	<0.001	41.03	1895	1896[h]	65; 91; 122
Ethyl tetradecanoate	5960 a	4228 b	4152 b	5262 a	<0.001	45.99	-	2040[h]	88; 101; 157
Octanoic acid	169961 a	146431 ab	138889 b	164568 ab	0.016	46.17	-	2051[h]	60; 73; 85; 101; 115; 144

[a] Retention index reference source, indicated by superscript. d, Chida et al. 2004; e, Lee and Noble 2006; f, Ferreira et al. 2001; g, Ruther 2000; h, Ferrari et al. 2004; i, Bianchi et al. 2007; j, Wada and Shibamoto 1997; k, Mariño et al. 1995; l, Lee et al. 2005; and m, Shimoda et al. 1996.

[b] Mean compound areas with identical letters are not significantly different via Tukey's honest significant difference test.

[c] * indicates a compound that was confirmed with authentic reference material.

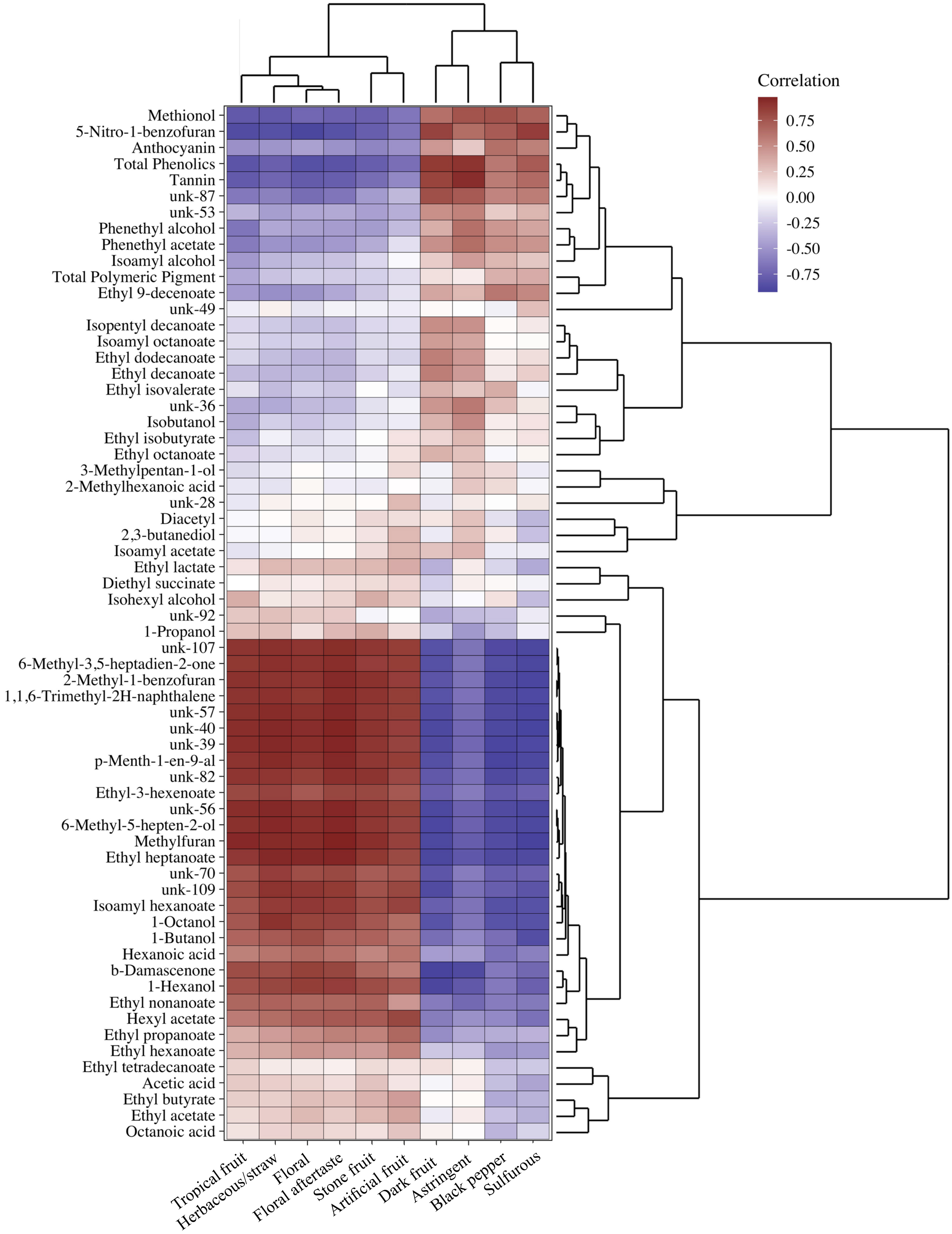

Figure 4 Correlation heat map showing the relationship between significant sensory attributes and the measured chemistry of Cabernet Sauvignon wines made with four different dosages of freeze-killed leaf material (0, 0.5, 2.0, 8.0 g/kg must).

fermentation. It is likely that the dried leaves do not remove aroma compounds, but only add additional aroma chemistry, thus, masking the dark fruit aromas with the addition of floral. Masking of fruity aroma by vegetative aroma is established in wine (Hein et al. 2009), but floral aromas have also demonstrated the ability to mask stale aromas in Pu-Erh tea (Zhang et al. 2019) and mask the aroma of esters in a model system (Xiao et al. 2019).

Although the complete mechanism by which these flavor and aroma compounds transfer from leaf to fermentation is not yet fully understood, our work demonstrates that the leaves must freeze for the taint to show. Additional fermentations using fresh leaves were caried out in parallel and did not produce wines with a frost tainted flavor profile (data not shown). We theorize that the cell vacuoles freeze, rupture, and then release their contents. In contrast, a fresh leaf would have the cellular vacuole intact and protected by the waxy leaf coating. Additionally, frozen leaves are extremely brittle and shatter into small fragments during harvest, which increases the leaf surface that contacts the ferment. Lastly, machine harvesting can produce small leaf fragments that are pervasive and nearly impossible to remove during processing.

