OENO One
vine & wine

DOI: https://doi.org/10.20870/oeno-one.2024.58.1.7736

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Descriptive temporal sensory properties and volatile composition of Pinot noir wines produced with contrasting alcoholic fermentation temperatures and cap management regimes

Emily S. Stoffel[1,2,3], Taylor M. Robertson[3], Anibal A. Catania[4], Luis F. Castro[2] and L. Federico Casassa[3*]

[1] E. & J. Gallo Winery, Healdsburg, CA, 95448, USA
[2] Food Science & Nutrition Department, California Polytechnic State University, San Luis Obispo, CA, 93407, USA
[3] Wine & Viticulture Department, California Polytechnic State University, San Luis Obispo, CA, 93407, USA
[4] Centro de Estudios de Enología, Estación Experimental Agropecaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (INTA), San Martín 3853, 5507 Luján de Cuyo, Mendoza, Argentina

*correspondence:
lcasassa@calpoly.edu

Associate editor:
Stamatina Kallithraka

Received:
2 September 2023

Accepted:
4 January 2024

Published:
29 January 2024

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ABSTRACT

Pinot noir is rapidly becoming a winemaking staple in the Central Coast of California. Therefore, it is important to understand how factors such as fermentation temperature and cap management affect the chemical and sensory impact of such wines. Herein, Pinot noir wines were made with three contrasting alcoholic fermentation temperature regimes (Cold, Cold/Hot, Hot) and combined factorially with two cap management regimes (with or without punch downs). The impact of these factors on colour, aroma, and the temporal retronasal aroma and mouthfeel profile was assessed by a trained sensory panel (n = 13) using descriptive analysis (DA) and time-intensity (TI), respectively. Combining a hot fermentation temperature and no punch downs led to wines with higher colour saturation and purple hue. In contrast, wines fermented at cold fermentation temperatures with no punch downs showed reduction aromas. In terms of individual main effects, wines fermented at cold temperatures had more orthonasal aroma, while wines fermented at hot temperatures had higher astringency. To understand the relationship between selected volatile aromas and perceived sensory results, a partial least square regression (PLSR) was conducted. PLSR indicated that cold fermentation temperature wines with punch downs were associated with esters, including ethyl hexanoate and ethyl heptanoate, as well as β-damascenone, which aligned with sensory results. The effect of salivary flow rate on the temporal sensory profile post-expectoration was also analysed. Low salivary flow rate panellists perceived both astringency and length significantly later and more intensely than high salivary flow rate panellists. However, the time of maximum intensity was perceived significantly later for high salivary flow rate panellists. Overall, fermentation temperature had a more significant impact on the sensory and volatile composition of Pinot noir wines than the cap management regime. However, cap management still appeared to affect sensory and volatile chemistry results through significant interactions. Therefore, the relationship between fermentation temperature and cap management was not straightforward from a volatile chemistry or sensory perspective. The importance of accounting for salivary flow rate in panellists when using time-based sensory analysis was also highlighted.

KEYWORDS: Pinot noir, descriptive analysis, time-intensity, salivary flow rate, fermentation temperature, cap management, volatile chemistry

INTRODUCTION

Alcoholic fermentation temperature and the choice of cap management protocol are arguably the two key winemaking decisions for the production of red wines. The choice of fermentation temperature dictates the rate of sugar consumption during alcoholic fermentation, the evolution and diversity of yeast populations, phenolic extraction, and ultimately, the sensory characteristics of the resulting wine (Casassa 2017, Massera *et al*. 2021, Pérez-Navarro *et al*., 2018, Sacchi *et al*. 2005, Şener, 2018). It is during alcoholic fermentation and maceration that phenolic extraction occurs, which influences colour, taste, and mouthfeel characteristics (Casassa and Harbertson, 2014). Generally, applying a warm-to-hot fermentation temperature regime (25 °C to 30 °C) favours higher phenolic extraction, thus resulting in a wine with higher astringency and colour (Sacchi *et al*., 2005). For example, an experiment examined the impact of cold (10 °C), hot (25 °C) and variable (seven days at 10 °C and seven days at 25 °C) fermentation temperatures in three clones of Pinot noir over two vintages (Reynolds *et al*., 2022). Hot fermentation temperatures produced wines with higher phenolics, including anthocyanins, tannins, and polymeric pigments compared to the other treatments. However, no formal sensory analysis was performed on these wines. Research in fermentation temperature has also been shown to influence the volatile chemistry and sensory aroma profile of the resulting wines. As an illustration of the latter, a study conducted on Merlot compared wines fermented at a low temperature (15 °C) against a control treatment (25 °C) using five different strains of cold-tolerant yeasts (Massera *et al*., 2021). Descriptive sensory analysis showed that wines with low fermentation temperatures, regardless of the yeast strain, had higher aroma intensity, specifically red fruit and banana aromas. Solid-phase microextraction supported these findings, as the low-temperature wines had a higher concentration of esters than the control wines. Similarly, an experiment on Pinot noir wines examined the impact on sensory and volatile composition of cold (20 °C), hot (30 °C) fermentation temperatures, and hot-cold, which consisted of a high-temperature short-time treatment of 90 °C for 1 minute on the must, then a fermentation temperature 15 °C of juice and must (Girard *et al*., 1997). Sensory descriptive analysis showed that hot fermentation temperatures (30 °C) led to higher intensities of cherry and currant orthonasal aromas, and a higher currant flavour compared to the other treatments. Analysis of volatile compounds showed that the hot treatment also had a higher concentration of acetic acid. The main overarching limitation of the previous studies was the reliance on chemical analysis or static sensory methods to assess the influence of fermentation temperature on the dynamic sensations of flavour and mouthfeel.

Research on the temporal sensory impact of cap management regimes has been recently conducted. For example, a study compared the impact of eight-week extended maceration, submerged caps, and punch downs in Merlot wines. Results indicated that punch downs produced wines with significantly lower levels of tannins relative to the other treatments (Frost *et al*., 2018b). Sensory descriptive analysis showed that the punch down treatment also had the lowest intensity score for astringency, but it was not significantly different from the submerged cap treatment. However, the Temporal Dominance of Sensations (TDS) curves of the wines post-expectoration (after 15 seconds) showed that punch down wines were not different from the other treatments, suggesting the treatments had similar levels of dominance but different intensities (Frost *et al*., 2018a). A limitation of the previous study was that it lacked a treatment where no cap management was performed to compare the other cap management techniques. It is important to note that the previously mentioned study utilised qualitative temporal sensory methods.

Research on sensory aspects resulting from fermentation temperature and cap management has traditionally relied almost exclusively on the use of descriptive analysis, with less emphasis placed on temporal sensory methods. One such dynamic sensory method is known as time-intensity (TI). This method is a time-based version of descriptive analysis whereby individuals rate the intensity of an attribute on a 10 or 15-cm line scale (Meilgaard *et al*., 2016). A major advantage of using TI is that the continuous quantification of the selected attributes provides a complete picture of how the attribute's intensity is perceived over time (Peyvieux and Dijksterhuis, 2001). However, the method is limited in that only one or two attributes can be measured at a time (Cliff and Heymann, 1993). Also, the limited number of attributes can lead to the sensory error of dumping or conflating unrelated sensory attributes (Clark and Lawless, 1994). Despite these limitations, TI is an established method in wine and has been predominately used on model wines or wines that were spiked with the target compounds to be studied. For example, TI was utilised to examine the impact of sucrose on astringency perception, and it was reported that this detection in model wines could be suppressed by increasing the level of sucrose (Ishikawa and Noble, 1995). Alternatively, research conducted on de-alcoholised Syrah wines utilised a factorial design for ethanol, tannin concentration, and three added flavour compounds to understand their impact on wine finish over time using TI (Baker and Ross, 2014). It was concluded that the high ethanol wines showed enhanced flavour persistence or length but only in wines containing a single compound (coconut and floral). Studies with model and spiked wines are useful for understanding the wine matrix in a controlled environment. However, these wines do not reflect the sensory characteristics resulting from the winemaking process.

Indeed, few studies have explored TI procedures while also attempting to further the knowledge of the sensory impact of specific winemaking techniques. One such study examined the impact of pre-fermentative skin contact time, whole cluster pressing, and fermentation on skins on the bitterness perception in Gewürztraminer and Riesling wines using TI. While there were no significant differences among treatments for the Riesling wines, Gewürztraminer wines fermented on the skins had significantly higher maximum intensity (I_{max})

and area under curve (AUC) for bitterness compared to the other treatments (Sokolowsky *et al*., 2015). However, this study was conducted on aromatic white wines, so the results cannot be extrapolated to red wines due to different levels of phenolics, particularly tannins and monomeric flavan-3-ols. Overall, understanding the temporal retronasal and mouthfeel profile of wine is important because consumers assess wine quality while tasting over other modalities such as colour or aroma (Charters and Pettigrew, 2007). As such, profiling sensory characteristics over time is the first step leading towards hedonic and quality assessment (Francis and Williamson, 2015).

