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Research Article

Frozen Leaf Material Causes "Frost Taint" in Cabernet Sauvignon

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

Author affiliation(s): [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)

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Abstract: 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 termed to be "rose tainted" or "frost tainted." Anecdotal evidence suggests that the inclusion of freeze killed leaf material (FKLM) into the fermentation is the source of the taint. We have investigated these claims by studying how the addition of FKLM into the fermentation affects the chemical and sensory profile of the resulting Cabernet Sauvignon wine. 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. A detailed analysis was performed capturing the treatment impact on the flavor and chemical profile of the experimental

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wines. Gas chromatography mass spectrometry identified 60 volatile and semi-volatile organic compounds that were correlated with the FKLM addition. Additionally, the phenolic chemistry showed reduced concentration of anthocyanin, tannin, and iron reactive phenolics. Descriptive sensory found that the addition of 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. We estimate that ~3 freeze killed leaves per vine will produce the taint characteristics. Overall, these results clearly show the impact of freeze killed grape vine leaves on Cabernet Sauvignon wine quality and provide convincing evidence of the taint 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 the 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 and brittle, and they are 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 also vary, one estimate finds that fresh leaf material

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comprises approximately 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 of greater the 5% by weight produced wine with strong floral aromas vs 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 (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. A recent study reported increases in multiple monoterpenes and norisoprenoids 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 on frozen MOG on Cabernet franc, the experiment uses fruit that experienced freezing temperatures which possibly confounds 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 were found to consistently vary across the two vintages. The Cabernet Sauvignon showed ethyl isobutyrate, ethyl heptanoate, ethyl decanoate, and citronellol to consistently change, and the Cabernet franc showed ethyl hexanoate, ethyl octanoate, isoamyl acetate, and nerol. Of these compounds, only nerol and citronellol are likely not fermentation derived. Although inconsistent across vintage and cultivar, Lan (2022a, 2022b) shows that frozen MOG does impact the chemical composition of Cabernet franc and Cabernet Sauvignon.

Given that frozen MOG has the capacity to modify wine chemistry, it is reasonable to assume that FKLM would also have the capacity to 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 October 21, 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 15ºC in plastic yard bags. Seven days later, on October 28, 2019, *Vitis vinifera* L. cv. Cabernet Sauvignon (clone 08) grapes were harvested from Cold Creek vineyard (Columbia Valley American Viticultural Area, Sunnyside, WA, USA). Fruit (1877.9 kg) was machine-harvested into half-ton bins from vines planted in 2009 with North-South row orientation and 2.4 x1.5 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, Güglingen, Germany). A total of 12 stainless steel-jacketed fermentors (Spokane Industries, Spokane, WA) were filled with 140 L of must. The average soluble solids across all 12 tanks were 28.6 Brix. All tanks were then adjusted to 25.0 Brix by watering-back with a 5g/L tartaric acid solution. 50 mg/L sulfur dioxide (in the form of $K_2S_2O_5$) was added to each tank post-crush.

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The 12 fermentors were randomly split 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, and 8.0 g/kg must. The addition rate is 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, post-crush, to reach a yeast assimilable nitrogen of 225 mg/L. Malolactic bacteria Lalvin VP41 (0.01g/L) was added to all 12 tanks 48 hours after initial yeast inoculation. All fermentation support products purchased through Scott Laboratories, Petaluma, CA.

Each tank was fitted with a variable capacity, stainless steel tank lids (Spokane Industries, Spokane, WA) and Cypress Integrated Fermentation Controller System 3.2 (IFCS) computers for temperature control, temperature monitoring, and reoccurring pump-overs (Cypress Semiconductor, San Jose, CA). The tanks were set to a maximum fermentation temperature of 30ºC. Pump-overs were performed every four hours for five minutes (six times daily) by a DDP 550 5 Chamber Diaphragm pump (Aquatec, Irvine, CA). Fermentation progress and temperature were monitored daily using a DMA 35N handheld densitometer (Anton-Paar, Graz, Austria) and by the IFCS computer. An Admeo Y15 Autoanalyzer (Admeo, Inc., Angwin, CA) measured residual sugar via enzymatic analysis on wine 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, San Jose, CA) and transferred to 60 L stainless steel sanitized 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 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 afterwards. Kegs were then stored in a temperature-

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controlled 12.8ºC (± 2ºC) room to settle out for 71 days. An XpressFill XF4100 4-spout bottling machine (XpressFill Systems LLC, San Luis Obispo, CA) bottled wines at ambient temperature into 750 mL green glass bottles (Glass Masilva USA AG Burgundy). 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 30x60 screwcaps (Scott Caps, Petaluma, CA). Bottles were placed in cardboard cases (12 bottles/case) and moved to a 12.8ºC (± 2ºC) room for storage. Approximately five cases per treatment were bottled. 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) measured were collected on the harvested fruit and wine as described by (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)), total polymeric pigments (A_{520nm}) were measured following established methods (Harbertson et al. 2015, 2003).