The experimental treatments showed a substantial effect between 0.5 and 2.0 g/kg of FKLM. Within this experiment, the mass of a single freeze-killed but intact Cabernet Sauvignon leaf was ~1.0 g, and one Cabernet Sauvignon cluster was ~185 g (data not shown). Thus, when expressed as leaf per pound of fruit, 0.5 and 2.0 g/kg of FKLM equates to 0.23 and 0.91 leaves/lb fruit. Although additional studies are needed, the detection threshold for frost taint is likely near 1.0 g/kg. This can be conceptualized by envisioning a single vine. If this vine produces 16 clusters, it will require three freeze-killed leaves to impact the wine flavor and aroma.

It remains to be investigated if mitigation can prevent frost taint from occurring. For example, can leaf removal, either immediately before a freeze or immediately before harvest, mitigate frost taint effectively? Various approaches should be rigorously evaluated, including leaf blowers, hand removal, pre-pruning, and hand-harvesting. Additionally, it is unclear how the length and temperature of the freeze impact the occurrence of taint, or what role is played by vine geometry and canopy management.

Conclusion

Using a replicated pilot scale experiment, the current study successfully modeled the impact of freezing temperatures prior to harvest on Cabernet Sauvignon wine. The FKLM added to the fermentation induced the development of floral, herbal, and straw-like aromas in the final wine. Additionally, the treatments stripped tannin from the fermentation, which led to a significant loss of astringent mouthfeel. Three marker compounds were also identified, which were strongly correlated with the frost taint characters and can be used to screen suspected wine lots for frost taint.

Acknowledgments

Jim Harbertson would like to acknowledge the Wine Research Advisory Committee, the Washington Wine Commission, the Washington Grape and Wine Research Program, and the WSU Agricultural Research Center. The authors would like to acknowledge our NIFA USDA Hatch Project #1016366. Dr Andrew Reynolds is thanked for helpful and informative discussions.

Supplemental Data

The following supplemental materials are available for this article at ajevonline.org:

Supplemental Table 1 Descriptive analysis mean square error results.

Supplemental Table 2 Polyphenolic measures, by fermentation replicate. CE, catechin equivalent; M3G, malvidin-3-glucoside equivalent; AU, absorbance unit.

Supplemental Figure 1 Cabernet Sauvignon clusters and leaves harvested from the same vineyard pre- (**A**) and post-frost (**B**) showing healthy and freeze-killed leaves.