Additionally, previous research has suggested the importance of accounting for the confounding factor of salivary flow rate. Salivary flow rate is an inherent physiological characteristic that is highly variable within humans (Neyraud *et al*., 2012). Both astringency and retronasal aromas can be impacted by the time and intensity of perception based on categorical salivary flow rate. Generally, individuals with a low salivary flow rate perceive astringency later and more intensely compared to those with a high salivary flow rate, who perceive astringency earlier and with lower intensity (Fischer *et al*., 1994; Ishikawa and Noble, 1995; Lesschaeve and Noble, 2005). The interactive effect, if any, between salivary flow rate and retronasal aroma perception is not as straightforward and appears to depend on the volatile compound assessed (Baker and Ross, 2014). For example, a study evaluated the impact of salivary flow rate on retronasal aroma in wine with added esters to Tempranillo and Verdejo-based rosé wines. A significant correlation was established between salivary flow rate and perceived intensity of aromas over time for the short-chain esters (isoamyl acetate, ethyl butanoate, ethyl hexanoate) studied. Panellists with higher salivary flow rates perceived the retronasal aromas more intensely than those with low salivary flow rates (Criado *et al*., 2019). Due to its impact on perception, it is important to account for salivary flow rate as a factor when conducting temporal sensory analysis on wine.

The present experiment aimed to determine the impact of three contrasting fermentation temperature regimes and two cap management protocols on the dynamic sensory properties and volatile composition of Pinot noir wines from the Central Coast of California (USA). A secondary objective was to assess the effect of salivary flow rate on the perception of astringency and retronasal aroma perception.

MATERIALS AND METHODS

1. Wines

Pinot noir grapes (clone 667) were sourced from the Bassi Vineyard in Avila Beach, California (USA). Winemaking treatments followed a three-by-two full factorial design structure. The first factor was alcoholic fermentation temperature at Cold (12 °C to 17 °C), Hot (28 °C to 32 °C), or Cold/Hot (seven days fermented cold followed by seven days fermented hot). The second factor was cap management in the form of two punch downs daily (PD) or no punch downs at all (No PD). All treatments were performed in triplicate. The winemaking protocol followed that of a previously published study (Reynolds *et al*., 2022), with the following modifications (Casassa *et al*., 2023; Stoffel *et al*., 2023b). Fruit was manually harvested and transported to Cal Poly Pilot Winery for processing the same day. Clusters were processed using a crusher–destemmer (Bucher Vaslin, Niederweningen, Switzerland), and the resulting musts went immediately into 60 L food-grade fermenters (Speidel, Swabia, Germany) at a rate of 50 kg per vessel. 50 mg/L of sulfur dioxide (SO_2) was added to each fermenter immediately post-crushing. Three different fermentation temperature regimes, combined with two contrasting cap management regimes, were established: Cold (targeting an average fermentation temperature of 12 °C), Cold/Hot (fermentation temperatures of 12 °C from day 0 to 7 and 28 °C from day 8 to 14), and Hot (targeting an average fermentation temperature of 28 °C throughout). In addition, two contrasting cap management regimes per fermentation temperature regime were established: no punch down (No PD), whereby the fermenters received no cap management during the fermentation/maceration period. Punch down (PD), whereby fermenters received two daily manual punch downs (9:00 and 17:00 hrs), each lasting one minute. All individual treatments were established in triplicate (n = 3). Musts were inoculated with dry yeast (Enartis D20, 153 Windsor, CA, USA) at a rate of 40 g/hL, 6 h after crush, and fermenters were taken to their respective fermentation conditions. Total maceration time was set to 14 days.

Cold fermentation temperature wines were placed in a temperature-controlled cold room set at 10 °C for the duration of the maceration period, while Hot fermentation regime wines were established by placing the fermenters in a temperature-controlled room set at 26 °C. The Cold/Hot fermentation regime treatments were established by placing the fermenters for seven days in a temperature-controlled cold room set at 12 °C and were then subsequently transferred to a warmer environment consisting in a temperature-controlled room set at 26 °C for the last seven days of the maceration period. Diammonium phosphate (DAP) was added on day two of alcoholic fermentation to all fermenters at a rate of 200 mg/L (Fermaid K, Scott Laboratories, Petaluma, CA, USA). Total soluble solids (°Brix) and daily temperature were monitored via a densitometer (Anton Paar DMA 35 Basic, Graz, Austria) during the duration of the 14-day maceration period, after which wines were pressed off the fermentation solids. Free-run wines were transferred to 18 L glass carboys. Wines were subsequently inoculated with *Oenococcus oenii* (VP41, Scott Laboratories, Paso Robles, CA, USA) at a rate of 20 g/hL to undergo malolactic fermentation. Malolactic fermentation was monitored via enzymatic analysis of L-malic acid and L-lactic acid with an enzymatic analyser (Admeo Y15, Angwin, CA, USA) using commercial enzymatic analysis kits (Biosystems, Barcelona, Spain). Malolactic fermentation was completed in all the wines (malic acid <0.1 g/L). Cold stabilisation occurred at 10 °C for 30 days, after which all wines were racked and adjusted to 30 mg/L SO_2. The wines were filtered through

8 µm cellulose filter pads (Vintner's Vault, Paso Robles, CA, USA) and readjusted to 30 mg/L SO_2. The wines were bottled in 750 mL bottles in February 2022 using DIAM 5 micro-agglomerated cork closure (G3 Enterprises, Modesto, CA, USA; oxygen transmission rate; 0.4 ng/bottle/year; oxygen initial release: 1.3 mg), stored in a vertical position and kept in cellar-like conditions (approximately 12 to 14 °C) until analyses. Wines received three months of bottle ageing prior to sensory analysis. The evolution of basic chemistry, colour, and phenolic results for the wines of the present study are detailed in a companion paper (Casassa *et al*., 2023).

2. Sensory analysis

The wines were subjected to general descriptive analysis and time-intensity sensory analysis. Panel training was conducted over five sessions, each lasting an hour and a half to two hours, in March and April 2022. The members of the sensory panel (n = 13) were recruited by e-mail, and the project received California Polytechnic State University Institutional Review Board approval (IRB protocol #2020-058). All panellists had previous experience with sensory panels or wine knowledge. The panel was composed of 62 % females and 38 % males, with an age range of 21 to 70 years old. Panellists were screened for PROP (6-n-propylthiouracil) sensitivity, visual disorders, and salivary flow rate. Sensitivity to PROP was assessed using 6-n-propylthiouracil (Fluka, Lot #: 1129436, Steinheim, Germany) following the procedure known as the PROP test (Tepper *et al*., 2001). The panel was composed of 15 % super-tasters, 54 % medium-tasters with 31 % non-tasters. Additionally, panellists were screened for colour perception deficiencies through pseudo-isochromatic colour testing plates (Ishihara maps). None of the panellists showed colour deficiencies based on this test. The stimulated saliva collection procedure and calculation of salivary flow rate were performed as previously reported (Stoffel *et al*., 2023a; Stoffel *et al*., 2023b). Saliva collection occurred in the afternoon at the beginning (15:00 or 17:30 hr) of three training sessions.

2.1. Panel training

Descriptive analysis and time-intensity training focused on the evaluation procedure, attribute definition, standard review, and assessment of intensity. Fifteen attributes, which encompassed colour, aroma, taste, and mouthfeel, were selected by the panel. Attributes with standard compositions used during training are shown in Supplementary Table 1. For time-intensity evaluation, two attributes were selected by the panel: overall astringency and retronasal aroma length (length). Overall astringency was defined as *"the overall sensation of drying, puckering, and roughening in the mouth and upper lip and its sub-qualities"*. Length was defined as *"lingering jam and dried fruit retronasal aromas after expectoration of the wine"*.