Sensory analysis: The effect of freeze killed leaf material on the flavor and aroma profile of the experimental wines was evaluated using descriptive analysis (Heymann and Lawless 2013). A panel of 13 judges (8 males; 5 females; 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, and thus after 4 session 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

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standards were finalized in session three; 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 individually evaluated for all 20 attributes using a 15 cm unstructured lines scale. Service order was determined used a randomized block design to control for possible carry over effect. Additionally, a one-minute rest between each wine, and a five-minute 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 3-digit blinding code. Panelists were provided with unsalted crackers, distilled water to rinse their pallet between samples. Data was collected using Compusense Software (Guelph, Ontario, Canada).

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, Santa Clara, CA). The GC/MS was equipped with an MPS Robotic autosampler (Gerstel, Linthicum, MD), a DB-WAX capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness (Agilent Technologies, Santa Clara). The method parameters and sampling protocols were adapted from (Hjelmeland et al. 2013). 10 mL of wine was pipetted into a 20 mL round bottom glass vial (Restek, Bellefonte, PA) with 3 g of NaCl. The vial was sealed with a screw cap and PTFE lined septum (Restek, Bellefonte, PA, 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 40ºC with agitation at 500 rpm for 5 mins; then a 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Burlington, MA), 23-gauge SPME fiber was inserted into the vial headspace. Agitation was slowed to 250 rpm and the fiber was exposed for 30 minutes. The SPME fiber was then desorbed at a 20:1 split with the inlet set to 240ºC. The SPME fiber was retained in the inlet for 3 mins. The GC oven

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temperature was programed to remain at 40oC for 5 mins; then increase at 3ºC/min up to 180 ºC; followed by a ramp of 30 ºC/min to final temperature of 240ºC, and finally held for 10 mins. 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 minutes. A solvent delay was set for the initial 2.5 mins, and the MSD was turned off from 3.40 to 3.80 minutes. The MSD was set to an electron energy of -70 eV, a source of 230 º C, and quadrupole temperature of 150 º 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 randomize order for a total of 36 injections – 3 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 then 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 7 of the 9 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, Wood Dale IL), 6-methyl-3,5-heptadien-2-one, 1,1,6,-trimethyl-1,2-dihydronapthalene (Santa Cruz Biotechnology Inc. Dallas, TX), 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, St. Louis, MO),

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propyl acetate, citronellol, ionone (Tokyo Chemical Industry Company, USA). Unknown compounds were given a unique identifier.

Data analysis: Statistical analyses were performed using R version 4.1.0 “Camp Pontanezen ” (R. C. Team 2021) and the R Studio IDE version 1.4.1717 (R. Team 2021). Graphics were prepared using the ggplot2 package (Wickham 2016). A significance of $\alpha = 0.05$ 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*, along with all two-, and three-way interactions were evaluated via the F-statistic. In the event where the main effect of *dose* and the *dose x judge* interaction were together 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 was 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 which significantly differed 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 honestly significant difference (hsd) and is displayed as letters above each bar. The addition of FKLM, at all dose levels, showed increased 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,

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only *floral*, *floral aftertaste*, *herbaceous/straw* showed a significant effect at 2.0 g/kg of freeze killed leaf matter. Lastly, no difference was found when comparing the 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 significantly altered at 8.0 g/kg FKLM.

Canonical variate analysis (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, followed by the 2.0 g/L FKLM dose, and lastly the 8.0 g/L FKLM dose. 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 iron reactive phenolics (mg/L CE) both decreased with the addition of FKLM, but anthocyanins and

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total polymeric pigments showed an effect of treatment that did not correlated 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 the 0.0 and 0.5 g/kg FKLM, and the low group comprised of 2.0 and 8.0 g/kg FKLM. Total iron reactive phenolics measures decreased with treatment and was strongly correlated with the dose rate ($\rho = -0.78$, df = 34, $p < 0.001$).

Volatile analysis. HS-SPME-GC/MS returned 126 tentative compounds across the 36 injections. From the 125, 60 unique compounds were carried forward; 46 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 which 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 honestly significant difference 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, which splits 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*, and the second cluster is comprised of four attributes: *dark*

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fruit aroma, *astringent mouthfeel*, *black pepper aroma*, and *sulfurous aroma*. The delineation between the two sensory clusters is clearly captured for each of the chemical measures. For example, total phenolics shows a negative correlation with all attributes in cluster 1 and a positive correlation with all attributes in cluster 2. While, p-Menth-1-en-9-al shows a similar but opposite relationship, positive correlations within cluster 1 attributes and negative correlations within cluster 2 attributes. For most of the measured chemistry, a similar diametric correlation between cluster 1 and cluster 2 is observed.

The chemistry dendrogram, shown to the right of the correlation heat map, clusters the 64 chemical measures into 2 groups (Figure 4). The hierarchical clustering of the chemical measures produced two primary clusters, the upper cluster of the figure 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 of Figure 4. 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 show 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 shows 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

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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 group 1 and 2 were non-significant ($-0.53 < \rho < 0.53$); thus, few measures within group 1 and 2 were able to provide meaningful evidence of how FKLM additions alter specific sensory attributes.