References

Bianchi F, Careri F, Mangia A and Musci M. 2007. Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: database creation and evaluation of precision and robustness. J Sep Sci 30:563-572. DOI: 10.1002/jssc.200600393

Boulton RB, Singleton VL, Bisson LF and Kunkee RE. 1999. Principles and Practices of Winemaking. Springer, Boston, MA.

Capone DL, Barker A, Pearson W and Francis IL. 2021. Influence of inclusion of grapevine leaves, rachis and peduncles during fermentation on the flavour and volatile composition of *Vitis vinifera* cv. Shiraz wine. Aust J Grape Wine Res 27:348-359. DOI: 10.1111/ajgw.12489

Chida M, Sone Y and Tamura H. 2004. Aroma characteristics of stored tobacco cut leaves analyzed by a high vacuum distillation and canister system. J Agric Food Chem 52:7918-7924. DOI: 10.1021/jf049223p

Ferrari G, Lablanquie O, Cantagrel R, Ledauphin J, Payot T, Fournier N et al. 2004. Determination of key odorant compounds in freshly distilled Cognac using GC-O, GC-MS, and sensory evaluation. J Agric Food Chem 52:5670-5676. DOI: 10.1021/jf049512d

Ferreira V, Aznar M, López R and Cacho J. 2001. Quantitative gas chromatography– olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality Spanish aged red wines. J Agric Food Chem 49:4818-4824. DOI: 10.1021/jf010283u

Fox J and Weisberg S. 2019. An R Companion to Applied Regression. Sage, Thousand Oaks, CA.

Frost SC, Blackman JW, Hjelmeland AK, Ebeler SE and Heymann H. 2018. Extended maceration and cap management impacts on the phenolic, volatile, and sensory profiles of Merlot wine. Am J Enol Vitic 69:360-370. DOI: 10.5344/ajev.2018.17062

Guerrini L, Masella P, Angeloni G, Calamai L, Spinelli S, Di Blasi S et al. 2018. Harvest of Sangiovese grapes: the influence of material other than grape and unripe berries on wine quality. Eur Food Res Technol 244:1487-1496. DOI: 10.1007/s00217-018-3063-y

Harbertson JF, Piccioto EA and Adams DO. 2003. Measurement of polymeric pigments in grape berry extract sand wines using a protein precipitation assay combined with bisulfite bleaching. Am J Enol Vitic 54:301-306. DOI: 10.5344/ajev.2003.54.4.301

Harbertson JF, Mireles M and Yue Y. 2015. Improvement of BSA tannin precipitation assay by reformulation of resuspension buffer. Am J Enol Vitic 66:95-99. DOI: 10.5344/ajev.2014.14082

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. DOI: 10.1021/jf960635a

Hein K, Ebeler SE and Heymann H. 2009. Perception of fruity and vegetative aromas in red wine. J Sens Stud 24:441-455. DOI: 10.1111/j.1745-459X.2009.00220.x

Heymann H and Lawless HT. 2013. Sensory Evaluation of Food: Principles and Practices. Springer Science & Business Media, New York.

Hjelmeland AK, King ES, Ebeler SE and Heymann H. 2013. Characterizing the chemical and sensory profiles of United States Cabernet Sauvignon wines and blends. Am J Enol Vitic 64:169-179. DOI: 10.5344/ajev.2012.12107

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. DOI: 10.5344/ajev.2012.11112

Huang P-JD, Cash JN and Santerre CR. 1988. Influence of stems, petioles and leaves on the phenolic content of Concord and Aurora blanc juice and wine. J Food Sci 53:173-175. DOI: 10.1111/j.1365-2621.1988.tb10202.x

Iland P. 2004. Chemical analysis of grapes and wine: techniques and concepts. Patrick Iland Wine Promotions PTYLTD, Campbelltown, SA, Australia.