Initial training sessions focused on the identification of standards and the detection of the attributes in the research wines. The remaining training sessions incorporated the use of intensity assessment of attributes with a 15 cm, unstructured line scale and practice with the TI evaluation procedure (Supplementary Figure 1). The TI task lasted sixty seconds, with intensity scores recorded continuously by the data collection software. If panellists no longer perceived the attribute being evaluated prior to 60 seconds, they were instructed to rate the intensity as zero for the remainder of the task. Finally, the last two training sessions had the panellists practice the use of the sensory software prior to formal evaluation. During training sessions, the evaluation of colour was conducted separately in clear ISO glasses, while all other attributes were assessed using black ISO glasses to mask the variation in colour among treatments and avoid bias due to colour.

2.2. Formal evaluations

Formal testing occurred over four sessions in May 2022. Formal evaluations were conducted in individual sensory cabinets. All replicates were evaluated in triplicate. For descriptive analysis testing, 30-mL aliquots of samples were served. For TI assessment, samples were served in 15-mL aliquots. All samples, regardless of the sensory method, were served at room temperature in clear ISO glasses with 4-digit random codes and an aluminium foil top. Wines were served monadically either under red lighting for aroma, taste, flavour, and mouthfeel assessment (Luna 3AO, 18:18W, Zaniboni Lighting, Clearwater, FL, USA) or under daylight setting (Luna 3, 26:26W, Zaniboni Lighting, Clearwater, FL, USA) for colour assessment. The order of serving was randomised according to William's Latin Square Design. The assessment used a 15 cm line scale anchored at 1 cm and 14 cm with the terms "low" and "high", respectively.

To lower bias, colour and the TI attributes were assessed separately from aroma, taste, and mouthfeel descriptive analysis attributes. This resulted in four separate tests with nine wines per test. The run order of the wines was randomised per test and per session. Panellists assessed wines following the evaluation procedures described during training, with a 10-minute break halfway through the testing session. Following each sample, there was a mandatory 15-second palate-cleansing break. Unsalted crackers (Nabisco unsalted tops, premium saltine crackers, East Hanover, NJ, USA) and water (Evian natural spring water, Evian, France) were provided for palate cleansing. All sensory data collection was carried out using RedJade Sensory Software (RedJade, Silicon Valley, CA, USA).

3. Volatile compound analysis

Untargeted volatile compound analysis was carried out on an 8890 gas chromatography system with a 7000D triple quadrupole GC/MS mass spectrometer detector (Agilent, Agilent Technologies Inc, Santa Clara, CA, 95051, USA) as well as an MPS autosampler (Gerstel, Linthicum, MD, 21090, USA) using a DB-Wax capillary column, 30 m, 0.250 mm, 0.25 µm (Agilent, Agilent Technologies Inc, Santa Clara, CA, 95051, USA). Helium was used as the carrier gas at a constant flow of 1.33 mL/min. Both solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) methods were utilised. SPME was used to quantify the compounds isoamyl alcohol, isobutanol, ethyl lactate,

TABLE 1. Time-intensity parameters and definitions.

Time Parameter	Definition
Onset time	Time of the first perception of an attribute
Onset value	Intensity of the first perception of an attribute
T_{max}	Time of maximum intensity
I_{max}	Maximum intensity rating
Area under curve	Area before and after the maximum intensity of perception
Extinction value	Intensity of attribute at last time measurement
Duration time	Duration of perception for an attribute

and phenylethyl alcohol. Sample preparation, sampling, and instrument analysis followed a previously outlined procedure (Hjelmeland *et al*., 2013). A modification to this procedure was that samples were heated to 40 °C and agitated for 10 minutes at 250 rpm before sampling by the SPME fibre. The SBSE method was based on a previously described procedure (Alves *et al*., 2005) with modifications for a DB-Wax capillary column (Stoffel *et al*., 2023b). Standards of the volatile compounds to be quantified were purchased from Acro's Organics (Thermo Fisher Scientific, Fair Lawn, NJ, USA), Alfa Aesar (Thermo Fisher Scientific, Fair Lawn, NJ, USA), Fischer Chemical (Thermo Fisher Scientific, Fair Lawn, NJ, USA), Sigma Aldrich (Sigma Aldrich, Sigma Aldrich Inc., St. Louis, MO, USA), Spectrum (Spectrum Chemical MFG. Corp., Gardena, CA, USA), and TCI (Tokyo Chemical Industry Co., LTD., Portland, OR, USA). Reference compound information, including CAS number, manufacturer, and purity, is provided in Supplementary Table 2.

Stock solutions of volatile standards to optimise compound separation and calculate the response factor for quantification were prepared in 95 % ethanol. Reference compounds were grouped according to typical concentration in wines to create ten stock solutions with no more than five reference compounds per stock solution and 50 µg/L of 2-undecanone (Sigma Aldrich, Sigma Aldrich Inc., St. Louis, MO, USA), which was used as the internal standard for all compounds (Hjelmeland *et al*., 2013). A model wine solution was utilised to optimise the separation of the volatile standards and calculate the response factor for each compound, which was based on a previously described procedure (Hjelmeland *et al*., 2013). Quantification of the volatile compounds in wine samples was conducted using the internal standard method as detailed in a previous procedure (Castro and Ross, 2015).

4. Statistical analysis

Data were analysed using a three-way ANOVA ($p < 0.05$) for the factors and interactions of fermentation temperature, cap management, as well as panellists who were designated as a random effect. The *post-hoc* comparison used for statistically significant variables was Fisher's LSD test. All ANOVA and *post-hoc* tests were conducted in XLSTAT 2022.1.2 (Addinsoft, Paris, France). A principal component analysis (PCA) using Pearson's correlation was constructed with R software (R Foundation for Statistical Computing, Vienna, Austria). Confidence ellipses were analysed using Hotelling's T2 test ($p < 0.05$) for each pair of products at 95 % confidence intervals and constructed with the SensoMineR package version 1.26 (Le and Husson, 2008). TI data were analysed as described above for DA but using selected time parameters (Table 1). Additionally, TI curves were constructed using the ggplot2 package (Wickham, 2016).

A two-way ANOVA for the main effects of fermentation temperature and cap management, as well as their interactions, was conducted on the SPME and SBSE data with Fisher's LSD as the *post-hoc* test. To understand the relationship between volatile chemistry and sensory results, a Partial Least Square Regression (PLSR) was conducted whereby sensory attributes were the dependent variable and volatile compounds analysed using SPME and SBSE methods were the independent variable (Noble and Ebeler, 2002). PLSR, ANOVA, and *post-hoc* tests for volatile chemistry data were conducted in XLSTAT 2022.1.2 (Addinsoft, Paris, France).

Panellist salivary flow rate data were analysed by a one-way ANOVA ($p < 0.05$) to determine the spread of variance in the panel. Based on the distance from above or below the overall mean, data was designated as high or low salivary flow rate (HF or LF) (Mialon and Ebeler, 1997). A separate one-way ANOVA ($p < 0.05$) was applied to the categorical salivary flow rate (LF or HF) to compare the salivary flow rate with length and overall astringency based on the selected time parameters (Table 1).

RESULTS AND DISCUSSION

1. Effect of fermentation temperature and cap management on perceived colour

Pinot noir is arguably one of the most fashionable varietals today, and as such, past and current research on many of its key chemistry and sensory aspects have been carried out (Casassa *et al*., 2021; Fang and Qian, 2006; Gao *et al*., 1997; Girard *et al*., 2001). In the present study, Pinot noir wines were fermented Cold (12 °C to 17 °C), Hot (28 °C to 32 °C), or Cold/Hot (seven days fermented cold followed by seven days fermented hot), with two punch downs per day or no punch

TABLE 2. Three-way analysis of variance (ANOVA) for the main effects of fermentation temperature and cap management as well as panellist with selected interactions showing the mean separation and *p*-values of descriptive sensory attributes of Pinot wines assessed by a trained panel (n = 13).