In contrast, heat map zones 3 and 4 contain a majority of the strong, significant correlations found between the 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 sub-clusters 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 visually clear with dark red and blue coloring. Overall, this sub-cluster contains the chemical measures which show the highest correlation with each sensory attribute. Additionally, the 13 compounds showing 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 greater then 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* were correlated with increased concentration of 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 previously been reported 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 the leaves of Muscat of Alexandria grapes (Wirth 2001), fresh

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distilled Calvados (Ledauphin 2003), and yuzu citrus fruit (Song et al. 2000). p-Menth-1-en-9-al is described as herbal, and it 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 highest flavor dilution values. Thus, these two compounds required very little concentration for impact in the yuzu citrus fruit. Lastly, 6-methyl-3,5-heptadien-2-one is also described as herbal, spicy, and wood and a component of basil (Lee et al. 2005) and green tea (Shimoda et al. 1996) (Shimoda 1995).

Previous work evaluating the effect of frozen MOG on wine chemistry reports multiple monoterpenes showing correlative relationships; specifically, geraniol, linalool, nerol, cis and trans rose oxide (Lan et al. 2022a, Lan et al. 2022b). In the presented study, the 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 relative humidly of eastern Washington, as compared to Ontario, Canada would produce MOG of different characteristics (Supplemental Figure 1).

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

In addition to the reduction of *astringent mouthfeel*, the reduction of *dark fruit aroma* was also observed with each increasing treatment. *Dark fruit aroma* is often associated with the presence of esters; for example, ethyl propanoate, ethyl-2-methylpropanoate, and ethyl-2-methylbutanoate have shown to be

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associated with dark berry aroma in red Bordeaux wine (Pineau et al. 2009). In the current work, the esters show little change with increased addition of FKLM. This key result suggests how the leaf material interacts with the aroma chemistry during the 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 the various impact chemistries transfers from the leaf to the fermentation is not yet fully understood, the presented work demonstrates that the leaves must freeze for the taint to show. Additional fermentations using fresh leaves were caried out in parallel, which 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 cellar 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 which are pervasive and may be 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 approximately 1.0 g, and one Cabernet Sauvignon cluster was approximately 185g (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/lbs. 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 3 freeze killed leaves to impact the wine flavor and aroma.

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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, effectively mitigate frost taint? Various approaches should be rigorously evaluated, including leaf blowers, hand removal, pre pruning, and hand harvesting. Additionally, it is not clear how the length and freeze temperature will impact the occurrence of the taint, or what role vine geometry and canopy management play.

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 freeze killed leaf material 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.

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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 Cheery Preserves + 25 mL base red wine
Dark Fruit	Dark fruity, slight woody and herbal aromatics associated with blackberry jam, blueberry jam, cassis, plum	0.25 Tbsp each of O Organic Blackberry Preserves + 0.25 Tbsp Bonne Maman Blackcurrant jam + 15 mL of crème de cassis + 10 mL of 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 of 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 of base wine

Cooked Veg	Pervasive aromas associated with cooked cabbage and canned asparagus	1 Tbsp of liquid from Jolly Green Giant canned asparagus + 1 Tbsp of cooked cabbage + 20 mL of 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)

*Franzia chillable red was used as the base wine

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Table 2 Basic wine chemistry measures for Cabernet Sauvignon wines made with different dosages of freeze killed leaf material (g/kg of must).

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

Table 3 Mean peak areas, confirmatory information of identified compounds (compound name, retention time, RT; retention index, RI) and statistical significance (p-value) from gas chromatography mass spectroscopy analysis of Cabernet Sauvignon wines made with different dosages of freeze killed leaf material (0 – 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]	645c	4256b	18572a	<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	8489b	10318a	9669ab	10351a	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*	46638ab	44028ab	39681b	47463a	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*	973975ab	1032325a	887889b	979752ab	0.034	9.61	1118	1132[f]	55; 70; 87
1-Butanol	4363b	4210b	5716ab	7304a	0.016	10.72	1143	1138[e]	56; 69
unk-28	7741a	9058a	7499a	8428a	0.668	12.73	1189	-	59; 74
Isoamyl alcohol*	3821739b	4478990a	4006295ab	3887873b	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*	44786c	57976b	54043bc	71530a	<0.001	16.30	1268	1264[g]	56; 69; 84; 101

unk-36	16788a	18030a	15830a	16149a	0.07	16.88	1281	-	71; 77; 105
Ethyl-3-hexenoate*	NDb	NDb	NDb	1920a	<0.001	17.58	1296	1290[h]	69; 88; 142
unk-39	NDc	NDc	4189b	35637a	<0.001	17.70	1299	1297[h]	69;125;140
unk-40	NDc	2411c	7677b	53203a	<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	9226b	10587a	9403ab	9773ab	0.044	18.91	1326	1325[i]	56; 69; 84
Ethyl heptanoate*	1478c	1734c	2725b	4636a	<0.001	19.07	1330	1332[j]	88; 101; 115
Ethyl lactate	63719b	75267a	67521ab	72899a	0.006	19.33	1336	1331[h]	45; 75
1-Hexanol*	105508d	126685c	145225b	162126a	<0.001	20.08	1353	1354[e]	56; 69; 84
unk-49	1090a	1293a	1199a	1112a	0.962	20.73	1368	-	59; 70; 74
unk-53	1958a	1147ab	NDb	718b	0.004	21.47	1384	-	69; 74; 87
5-Nitro-1-benzofuran	3065a	2858ab	2337ab	1366b	0.028	22.21	1401	-	135; 151; 179
unk-56	NDc	NDc	5760b	37077a	<0.001	22.47	1408	-	67; 85; 119; 137; 151; 166
unk-57	NDb	NDb	973b	16197a	<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	21522b	21197b	22263b	28895a	<0.001	24.50	1456	1450[e]	70; 99; 117
6-Methyl-5-hepten-2-ol*	NDc	NDc	7927b	49266a	<0.001	24.82	1463	1473[k]	69; 95; 110; 128
unk-70	NDb	NDb	NDb	2447a	<0.001	26.38	1501	-	55; 67; 81; 93; 121; 136
Ethyl nonanoate	4120b	4149b	5225a	5274a	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	NDb	NDb	548b	13414a	<0.001	28.41	1551	-	68; 81; 97
1-Octanol	4525b	4731b	6239b	9447a	<0.001	28.66	1558	1547[h]	56; 70; 84
unk-87	10928a	11539a	1549b	1974b	0.001	29.27	1573	-	45; 57
2-Methyl-1-benzofuran*	NDb	NDb	NDb	5539a	<0.001	29.35	1575	-	55; 77; 103; 131