Lan Y, Wang J, Aubie E, Crombleholme M and Reynolds AG. 2022a. The effects of frozen materials other than grapes on red wine volatiles. Mitigation of floral taints by yeast strains. Am J Enol Vitic 73:125-141. DOI: 10.5344/ajev.2022.22005

Lan Y, Wang J, Aubie E, Crombleholme M and Reynolds AG. 2022b. Effects of frozen materials other than grapes on red wine aroma compounds. Impacts of harvest technologies. Am J Enol Vitic 73:142-155. DOI: 10.5344/ajev.2022.22006

Lee S-J and Noble AC. 2006. Use of partial least squares regression and multidimensional scaling on aroma models of California Chardonnay wines. Am J Enol Vitic 57:363-370. DOI: 10.5344/ajev.2006.57.3.363

Lee S-J, Umano K, Shibamoto T and Lee K-G. 2005. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem 91:131-137. DOI: 10.1016/j.foodchem.2004.05.056

Mariño NC, Tamames EL and García Jares YCM. 1995. Contribution to the study of the aromatic potential of three Muscat *Vitis vinifera* varieties: identification of new compounds. Food Sci Technol Int 1:105-116. DOI: 10.1177/108201329500100206

Mendiburu F. 2021. agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-5.

Owen JG and Chmielewski MA. 1985. On canonical variates analysis and the construction of confidence ellipses in systematic studies. Syst Zool 34:366-374. DOI: 10.2307/2413155

Parenti A, Spugnoli P, Masella P, Guerrini L, Benedettelli S and Di Blasi S. 2015. Comparison of grape harvesting and sorting methods on factors affecting the must quality. J Agric Eng 46:19. DOI: 10.4081/jae.2015.456

Petrucci VE and Siegfried R. 1976. The extraneous matter in mechanically harvested wine grapes. Am J Enol Vitic 27:40-41. DOI: 10.5344/ajev.1976.27.1.40

Pineau B, Barbe J-C, Van Leeuwen C and Dubourdieu D. 2009. Examples of perceptive interactions involved in specific "red-" and "black-berry" aromas in red wines. J Agric Food Chem 57:3702-3708. DOI: 10.1021/jf803325v

Ruther J. 2000. Retention index database for identification of general green leaf volatiles in plants by coupled capiliary gas chromatography-mass spectrometry. J Chromatogr A 890:313-319. DOI: 10.1016/S0021-9673(00)00618-X

Shimoda M, Shigematsu H, Shiratsuchi H and Osajima Y. 1995. Comparison of volatile compounds among different grades of green tea and their relations to odor attributes. J Agric Food Chem 43:1621-1625. DOI: 10.1021/jf00054a038

Shimoda M, Wu Y and Osajima Y. 1996. Aroma compounds from aqueous solution of haze (*Rhus succedanea*) honey determined by adsorptive column chromatography. J Agric Food Chem 44:3913-3918. DOI: 10.1021/jf9601168

Song HS, Sawamura M, Ito T, Kawashimo K and Ukeda H. 2000. Quantitative determination and characteristic flavour of *Citrus junos* (yuzu) peel oil. Flavour Fragr J 15:245-250.

Soria AC, Sanz J and Martínez-Castro I. 2009. SPME followed by GC–MS: a powerful technique for qualitative analysis of honey volatiles. Eur Food Res Technol 228:579-590. DOI: 10.1007/s00217-008-0966-z

Team R. 2021a. RStudio: Integrated Development Environment for R. R Studio, Boston, MA.

Team R. 2021b. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Wada K and Shibamoto T. 1997. Isolation and identification of volatile compounds from a wine using solid phase extraction, gas chromatography, and gas chromatography/mass spectrometry. J Agric Food Chem 45:4362-4366. DOI: 10.1021/jf970157j

Ward SC, Petrie PR, Johnson TE, Boss PK and Bastian SEP. 2015. Unripe berries and petioles in *Vitis vinifera* cv. Cabernet Sauvignon fermentations affect sensory and chemical profiles. Am J Enol Vitic 66:435-443. DOI: 10.5344/ajev.2015.15016

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Wirth J, Guo W, Baumes R and Günata Z. 2001. Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from *Vitis vinifera* Muscat of Alexandria and Shiraz cultivars. J Agric Food Chem 49:2917-2923. DOI: 10.1021/jf001398l

Xiao Z, Luo J, Niu Y, Wang P, Wang R and Sun X. 2019. Olfactory impact of esters on rose essential oil floral alcohol aroma expression in model solution. Food Res Int 116:211-222. DOI: 10.1016/j.foodres.2018.08.015

Zhang T, Ni H, Qiu X-J, Li T, Zhang L-Z, Li L-J et al. 2019. Suppressive interaction approach for masking stale note of instant ripened Pu-Erh tea products. Molecules 24:4473. DOI: 10.3390/molecules24244473