Treatments	Saturation	Purple hue	Overall aroma intensity	Jasmine	Red jam	Banana	Dried fruit	Meaty	Herbal	Mineral	Mushroom	Soil	Reduction	Body	Acidity	Bitter
Fermentation Temperature																
Cold	4.13 b*	5.61 b	8.97 a	3.92	5.79	5.13 a	6.84	4.28	3.76	4.21	4.22 a	3.67	4.13 a	4.87	7.41	4.53
Cold/Hot	6.99 a	8.52 a	7.94 b	3.92	5.78	4.57 b	6.84	3.96	4.25	4.71	3.56 b	3.62	2.65 b	5.25	7.54	4.68
Hot	7.43 a	8.45 a	7.55 b	4.00	6.23	4.18 b	7.00	3.71	4.16	4.56	3.51 b	3.60	2.58 b	5.00	7.74	4.37
p-value**	**<0.0001**	**<0.0001**	**<0.0001**	0.942	0.235	**0.003**	0.817	0.069	0.195	0.158	**0.009**	0.954	**<0.0001**	0.242	0.502	0.565
Cap Management																
PD	6.51 b	5.57 b	8.09	4.15	6.13	4.43	6.92	3.84	4.16	4.45	3.59	3.59	2.85 b	5.05	7.65	4.54
No PD	8.54 a	6.79 a	8.22	3.74	5.74	4.82	6.86	4.13	3.95	4.54	3.93	3.67	3.39 a	5.02	7.47	4.51
p-value	**<0.0001**	**<0.0001**	0.449	0.056	0.115	0.096	0.792	0.150	0.360	0.691	0.111	0.701	**0.006**	0.897	0.442	0.879
Fermentation Temperature x Cap Management																
p-value	**<0.0001**	**<0.0001**	**<0.0001**	0.562	0.177	**0.012**	0.809	0.080	0.461	0.570	**0.035**	0.991	**<0.0001**	0.535	0.825	0.920
Fermentation Temperature x Panellist																
p-value	**0.003**	**<0.0001**	**0.001**	0.659	**0.001**	**<0.0001**	**0.000**	**0.028**	**0.002**	0.313	**0.018**	**0.010**	0.259	**0.017**	0.941	**0.010**
Cap Management x Panellist																
p-value	**0.002**	0.812	0.248	0.075	0.056	0.570	0.070	**0.038**	**<0.0001**	**0.001**	0.303	**0.030**	**<0.0001**	**0.036**	0.747	0.578

*Different letters within columns indicate a significant difference for Fisher's least significant differences test ($p < 0.05$).

** Significant *p*-values are shown in bold fonts.

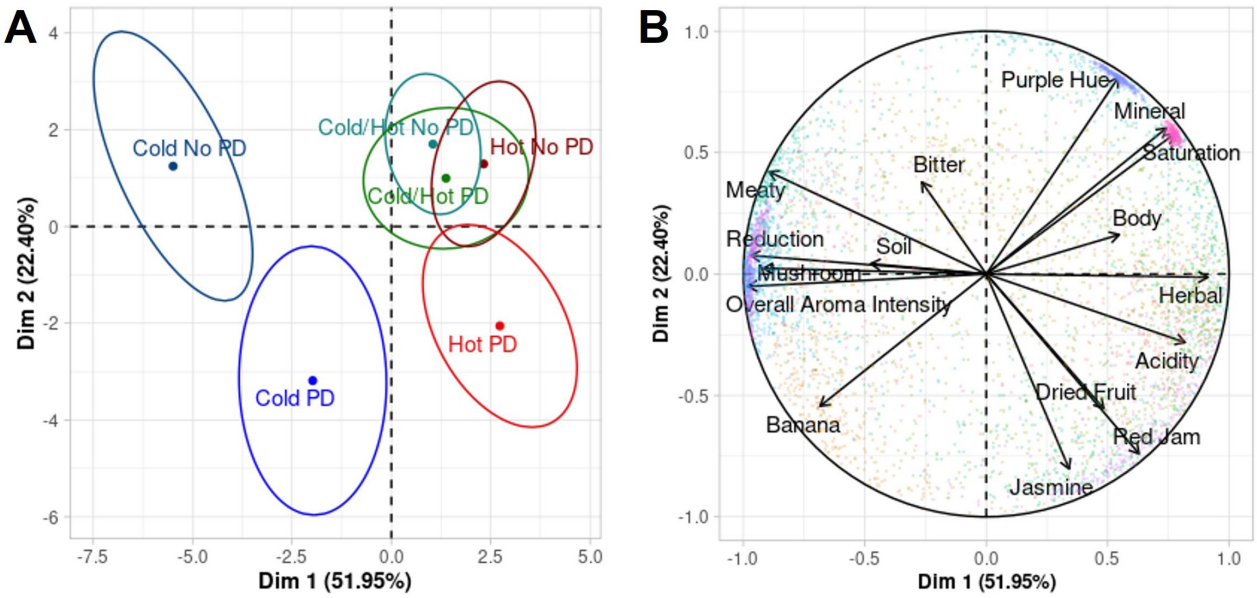


FIGURE 1. Principal component analysis of descriptive sensory data of Pinot noir wines evaluated by a trained sensory panel (n = 13). A: Confidence ellipses constructed using Hotelling's T2 test ($p < 0.05$) for each pair of products indicate 95 % confidence intervals. B: Sensory attribute loadings. Coloured dots represent individual data points with dots of the same colour corresponding to the replications of one sensory descriptor.

downs to understand the combined effect of fermentation temperature and cap management protocol on the sensory profile of the resulting wines. A three-way ANOVA of descriptive analysis results is shown in Table 2. Both colour parameters assessed, saturation and purple hue, were statistically significant for the main effects of fermentation temperature and cap management, as well as their interaction. The Cold/Hot and Hot wines had significantly higher saturation ($p < 0.0001$) and purple hue ($p < 0.0001$). For colour saturation, these results aligned with the chemical findings of a previous study that focused on the effect of fermentation temperature on three clones of Pinot noir wines reported that increasing fermentation temperature (10 °C to 25 °C) led to an increase in saturation according to CIE Lab results (Reynolds *et al*., 2022). A separate study that assessed both the chemical and sensory impacts of fermentation temperature and yeast type in Pinot noir from Canada showed that the high fermentation temperature (30 °C) and the yeast *Saccharomyces bayanus* produced the highest colour saturation according to CIE Lab results (Girard *et al*., 2001).

As for purple hue, past literature regarding fermentation temperature has focused almost exclusively on red hue assessment in sensory testing, or else a* within CIE Lab colour space (Reynolds *et al*. 2001). For red hue, it has generally been reported that a higher fermentation temperature (25 °C) led to a higher red hue (Sacchi *et al*. 2005, Şener 2018). However, a study that compared the chemical and sensory effects of a cold soak versus a control in six different varieties, including Pinot noir, utilised violet hue as an attribute for sensory assessment (Casassa and Sari, 2015). Sensory evaluation through descriptive analysis showed that the control wines, which were fermented at 24.5 °C, had significantly higher violet (purple) hue intensity compared to the cold soak treatment, which underwent a four-day cold soak at 9 °C followed by alcoholic fermentation conditions as the control. These treatments resemble the Hot and Cold/Hot treatments of the present study. Therefore, it stands to reason that herein, the wines subjected to the Cold fermentation treatment had the lowest purple hue, while the wines fermented at a Hot temperature had the highest purple hue, respectively.

For cap management, the No PD protocol wines led to significantly higher colour saturation ($p < 0.0001$) and purple hue ($p < 0.0001$). A principal component analysis (PCA) was conducted to understand the relationship between winemaking treatments and sensory attributes (Figure 1A,B). Dimension 1 (eigenvalue: 8.31; variability: 51.9) and dimension 2 (eigenvalue: 3.58; variability: 22.4) of the resulting PCA indicated that 74 % of the variation within the dataset was explained by the first two components. Indeed, both Cold/Hot and Hot wines with No PD protocols were associated with saturation and purple hue as well as the Cold/Hot PD wines. However, a previous experiment reporting on the effect of pump-over frequency and volume in Cabernet Sauvignon fermented at 25 °C showed different results from those reported in the present study (Lerno *et al*., 2018). The results of the previous study showed no significant differences between wines that did not receive pump-overs and those that received high volume and frequent pump-overs in all phenolics measured, including malvidin 3-glucoside.

The authors surmised that this was due to a natural mixing effect with the bottom of the cap by the carbon dioxide produced during alcoholic fermentation, as well as the small volume of the fermenters (100 L). The treatments of the past study resembled the Hot treatments of the present study; therefore, the mixing effect and the relatively small fermenter volume could explain the higher colour extraction resulting in high saturation and purple hue seen in the present study for the Hot No PD wines, that is, in the absence of cap management. This explanation paired with the fact that Pinot noir wines are known to have low colour (Mazza, 1995), could explain the discrepancies between studies. Indeed, Table 2 demonstrates a significant interaction between fermentation temperature and cap management for both saturation ($p < 0.0001$) and purple hue ($p < 0.0001$). Therefore, the results of the present study suggested that a combination of Cold/Hot and Hot fermentation temperatures combined with No PD protocol produced wines with increased intensity perception of purple hue and saturation for Pinot noir.