6-Methyl-3,5-heptadien-2-one*	NDb	NDb	NDb	9067a	<0.001	29.46	1578	1587[l]	55; 81; 109; 124
unk-92	2503a	1200a	1990a	2690a	0.327	30.33	1600	-	42; 56; 86
p-Menth-1-en-9-al	NDb	NDb	565b	12099a	<0.001	31.20	1622	1620[m]	60; 79; 94; 152
Ethyl decanoate	3115933a	2784211ab	2558398b	2703806b	0.002	31.73	1636	1630[f]	70; 88; 101. 115; 155; 200
Isoamyl octanoate	39241a	31711b	29682b	32912b	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	43343c	59009a	51065b	52114b	<0.001	32.97	1669	1690[j]	74; 101; 129
Ethyl 9-decenoate	13824b	33727a	19318b	10116b	<0.001	33.62	1686	1685[h]	69; 88; 101; 110; 135;152
Methionol	5242b	7374a	5094b	2404c	<0.001	34.32	1705	1738[f]	57; 61; 75; 106
1,1,6,trimethyl-2H-naphthalene (TDN)*	NDb	NDb	NDb	2966a	<0.001	34.99	1723	1722[h]	142; 157; 172
unk-107	NDb	NDb	NDb	19326a	<0.001	35.12	1727	-	43; 67; 81; 96
unk-109	NDb	525b	838b	3415a	<0.001	36.39	1762	-	41; 55; 70; 82
Phenethyl acetate*	56703b	70654a	51774b	52336b	<0.001	37.72	1799	1803[e]	43; 65; 77; 91; 104
ß-Damascenone*	5169b	5150b	6707a	7195a	<0.001	37.94	1805	1832[f]	69; 77; 91; 105; 121; 190
Hexanoic acid	56967ab	50521b	53309b	65971a	0.001	39.04	1837	1840[e]	60; 73; 87
Ethyl dodecanoate	385821a	317100b	288677b	317128b	<0.001	39.17	1841	1835[h]	88; 101; 115; 157; 183; 228
Isopentyl decanoate	16411a	12273bc	9669c	12500b	<0.001	39.83	1860	1863[h]	70; 155; 173
Phenethyl alcohol*	1724285b	2061510a	1799951b	1692450b	<0.001	41.03	1895	1896[h]	65; 91; 122
Ethyl tetradecanoate	5960a	4228b	4152b	5262a	<0.001	45.99	-	2040[h]	88; 101; 157
Octanoic acid	169961a	146431ab	138889b	164568ab	0.016	46.17	-	2051[h]	60; 73; 85; 101; 115; 144

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[a] Retention index reference source, indicated by superscript: d, Chida et al. 2004; e, Lee and Noble 2003; 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; m, Shimoda et al. 1996.

[b] Mean compound area with identical letters are not significantly different via Tukey's honestly significantly difference test.

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

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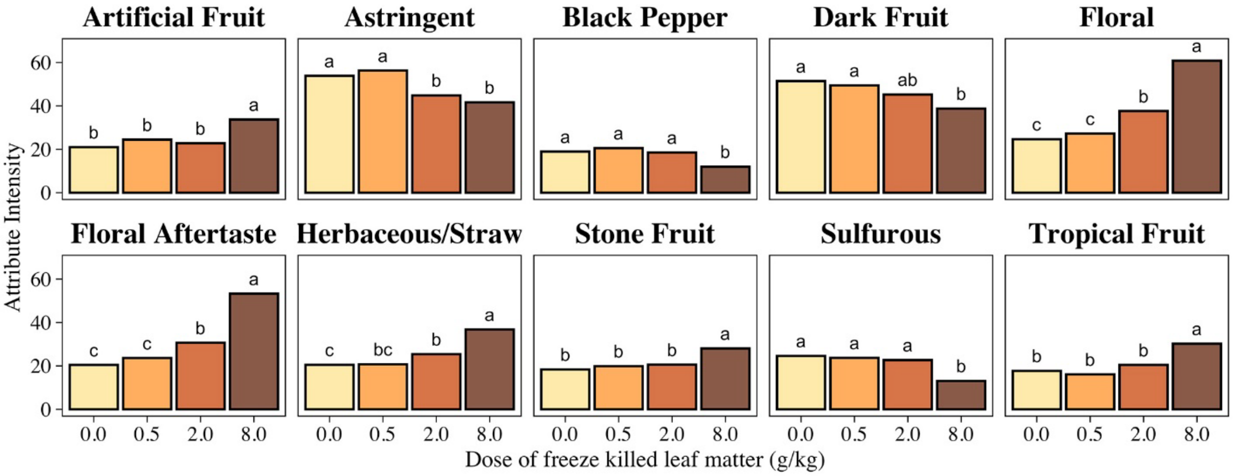