2. Effect of fermentation temperature and cap management on volatile chemistry and aroma

The three-way ANOVA indicated that four aroma attributes had statistically significant *p*-values (Table 2). Overall aroma intensity was statistically significant for the main effect of fermentation temperature ($p < 0.0001$), with a statistically significant interaction between fermentation temperature and cap management ($p < 0.0001$). Although the main effect of cap management was not statistically significant, there was a tendency for No PD wines to have higher average overall aroma intensity scores compared to PD wines. As such, Cold wines showed the highest perceived overall aroma intensity ($p < 0.0001$) and were significantly different from all other fermentation temperatures wines for that attribute (Table 2). Based on the herein instituted definition of overall aroma intensity, i.e., "*the intensity of all aromas in the wine prior to swirling*", the high overall aroma intensity in Cold wines could have been attributed to dissimilar attributes such as banana ($p = 0.003$), mushroom ($p = 0.009$), or reduction aromas ($p < 0.0001$) perceived in the wines as those attributes had significantly higher intensity scores compared to the Cold/Hot and Hot fermentation temperature wines. The PCA showed that the confidence ellipses of Cold wines were separated from the other treatments (Figure 1A,B). Both Cold wines were associated with overall aroma intensity. The Cold No PD wines were characterised by mushroom, meaty, and reduction aromas, while Cold PD wines were characterised primarily by banana aroma. Cold PD wines were different from the other treatments, including Cold No PD wines, as the confidence ellipses did not overlap (Figure 1A).

Indeed, the banana aroma was associated with Cold PD wines (Figure 1) and was significantly higher in intensity compared to the other fermentation temperatures (Table 2). However, there was a significant interaction between cap management and fermentation temperature for the banana aroma attribute ($p = 0.012$) (Table 2). While the main effect of cap management was not significantly different ($p = 0.096$), there was a tendency for the No PD wines to display higher banana aroma intensity. The banana aroma attribute was most likely attributed to the presence of esters. Table 3 shows a two-way ANOVA of selected volatile compounds (µg/L) analysed by SPME and SBSE methods whereby Cold wines had significantly higher concentrations of isoamyl acetate ($p = 0.002$), which is responsible for the banana odorant (Criado *et al*., 2019), compared to the other fermentation temperatures. However, the interaction between fermentation temperature and cap management was significant for isoamyl acetate ($p = 0.002$), indicating that while cap management was not statistically significant on its own, there was a tendency of the No PD wines to have a higher relative concentration of isoamyl acetate. This was the case with many of the esters assessed, with the exceptions of ethyl butyrate and ethyl lactate, both of which had significantly higher concentrations in PD wines. Additionally, diethyl succinate and phenylethyl acetate tended to have a higher concentration in PD wines, although it was not statistically significant. Indeed, the relationship between volatile chemistry and sensory perception in wine is often tenuous, and frequently, it is a mixture of volatile compounds that contributes to a specific perceived aroma in the wines (Prusova *et al*., 2022), a concept that has been termed odour (to contrast with "odorants", i.e., individual molecules). Bearing in mind the latter, the relationship between volatile chemistry and orthonasal descriptive analysis attributes, as well as the length TI attribute, were analysed through a PLSR (Figure 2A,B). Factor 1 (R^2X: 0.59; R^2Y: 0.40; Q^2: 0.08) and Factor 2 (R^2X: 0.75; R^2Y: 0.73; Q^2: 0.20) explained 73.0 % of the variation within the dataset. The x-axis roughly discriminated between PD and No PD wines but also was grouped as a function of fermentation temperature. As such, the Cold fermentation temperatures wines were more correlated with esters compared to the other fermentation temperature wines. Esters are produced as a result of yeast metabolism during alcoholic fermentation and are divided into two classes (acetate esters and ethyl esters) based on the pathway of their formation. Many factors affect ester formation, including yeast type, yeast nutrition, and fermentation temperature (Prusova *et al*., 2022). Low fermentation temperatures tend to result in a higher concentration of esters, likely due to the increased esterification of fatty acids, which can be toxic to yeast and create stuck fermentations (Besada-Lombana *et al*., 2017). Alternatively, higher fermentation temperatures tend to have a lower concentration of esters due to the volatility of the compounds (Killian and Ough, 1979). Indeed, the results of the present study align with previous literature regarding fermentation temperature. For example, a study on Merlot fermented cold (15 °C) compared to a control (25 °C) showed that the cold fermentation wines had a stronger association with esters, including ethyl hexanoate, compared to the control (Massera et al., 2021). For Pinot noir wines specifically, another study that examined the impact of fermentation temperature and yeast type found that the cold-modified treatment (15 °C with the addition of frozen pomace and EC-1118 yeast) was more associated

TABLE 3. Two-way analysis of variance (ANOVA) for the main effects of fermentation temperature and cap management with interaction showing the mean separation and *p*-values for the relative concentration of volatile compounds (µg/L) in Pinot noir wines.

Compounds	Fermentation Temperature				Cap Management			Fermentation Temperature x Cap Management
	Cold	Cold/Hot	Hot	*p*-value**	PD	No PD	*p*-value	*p*-value
Esters								
Ethyl butyrate	250 a*	184 b	142 b	**0.000**	1.63 10^3 a	220 b	**0.031**	**<0.0001**
Isoamyl acetate	424 a	223 b	187 b	**0.002**	228	328	0.137	**0.002**
Ethyl hexanoate	829 a	696 b	404 c	**<0.0001**	617	669	0.579	**<0.0001**
Ethyl lactate	3.2110^4	3.57 10^4	4.73 10^4	0.355	5.10 10^4 a	2.58 10^4 b	**0.001**	**0.005**
Ethyl heptanoate	2.22 a	0.505 b	n.d.***	**0.000**	0.903	0.915	0.985	**0.001**
Ethyl octanoate	523 a	249 ab	167 b	**0.042**	253	373	0.355	0.204
Ethyl decanoate	23.8	5.02	5.70	0.178	7.19	15.8	0.369	0.420
Diethyl succinate	497 b	467 b	1,007 a	**0.000**	724	590	0.369	**0.000**
Phenylethyl acetate	n.d.	2.39 b	14.2 a	**0.000**	7.27	3.80	0.365	**0.001**
Ethyl cinnamate	1.55 a	0.260 b	0.287 b	**0.009**	0.432	0.965	0.223	0.054
Nor-isoprenoids								
β-damascenone	3.97 a	2.75 b	2.05 c	**<0.0001**	2.77	3.08	0.489	**<0.0001**
Terpenes								
Citronellol	10.0	7.51	6.49	0.270	6.77	9.25	0.176	0.109
trans-farnesol	1.17 b	1.14 b	2.85 a	**0.010**	1.70	1.74	0.934	0.140
Alcohols								
1-hexanol	4.2010^3	4.32 10^3	2.39 10^3	0.091	4.59 10^3 a	2.68 10^3 b	**0.014**	**0.001**
1-octanol	1.17 c	7.71 b	14.3 a	**<0.0001**	8.00	7.43	0.848	**0.000**
1-nonanol	6.18 a	3.23 b	8.93 a	**0.002**	5.30	6.92	0.296	**<0.0001**
Isobutanol	1.89 10^4	1.83 10^4	1.7110^4	0.838	2.0910^4 a	1.53 10^4 b	**0.012**	0.150
Isoamyl alcohol	3.10 10^5	9.96 10^5	3.56 10^5	0.099	5.78 10^5	5.30 10^5	0.877	0.516
Benzyl alcohol	868	1.56 10^3	2.11 10^3	0.258	1.80 10^3	1.23 10^3	0.367	**0.009**
Phenylethyl alcohol	3.23 10^4	9.56 10^4	7.56 10^4	0.387	9.03 10^4	4.53 10^4	0.237	0.544

*Different letters within columns indicate a significant difference for Fisher's least significant differences test ($p < 0.05$).

** Significant *p*-values are shown in bold fonts.

***Volatile compounds were not detected during analysis.

with tropical fruit aroma compared to the ambient (20 °C) and high treatments (30 °C) using the same yeast. According to the volatile chemistry data of the previous study, both the cold-modified treatment and the cold treatment (15 °C) had higher concentrations of ethyl butyrate, ethyl hexanoate, ethyl heptanoate, and ethyl 9-decenoate compared to the other fermentation temperature treatments regardless of yeast type (Girard et al., 2001). In the present study, the banana aroma was associated with Cold PD wines and correlated with a combination of esters, particularly ethyl hexanoate (0.77) and ethyl heptanoate (0.84), as well as the C13-norisoprenoid, β-damascenone (0.76) according to Pearson's correlation (Figure 2A,B).

All three of these compounds were significantly higher in relative concentration for Cold wines compared to the other fermentation temperature wines, but these compounds did have statistically significant interactions between fermentation temperature and cap management (Table 3). The No PD wines for the factor of cap management tended to have higher relative concentrations of ethyl hexanoate, ethyl heptanoate, and β-damascenone. This trend was likely due to the reductive environment and the resulting low or no oxygen dissolution created in the Cold No PD wines during fermentation, as these wines did not receive any cap management. In line with the prior results, a study was conducted on the effect of redox state on volatile compound formation using micro-ferments in Erlenmeyer flasks that were either reductive and enclosed with a Muller valve or oxidative, which were enclosed with a cotton ball. The results indicated that the reductive fermentation environment (Muller valve closure) had higher concentrations

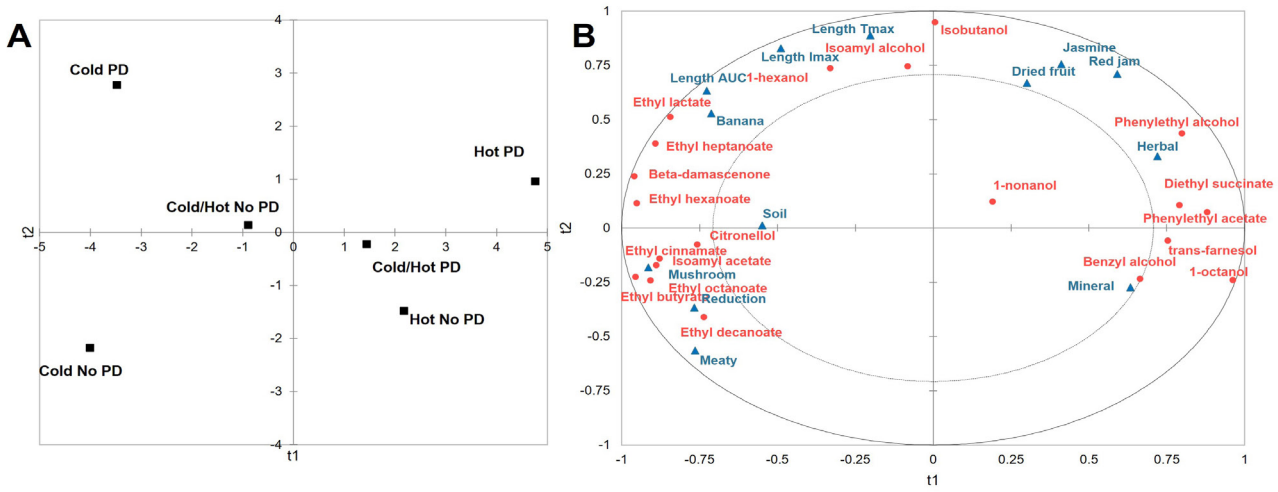


FIGURE 2. Partial least square regression analysis of volatile chemistry and sensory attributes A: Wine treatments (■). B: Correlation loading of the relationship between the volatile chemistry (●) and sensory attributes (▲).

of esters compared to the ferments which used a cotton ball as a closure (Fariña *et al*., 2012). Therefore, the interaction of fermentation temperature and cap management demonstrated in several esters was likely due to the combination of a cold fermentation temperature and a reductive environment. Interestingly, the Cold No PD wines were associated with several esters, including ethyl butyrate, ethyl octanoate, ethyl decanoate, isoamyl acetate, and ethyl cinnamate (Figure 2). Both isoamyl acetate and ethyl cinnamate are compounds related to the winemaking practice of carbonic maceration (Tesniere and Flanzy, 2011). This association is unsurprising as the Cold No PD wines had minimal exposure to oxygen as there was no cap management, which may have led to partial carbonic maceration. However, these esters were not in the same quadrant as any fruit-related sensory descriptors and showed only moderate to poor correlation with the banana aroma (ethyl butyrate: 0.52; ethyl octanoate: 0.63; ethyl decanoate: 0.46; isoamyl acetate: 0.59; ethyl cinnamate: 0.56) (Figure 2). A possible explanation for the association of Cold No PD wines with esters, which did not correspond to any fruit-related sensory descriptors, may be due to a masking effect of reductive aromas. This assertion is supported by a previous study (Franco-Luesma *et al*., 2016). In it, the influence on the aroma profile of model white, red, and red with oak wines with added hydrogen sulfide and methanethiol was tested. Through Rate-All-That-Apply (RATA) sensory analysis, it was determined that both sulfur compounds masked fruit and floral aromas in both white and red wines.

Accordingly, the reduction aroma descriptor was one of the most significant sensory characteristics in the wines of the present study. The aroma descriptor reduction was statistically significant for the main effects of fermentation temperature ($p < 0.0001$), cap management ($p = 0.006$), and their interaction ($p < 0.0001$) (Table 2). According to the results of Fisher's LSD test, the reduction aroma was significantly higher in Cold and No PD wines. Additionally, the mushroom aroma was statistically significant for the factor of fermentation temperature ($p = 0.009$), where Cold wines were higher than other fermentation temperatures. The mushroom attribute also had a significant interaction between fermentation temperature and cap management ($p = 0.035$), whereby No PD wines tended to have higher intensity (Table 2). Additionally, although not statistically significant, the aroma attribute meaty ($p = 0.069$) tended to have higher intensity in Cold and No PD wines. Moreover, the meaty aroma was associated with Cold No PD wines in the PLSR (Figure 2). Mushroom, reduction, and meaty aroma attributes are commonly associated with the presence of hydrogen sulfide and other related volatile sulfur compounds (Smith *et al*., 2015). Reduction, primarily due to hydrogen sulfide, occurs naturally during alcoholic fermentation but can be exacerbated by low availability of yeast nutrients (Ugliano *et al*., 2011), reduced or sub-optimal oxygen exposure (Bekker *et al*., 2016), yeast strain selection (Acree *et al*., 1972), fermentation temperature (Bohlscheid *et al*., 2011) or a combination of the mentioned factors. In the present study, the Cold fermentation temperature wines had higher perceived intensity of reduction aromas compared to the other fermentation temperature regimes. Previous research reported that under non-limiting nutritional conditions, the maximal sum of hydrogen sulfide produced during alcoholic fermentation was enhanced at 30 °C with lower levels either at 15 °C and 22 °C (Bohlscheid et al., 2011). However, under our experimental conditions, the opposite was found, at least from a sensory standpoint. Indeed, Cold fermentation temperature wines showed more perceivable levels of reduction, presumably caused by hydrogen sulfide and/or other volatile sulfur compounds. A study conducted on Pinot noir in British Columbia demonstrated that the cold fermentation treatment (20 °C) had higher levels of 3-(methythio)-1 propanol, also

known as methionol (Girard *et al*., 1997). This compound is associated with the aroma of cauliflower in wines but is less volatile compared to hydrogen sulfide (Moreira *et al*., 2011). Interestingly, in the present study, reduction, mushroom, and meaty aromas showed a strong association with the Cold No PD wines, but neither hydrogen sulfide nor other volatile sulfur compounds were quantified. Another possible explanation for the presence of reduction aromas in Cold fermentation temperature wines could be that cold fermentation temperatures may have resulted in a slower rate of carbon dioxide release which might have caused the accumulation of hydrogen sulfide. On the other hand, Hot fermentation temperature wines showed zero to low levels of perceived reduction in their sensory profile. The Hot fermentation temperature regime might have produced wines with low levels of volatile sulfur compounds due to the thiol-scavenging properties of electrophilic quinones, which react with thiols such as hydrogen sulfide, decreasing volatility (Casassa, 2017). The higher temperature of these wines may have led to enhanced phenolic extraction and a higher rate of quinone formation, leading to the above outcome. Again, this was empirically demonstrated by the lack of reduction aromas in the wines that underwent Hot fermentation temperature regimes (Table 2). Overall, the sensory and volatile chemistry results of the present study suggested that while fermentation temperature was more impactful statistically speaking, cap management still affected these aspects of the wines, as evidenced by the numerous interactions.