Figure 1 Mean attribute intensity for each significant attribute showing an effect of freeze killed leaf material dosage (0, 0.5, 2.0, 8.0 g/kg must) on Cabernet Sauvignon wines. Within each attribute, treatment additions are not significantly different by Tukey's honestly significantly difference test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

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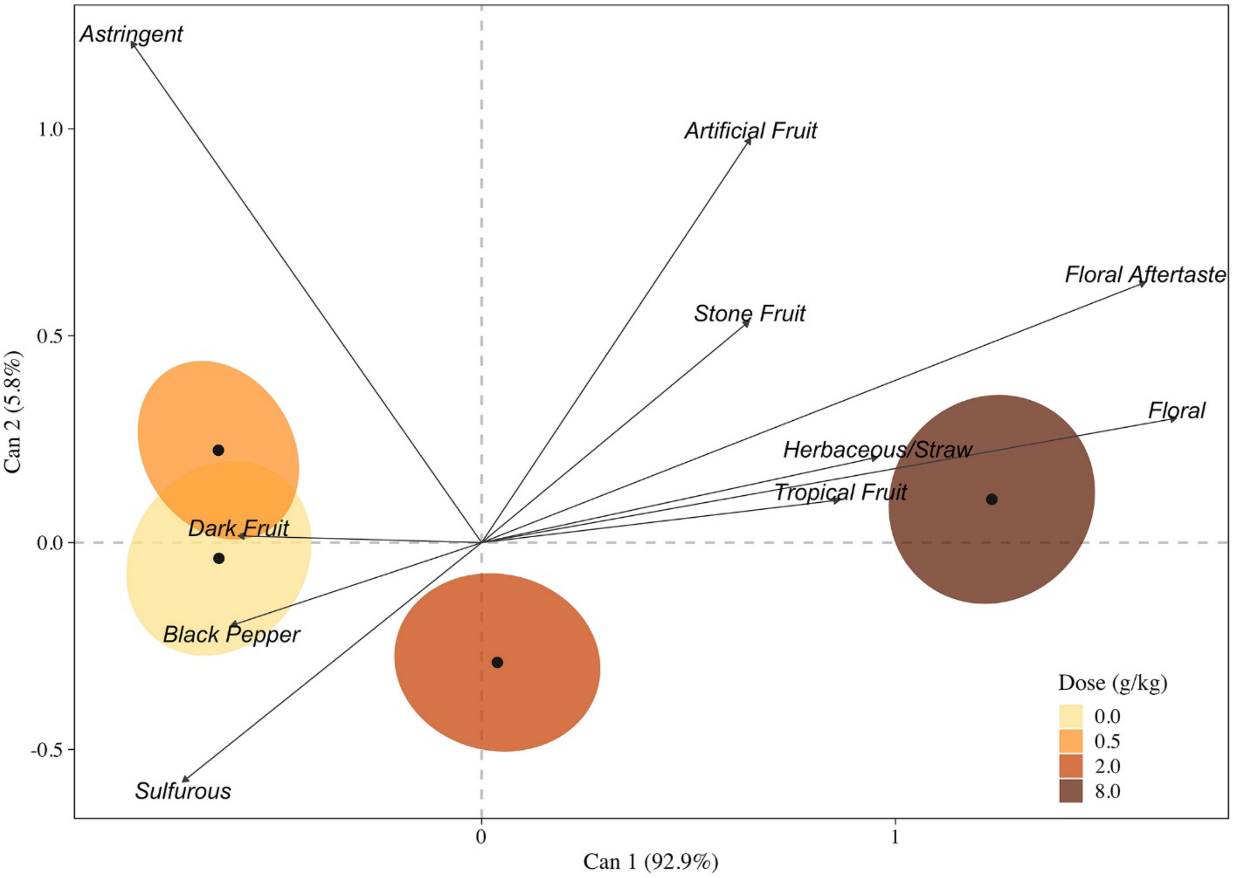

Figure 2 Canonical variate analysis (CVA) of Cabernet Sauvignon wines made from four different dosages of freeze killed leaf material (0, 0.5, 2.0, 8.0 g/kg must). The canonical variate scores for the four treatments are color coded and enclosed by a 95% confidence ellipse.

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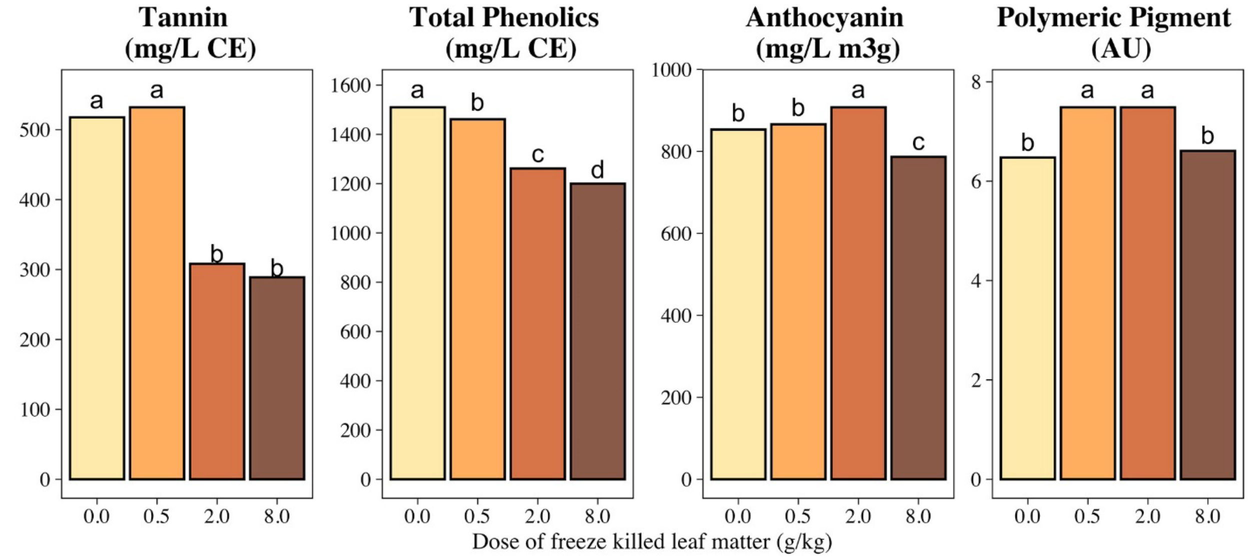

Figure 3 Mean concentration of four phenolic measures of Cabernet Sauvignon wines made with four different dosages of freeze killed leaf material (0, 0.5, 2.0, 8.0 g/kg must). Within each attribute, treatment additions are not significantly different by Tukey's honestly significantly difference test if they are labeled with the same letter. Tannins and total iron reactive phenolics (listed as total phenolics for space reasons) are measured in catechin equivalents (CE) whereas anthocyanins are expressed on a malvidin-3-glucoside equivalent (m3g) and polymeric pigments in A_{520nm} absorbance units (AU).

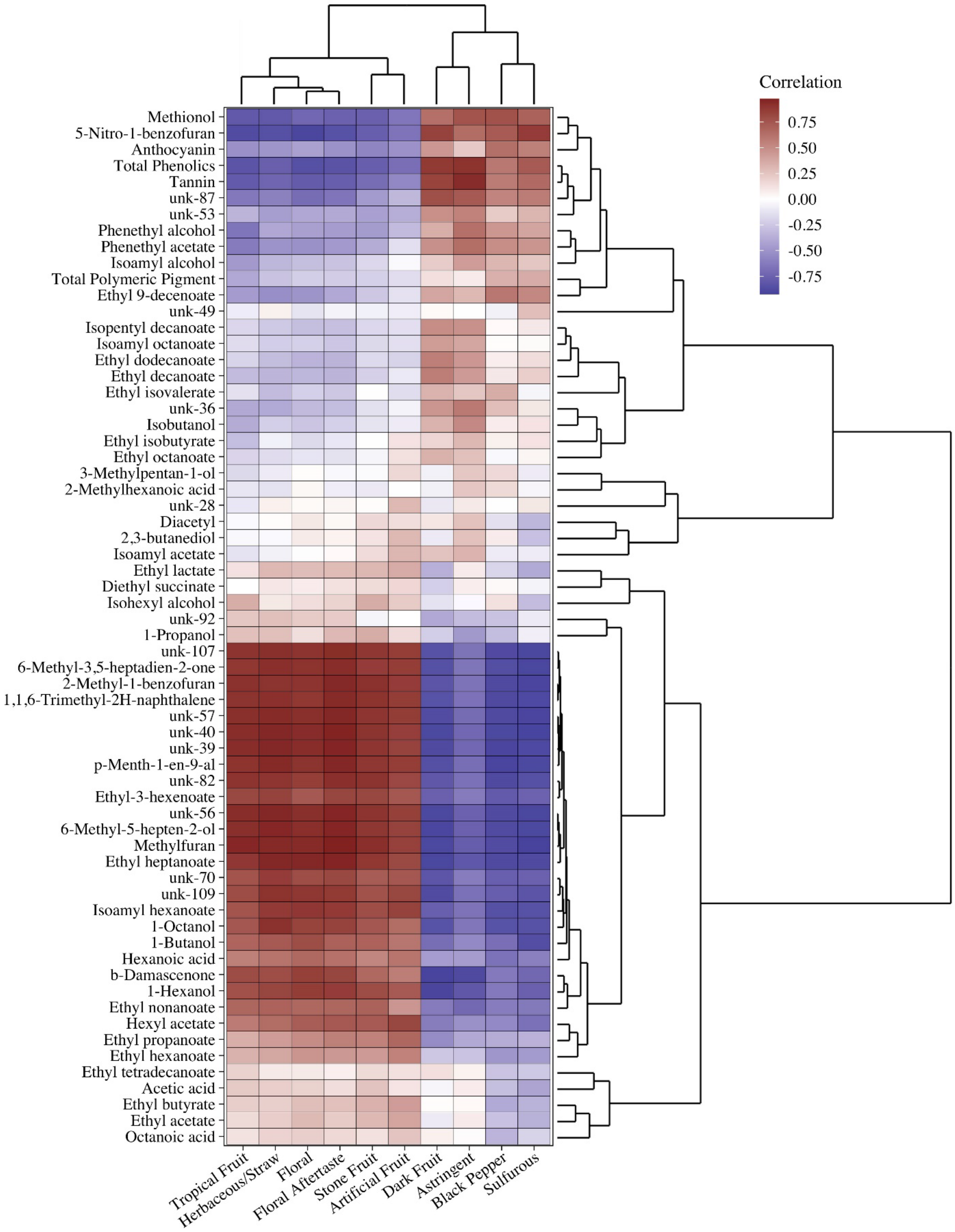
Correlation
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
Methionol
5-Nitro-1-benzofuran
Anthocyanin
Total Phenolics
Tannin
unk-87
unk-53
Phenethyl alcohol
Phenethyl acetate
Isoamyl alcohol
Total Polymeric Pigment
Ethyl 9-decenoate
unk-49
Isopentyl decanoate
Isoamyl octanoate
Ethyl dodecanoate
Ethyl decanoate
Ethyl isovalerate
unk-36
Isobutanol
Ethyl isobutyrate
Ethyl octanoate
3-Methylpentan-1-ol
2-Methylhexanoic acid
unk-28
Diacetyl
2,3-butanediol
Isoamyl acetate
Ethyl lactate
Diethyl succinate
Isohexyl alcohol
unk-92
1-Propanol
unk-107
6-Methyl-3,5-heptadien-2-one
2-Methyl-1-benzofuran
1,1,6-Trimethyl-2H-naphthalene
unk-57
unk-40
unk-39
p-Menth-1-en-9-al
unk-82
Ethyl-3-hexenoate
unk-56
6-Methyl-5-hepten-2-ol
Methylfuran
Ethyl heptanoate
unk-70
unk-109
Isoamyl hexanoate
1-Octanol
1-Butanol
Hexanoic acid
b-Damascenone
1-Hexanol
Ethyl nonanoate
Hexyl acetate
Ethyl propanoate
Ethyl hexanoate
Ethyl tetradecanoate
Acetic acid
Ethyl butyrate
Ethyl acetate
Octanoic acid
Tropical Fruit
Herbaceous/Straw
Floral
Floral Aftertaste
Stone Fruit
Artificial Fruit
Dark Fruit
Astringent
Black Pepper
Sulfurous