3. Effect of fermentation temperature and cap management on overall astringency and length

Pinot noir is known to produce wines with relatively low tannin content (Harbertson *et al*., 2008). This phenomenon has been attributed to the fact that Pinot noir grapes have higher tannins in the seeds compared to the skins, which are thought to be more difficult to extract (Sparrow *et al*., 2015). An alternative theory is that Pinot noir grapes produce tannins with low molecular weight, which lead to low astringency wines as the proline-rich-proteins in saliva do not react with them readily as with high molecular weight tannins (Sun *et al*., 2013). However, a warm (25 °C or greater) fermentation temperature has been thought to lead to more phenolic extraction and, thus, enhanced tannin extraction for astringency perception (Casassa, 2017). Indeed, in a previous study on Pinot noir, significantly higher levels of tannins were reported in the hot (25 °C) fermentation temperature wines (Reynolds *et al*., 2022). As such, the present study sought to determine if the factors of fermentation temperature or cap management could enhance the astringency perception of Pinot noir wines. Astringency time-intensity curves for each treatment are shown in Figure 3A. Hot No PD wines had the highest astringency intensity compared to all other treatments, while Cold No PD wines had the lowest. In the three-way ANOVA, the TI parameters for overall astringency showed that only maximum intensity (I_{max}) (p = 0.035) was statistically significant for fermentation temperature (Table 3). Hot fermentation temperature wines had the highest maximum intensity for overall astringency and were significantly higher than Cold wines. For cap management, none of the TI parameters were significantly affected by it, but the interaction between fermentation temperature and cap management was only statistically significant for the time parameter I_{max} (p = 0.006). These results indicated that there was a lack of significant difference in astringency perception between wines except for Hot No PD wines, which had higher astringency intensity. Additionally, the cap management protocol may have influenced the astringency profile of the wines, but no clear trend in astringency attributes was observed as a function of the cap management regime. This reinforces the prevailing effect of fermentation temperature over the cap management regime on the temporal perception of astringency for Pinot noir wines.

Length (retronasal aroma) was defined by the panel as "*the lingering flavours of red jam and dried fruit post-expectoration*". The TI curves indicated that Cold PD

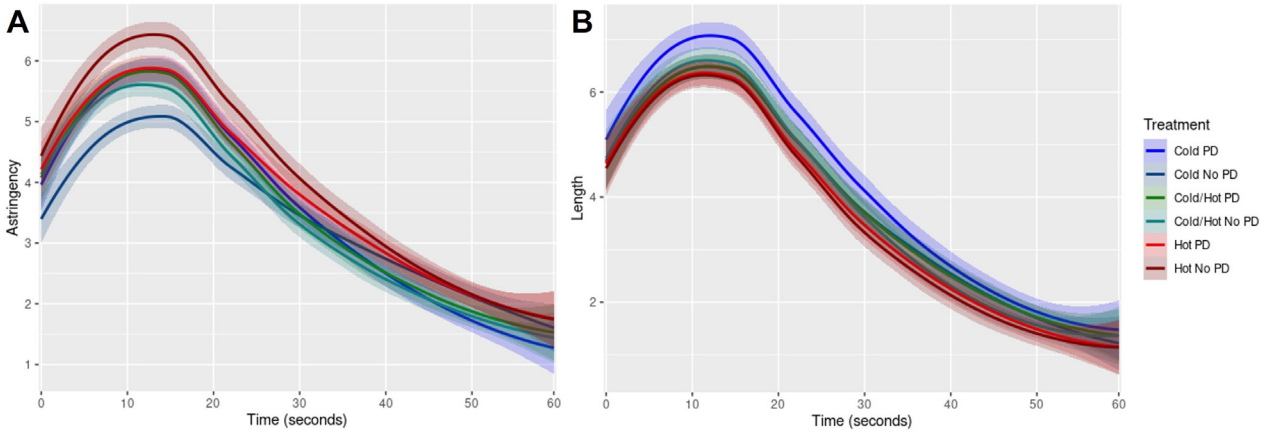


FIGURE 3. Time-intensity curves for overall astringency and length of Pinot noir wines evaluated by a trained sensory panel (n = 13). Each line represents the average intensity response, with shadows representing confidence intervals for each treatment. Time zero is equivalent to ten seconds post-expectoration. A: Overall astringency. B: Length.

TABLE 4. Three-way analysis of variance (ANOVA) for the main effects of fermentation temperature and cap management as well as panellist with selected interactions showing the mean separation and *p*-values of overall astringency of Pinot wines assessed by a trained panel (n = 13).

Treatments	Onset Time (seconds)	Onset Value (intensity)	Time of maximum intensity (T_{max}) (seconds)	Maximum intensity (I_{max})	Area Under Curve	Extinction Value (intensity)	Duration Time (seconds)
Fermentation Temperature							
Cold	2.03	5.37	8.34	7.19 b*	215	1.45	45.6
Cold/Hot	1.99	5.64	7.72	7.60 ab	218	1.46	46.0
Hot	1.91	5.63	7.22	7.91 a	242	1.76	47.0
p-value**	0.701	0.723	0.540	**0.035**	0.111	0.295	0.627
Cap Management							
PD	1.98	5.59	7.71	7.64	226	1.52	46.6
No PD	1.98	5.51	7.81	7.50	225	1.59	45.8
p-value	0.979	0.774	0.904	0.541	0.931	0.683	0.494
Fermentation Temperature x Cap Management							
p-value	0.725	0.793	0.585	**0.006**	0.361	0.574	0.556
Fermentation Temperature x Panellist							
p-value	0.885	**0.008**	0.202	**0.007**	0.697	0.074	0.937
Cap Management x Panellist							
p-value	0.730	0.239	0.954	0.794	0.541	0.654	0.880

*Different letters within columns indicate a significant difference for Fisher's least significant differences test ($p < 0.05$).

** Significant *p*-values are shown in bold fonts.

TABLE 5. Three-way analysis of variance (ANOVA) for the main effects of fermentation temperature and cap management as well as panellist with selected interactions showing the mean separation and *p*-values of the length of Pinot wines assessed by a trained panel (n = 13).

Treatments	Onset Time (seconds)	Onset Value (intensity)	Time of maximum intensity (T_{max}) (seconds)	Maximum intensity (I_{max})	Area Under Curve	Extinction Value (intensity)	Duration Time (seconds)
Fermentation Temperature							
Cold	1.77	5.78	5.94	8.61	248	1.34	46.8
Cold/Hot	1.92	5.95	5.53	8.40	236	1.34	46.3
Hot	1.89	5.51	5.65	8.17	224	1.10	45.2
p-value*	0.541	0.504	0.814	0.246	0.123	0.336	0.486
Cap Management							
PD	1.85	5.65	5.96	8.56	241	1.30	46.2
No PD	1.87	5.83	5.45	8.22	230	1.21	46.0
p-value	0.861	0.557	0.349	0.112	0.265	0.552	0.851
Fermentation Temperature Cap Management							
p-value	0.842	0.840	0.680	0.096	0.255	0.757	0.908
Fermentation Temperature Panellist							
p-value	0.927	0.097	0.776	**0.026**	0.240	0.166	0.810
Cap Management Panellist							
p-value	0.394	0.125	0.658	**0.012**	0.860	**0.029**	0.800

* Significant *p*-values are shown in bold font.

wines had the highest length intensity compared to all other treatments, which persisted from post-expectoration to 50 seconds (Figure 3B). In the three-way ANOVA, the time parameters did not show a significant difference between treatments (Table 5). The lack of differences in length perception for the present study aligns with those from a past study that examined the effect of fermentation temperature on Pinot noir (Girard *et al.*, 1997). In the previous experiment,

descriptive analysis results from retronasal evaluation indicated that the cold (20 °C), hot (30 °C), and hot-cold treatments were not significantly different from each other for cherry flavours.

However, in the present study, there appeared to be discrepancies in the understanding of the definition of length based on the PLSR results. The length parameter AUC was in the same quadrant as several esters, which were also associated with the banana aroma and the treatment Cold PD but not red jam and dried fruit, as the definition states (Figure 2A,B). Indeed, neither the length attribute (Table 5) nor the orthonasal aroma attributes of dried fruit or red jam were significantly different among treatments (Table 2). While the panel agreed upon the definition of length, other retronasal aroma attributes could have influenced how length was perceived. This suggests that perceived length in wines is a very complex physiological and psychological construct. Indeed, the definition of length herein instituted likely limited the scope of the present results, and the use of specific retronasal aroma attributes may have yielded more decisive results. However, since the temporal sensory method used was TI, it was limited to a maximum of two attributes evaluated (Cliff and Heymann, 1993). This restriction for TI helps to control panellist fatigue but limits what can be learned about the other attributes present in the retronasal profile. Therefore, future studies aimed at understanding the dynamic effect of retronasal aroma length should use other temporal methods that allow for more attributes to be assessed.