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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).

Supplemental Table 1 Descriptive analysis mean square error results

Factor	df	Artificial Fruit	Astringent	Baking Spices	Bitter	Black Pepper	Citrus	Cooked Veg
Judge	12	6552.3	3904.1	4341.9	9919.1	4475.2	5510.2	3411.3
Trt	3	3763.6	5797.7	42.5	216.5	1678.2	1306.8	327.2
senRep	2	559.5	420.2	75.9	362.3	10.6	434.0	217.1
Trt(fermRep)	8	336.6	319.7	78.4	345.5	154.3	130.5	93.9
Judge:Trt	36	849.5	645.2	206.3	195.1	291.4	731.9	150.1
Judge:senRep	24	412.2	639.1	102.1	340.3	172.1	250.6	329.0
Judge:Trt(fermRep)	96	222.9	241.6	78.4	211.9	155.5	121.2	88.2
senRep:Trt	6	128.9	313.5	129.3	309.8	256.4	212.5	148.4
senRep:Trt:fermRep	16	199.4	245.8	125.4	183.1	245.1	143.6	78.6
Residuals	264	292.1	314.5	109.6	170.8	162.1	160.4	100.4

	df	Dark Fruit	Dried Fruit	Floral	Floral Aftertaste	Fresh Green Veg	Herbaceous Straw	Hot
Judge	12	12209.1	6530.6	4261.5	5873.9	7031.6	7626.5	10699.6
Trt	3	3671.1	341.5	31784.5	25683.7	417.7	6808.4	241.2
senRep	2	471.5	442.8	266.8	205.7	35.5	38.5	32.1
Trt(fermRep)	8	124.8	266.9	686.6	321.8	122.2	154.8	224.3
Judge:Trt	36	779.0	494.9	1087.5	1081.5	282.1	1256.3	112.7
Judge:senRep	24	430.4	273.7	680.8	510.6	138.3	316.6	269.7
Judge:Trt(fermRep)	96	245.5	152.3	401.9	219.1	105.3	203.7	106.1
senRep:Trt	6	238.0	89.1	457.5	419.0	175.1	122.6	293.6
senRep:Trt:fermRep	16	510.7	119.4	357.0	239.0	172.5	147.0	188.5
Residuals	264	390.1	154.8	412.3	338.6	145.2	194.3	175.3

	df	Oxidized	Red Fruit	Sour	Stone Fruit	Sulfurous	Sweet	Tropical Fruit
Judge	12	2476.8	14161.4	9004.4	5237.2	6596.0	5684.7	4832.7
Trt	3	90.5	488.7	266.4	2188.5	3348.7	191.9	4696.4

senRep	2	141.2	262.1	257.7	134.6	151.5	166.5	722.2
Trt(fermRep)	8	31.8	513.5	755.7	173.6	234.5	183.6	215.9
Judge:Trt	36	73.4	679.6	306.4	654.3	482.9	383.9	893.2
Judge:senRep	24	64.3	393.7	518.1	148.1	276.6	190.1	394.4
Judge:Trt(fermRep)	96	50.9	377.6	290.6	147.9	223.1	142.9	214.0
senRep:Trt	6	110.4	339.6	235.8	52.5	312.7	71.5	391.6
senRep:Trt:fermRep	16	72.1	584.9	573.0	143.4	76.2	167.6	219.8
Residuals	264	79.4	311.8	267.6	167.4	209.6	168.9	212.3