4. Effect of salivary flow rate on the perception of overall astringency and length

Previously, the determination of salivary flow rate was carried out by collecting stimulated saliva through the ingestion of a citric acid solution (Baker and Ross, 2014; Ishikawa and Noble, 1995). This method has been favoured for wine sensory research as it was thought to stimulate the parotid gland, which is the only saliva gland to excrete proline-rich-proteins (PRPs) which interact with tannins to elicit astringency (Benn and Thomson, 2014). However, it has been reported that the use of a citric acid solution leads to a mean contribution of 45 % parotid gland and 45 % submandibular glad for salivary flow rate, while mechanical stimulation (chewing) causes a mean contribution of 58 % parotid gland and 33 % submandibular gland for salivary flow rate. (Aps and Martens, 2005). Considering that chewing also stimulates the parotid gland, this method should be valid for use in wine sensory research. Indeed, the present study collected stimulated saliva through mechanical stimulation (chewing), and this method has been employed in previous wine sensory studies with comparable results (Dinnella *et al*., 2009; Criado *et al*., 2019; Lyu *et al*., 2021).

As such, Supplementary Table 3 shows that there was a 5-fold difference between the maximum salivary flow rate (3.28 mL/min) and the minimum salivary flow rate (0.60 mL/min) within panellists. The average salivary flow rate was 1.91 mL/min. Based on the mean, there were six high salivary flow rate (HF) panellists (2.04 to 3.28 mL/min) and seven low salivary flow rate (LF) panellists (0.60 to 1.87 mL/min), (Mialon and Ebeler, 1997). The results of

TABLE 6. One-way analysis of variance (ANOVA) showing mean separation and *p*-values of salivary flow rate and overall astringency time parameters of Pinot noir wines assessed by a trained panel (n = 13).

Salivary flow rate	Onset Time (seconds)	Onset Value (intensity)	Time of maximum intensity (T_{max}) (seconds)	Maximum intensity (I_{max})	Area Under Curve	Extinction Value (intensity)	Duration Time (seconds)
Low flow rate	2.11 a*	7.05 a	6.83 b	8.76 a	287 a	1.81 a	54.4 a
High flow rate	1.82 b	3.79 b	8.84 a	6.18 b	153 b	1.25 b	36.7 b
p-value**	**0.009**	**<0.0001**	**0.015**	**<0.0001**	**<0.0001**	**0.002**	**<0.0001**

*Different letters within columns indicate a significant difference for Fisher's least significant differences test ($p < 0.05$).

** Significant p-values are shown in bold fonts.

TABLE 7. One-way analysis of variance (ANOVA) showing mean separation and *p*-values of salivary flow rate and length time parameters of Pinot noir wines assessed by a trained panel (n = 13).

Salivary flow rate	Onset Time (seconds)	Onset Value (intensity)	Time of maximum intensity (T_{max}) (seconds)	Maximum intensity (I_{max})	Area Under Curve	Extinction Value (intensity)	Duration Time (seconds)
Low flow rate	1.81	6.96 a*	5.00 b	9.16 a	272 a	1.47 a	50.3 a
High flow rate	1.91	4.32 b	6.53 a	7.49 b	193 b	1.02 b	41.2 b
p-value**	0.343	**<0.0001**	**0.005**	**<0.0001**	**<0.0001**	**0.003**	**<0.0001**

*Different letters within columns indicate a significant difference for Fisher's least significant differences test ($p < 0.05$).

** Significant p-values are shown in bold fonts.

the present study aligned with patterns previously reported in the literature for salivary flow rates that utilised TI (Baker and Ross, 2014; Criado *et al*., 2019; Fischer *et al*., 1994; Ishikawa and Noble, 1995). For overall astringency, LF panellists had significantly higher intensity due to their high I_{max} ($p < 0.0001$) and AUC ($p < 0.0001$), as shown in Table 6. Additionally, for LF panellists, overall astringency showed a longer duration ($p < 0.0001$) and higher extinction value ($p = 0.002$), meaning the astringency sensation persisted longer compared to HF panellists. Finally, onset time ($p = 0.009$) and value ($p < 0.0001$) occurred later and higher compared to the LF panellists, respectively. The pattern of LF panellists perceiving astringency more intensely than HF panellists has been shown before in several studies exploring the relationship between salivary flow rate and astringency perception post-expectoration (Fischer *et al*. 1994, Ishikawa and Noble 1995, Lesschaeve and Noble 2005). However, T_{max} ($p = 0.009$) occurred significantly later in HF individuals compared to LF individuals, which is contrary to previous research.

As for retronasal aromas, LF individuals experienced perceived length with significantly higher intensity throughout testing as indicated by the higher onset value ($p < 0.0001$), I_{max} ($p < 0.0001$), and AUC ($p < 0.0001$) (Table 7). LF individuals also perceived length longer compared to HF individuals according to the duration time ($p < 0.0001$). These results are counter to a previous study evaluating the impact of ethanol, tannin, and flavour addition in model wines, which reported that for single compound treatments, the maximum intensity was not significantly different between salivary flow rates. However, in said study, LF individuals did have a significantly higher duration and AUC (Baker and Ross, 2014). This may be because none of the flavours used in the past study were esters, which are related to the perception of fruit flavours. Another study examined the effect of esters and the relationship between perception of fruit flavours and salivary flow rate. In it, HF individuals perceived short-chain esters (isoamyl acetate, ethyl hexanoate, ethyl butanoate) more intensely than LF panellists (Criado *et al*., 2019). This contrasts with the results of the present study, whereby LF individuals had significantly higher I_{max} and AUC values compared to HF panellists for length. Also, the length AUC and I_{max} parameters of the present study were associated with ethyl heptanoate and ethyl hexanoate, both of which are considered short-chain esters as they have less than a ten-carbon chain (Su *et al*., 2016). The discrepancies seen between studies may be due to the length of the evaluation period. In the present study, the salivary flow rate data represented an average over a total of 60 seconds beginning at post-expectoration, while the previous study had a longer evaluation time (120 seconds) and determined their findings based on a single time point of 60 seconds post-expectoration. Regardless, the results of the present study have further indicated the impact of salivary flow rate on the timing and intensity of astringency and length perception. Thus, the salivary flow rate should be accounted for in future studies using time-based sensory methods to examine in-mouth and post-expectoration sensations.

CONCLUSIONS

Pinot noir wines produced with three contrasting fermentation temperature protocols and two cap management regimes underwent both descriptive analysis and time-intensity sensory analysis to understand the separate and combined effect of both factors. Cold fermentation temperatures led to wines with higher overall aroma intensity and fruity aromas, likely due to the presence of esters but, at the same time, these wines were less astringent and showed more reduction aromas. Conversely, the hot fermentation temperature regimes led to wines with more colour saturation and astringency, with more duration of the latter, but with less aromatic persistence and length. However, there were numerous significant interactions for both colour attributes and orthonasal aromas. The absence of cap management led to wines with higher perceived colour saturation and purple hue, especially when fermented at warm temperatures. For reduction aromas, a significant interaction between fermentation temperature and cap management technique was uncovered, whereby perceived reduction was higher in wines fermented at cold temperatures with no cap management. The banana and mushroom aroma attributes, while not significant, had a tendency towards higher intensity with no cap management.

The present study has demonstrated conclusively that the use of contrasting fermentation temperature protocols produced Pinot noir wines with vastly different sensory features. It should also be noted that the outcomes of applying different cap management protocols and fermentation temperature regimes during alcoholic fermentation will vary depending on fermentation volume, the geometry and material of the fermentor, grape variety, and growing conditions. Herein, the absence of cap management coupled with a cold fermentation led to reduction aromas, which may have masked esters that were otherwise particularly abundant in cold-fermented wines. Therefore, rather than no cap management at all, it is suggested that gentler cap management regimes, in frequency and intensity, may be beneficial for enhancing the aromatic composition and intensity of Pinot noir wines. Alternatively, oxygen or nitrogen can be dissolved using a sintered porous sparger during the maceration of Pinot noir wines undergoing a minimal cap management protocol to counter the production of reductive aromas.

Finally, from a winemaker's perspective, the contrasting sensory profiles resulting from extreme fermentation temperature regime protocols should be considered to produce blends of enhanced sensory complexity or, else, to craft wines abiding to specific and targeted styles and sensory specifications.

ACKNOWLEDGEMENTS

The authors would like to thank the sensory panellists for their diligent work and dedication to this project. Mike Sinor (Sinor-Lavallée, Avila Beach, CA) is thanked for the generous donation of the fruit for this project. The authors declare no conflict of interest.

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