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Supplemental Table 2 Polyphenolic measures, results by fermentation replicate

Dose (g/kg FLKM)	Fermentation Replicate	Tannin (mg/L GAE)	Total Phenolics (mg/L GAE)	Anthocyanin (mg/L M3G)	Polymeric Pigment (AU)
0.00	1.00	523 (15)	1506 (34)	786 (6)	6.6 (0.0)
0.00	2.00	490 (7)	1460 (6)	874 (13)	6.6 (0.1)
0.00	3.00	539 (11)	1564 (9)	900 (11)	6.2 (0.1)
0.50	1.00	543 (17)	1464 (25)	834 (5)	7.4 (0.2)
0.50	2.00	536 (13)	1460 (50)	876 (11)	7.7 (0.2)
0.50	3.00	517 (11)	1460 (21)	888 (4)	7.4 (0.1)
2.00	1.00	367 (22)	1336 (9)	890 (34)	7.7 (0.1)
2.00	2.00	290 (3)	1236 (54)	919 (9)	7.5 (0.2)
2.00	3.00	268 (7)	1213 (36)	914 (3)	7.3 (0.1)
8.00	1.00	275 (3)	1203 (19)	700 (11)	6.8 (0.2)
8.00	2.00	313 (11)	1235 (32)	799 (19)	6.5 (0.1)
8.00	3.00	279 (17)	1162 (13)	861 (28)	6.6 (0.0)

*GAE – Gallic acid equivalent; Standard error found in parenthesis

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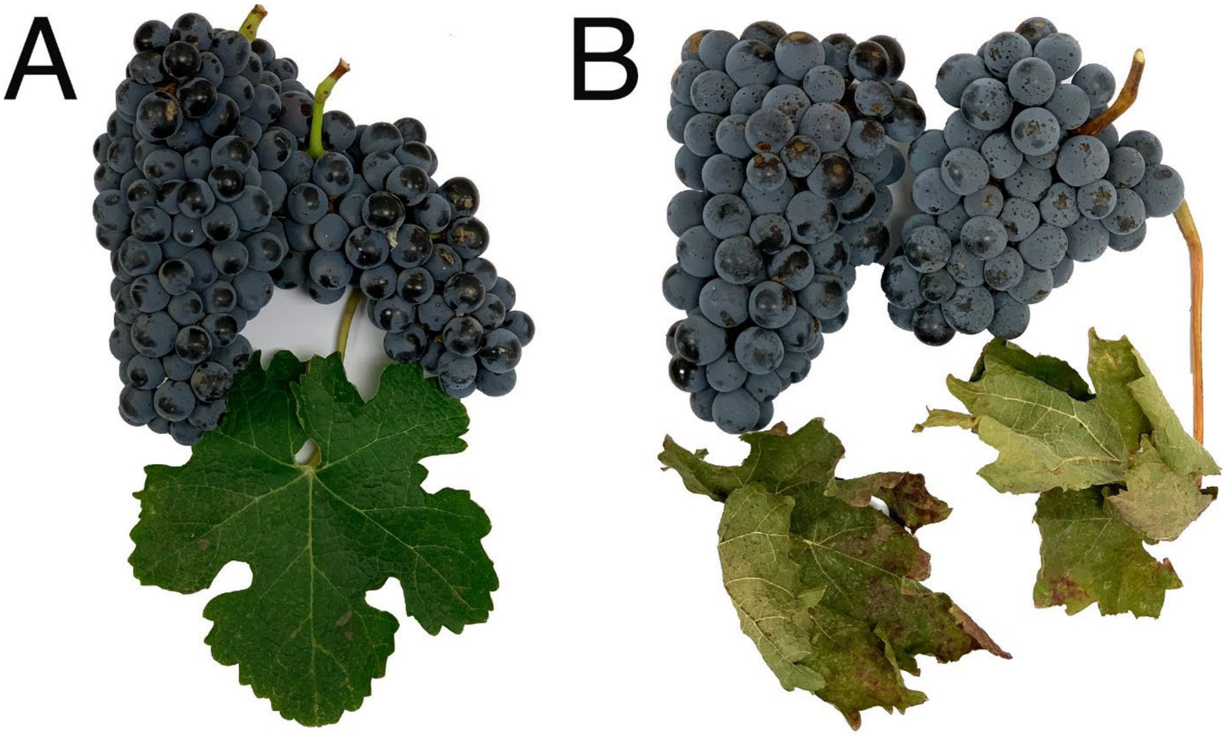

Supplemental Figure 1 Cabernet Sauvignon clusters and leaves harvested from the same vineyard pre- (A) and post-frost (B) showing healthy and freeze killed leaves.