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Understanding ashy flavour recognition thresholds in Pinot noir wines

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ABSTRACT

Wildfires have become a growing concern in wine-producing regions globally, as they produce harmful levels of smoke that can carry volatile compounds over vast distances. When vineyards are exposed to this smoke, grapes absorb these compounds, which are then found in the resulting wine. These wines are described as having smoky, burnt, and dirty flavours, along with an ashy aftertaste that is considered a unique, negative attribute of wildfire-affected wines. While volatile phenols, along with their glycosides, are known contributors to this ashy attribute, thiophenols have additionally been identified to be influential in the perception of this flavour. This work aimed to determine at what concentration of thiophenols Pinot noir takes on this perceptual "ashy" property at various levels of smoke-related volatile phenols (45 µg/L, 135 µg/L, 450 µg/L). Using an adaptive staircase procedure, the threshold level of a mixture of smoke-related thiophenols was determined in different styles of Pinot noir wine. Threshold estimates were found based on the fitted psychometric curves from the individual thresholds collected, allowing different levels of risk, or percentage of consumers to recognize ashy flavour, to be estimated. The results indicated that there were some wine style differences observed at the lowest phenol concentration; however, at higher concentrations, more representative of smoke exposure differences were no longer found. Concentrations of volatile phenols at 135 µg/L are at risk of ashy recognition starting at a total thiophenol concentration of 20 ng/L, while at 450 µg/L if phenols are at risk at a total thiophenol concentration of 10 ng/L. Not only is the determined threshold level important, but additionally, understanding the wide range of sensitivities that exist within the population. This knowledge is imperative to the wine industry to be able to understand the potential of a wine to exhibit this negatively regarded ashy flavour. In looking to better understand the impact of wildfires on wines, it is vital to understand how consumers perceive these wines and relate them to the volatile composition to be able to make rapid sensory-directed decisions to minimise waste and avoid unnecessary losses.

KEYWORDS: smoke taint, sensory, adaptive staircase, phenols, thiophenols

INTRODUCTION

Smoke is a complex substance that contains over 500 volatile compounds, which can impart aromas and flavours to foods and beverages (Toth & Wittkowski, 1985). The volatile compounds from wood fire smoke are predominantly volatile phenols, such as guaiacol and syringol (Simoneit, 2002). These phenolic compounds have been found to impart smoky aromas to foods that have come in contact with woodfire smoke (Wang & Chambers IV, 2018). However, with the increased occurrence of unintentional smoking of wine grapes as a result of wildfires, there can also be negative sensory changes. First recorded as a problem in 2003 from the Canberra bushfires in Australia, wildfire smoke can lead to negative sensory qualities in wines produced from grapes that have been exposed to wildfire smoke (Høj *et al*., 2003). Grapes absorb the volatile compounds contained within smoke, which can lead to elevated concentrations in the resulting wine (Jiang *et al*., 2021). Along with volatile phenols, an increase in phenolic glycosides occurs, which is thought to be produced from the grape's stress response to the introduction of these foreign phenolic compounds (Hayasaka *et al*., 2010). While these phenolic glycosides are non-volatile, during the wine-making process and upon ingestion, they can be broken down, releasing the volatile phenol, once again making them perceptible and contributing to the lasting nature of smoke-related flavours (Mayr *et al*., 2014).

Smoke-affected wines are described as having smoky, burnt, and dirty aromas and flavours, with a distinctive ashy aftertaste (Parker *et al*., 2012). While there are elevated concentrations of volatile phenols in wildfire-affected wines, these compounds are not unique to this situation. Many of the same volatile phenols in smoke-affected wines can be found at similar concentrations in wines aged in oak, particularly in heavily toasted barrels (Parker *et al*., 2024a). Additionally, when these compounds have been added to non-smoke-impacted wines to achieve a similar composition as a smoke-impacted wine, the sensory experience is not the same, leading to the question of what else is leading to these unpleasant smoke-related aromas and flavours (Krstic *et al*., 2015).

Tomasino *et al*. (2023) identified the presence of thiophenols in wildfire-affected wines. These compounds have been found to elicit meaty and rich aromatics and are associated with burnt meats (Khan *et al*., 2015). Other thiol-containing compounds commonly found in wine, such as 3-sulfanylhexanol, provide tropical aromatics, and methanethiol, which contribute to rotten cabbage characters (Parker *et al*., 2024b). These compounds are perceptible at the parts per trillion level, making a small amount impactful to the resulting flavour profile of the wine (Chen *et al*., 2019).

Thresholds are defined as the limits to our sensory capabilities, which include detection thresholds, recognition thresholds, and others. The basic definition of a sensory threshold is that there is a concentration range that exists below a certain level when a stimulus is not detectable, and above which it will be readily detected (ASTM, 2019). Humans can detect billions of volatile compounds through the olfactory system, via both the orthonasal and retronasal pathways (Sell, 2019). Detection thresholds of the individual volatile phenols present within smoke-affected wines have been studied, with a broad range of values dependent on method and matrix presented, for example, within water, red wine, and model alcohol solution) (Boidron *et al*., 1988; Eisele & Semon, 2006; Ferreira *et al*., 2000; Parker *et al*., 2012). These thresholds have generally been aroma thresholds, or the orthonasal intake of volatile compounds. Olfaction is unique as it is a dual sense modality, as it takes in volatiles via the nose and within the mouth (Rozin, 1982). While these routes carry volatiles to the same system, there are differences in processing, leading to differences in the resulting perception (Özay *et al*., 2021). In recent works, Parker *et al*. (2012) have determined the retronasal threshold in red wine to be 27 µg/L. However, as smoke-impact is not solely due to guaiacol, this leads to the need to understand thresholds from both pathways for other influential volatile compounds, especially when considering that wine is meant to be consumed.

Although humans can detect a wide range of odorants, the ability to recognise and identify them is much more restricted. This limitation arises from the difficulty in retrieving a perceived flavour from memory, which is based on the intensity and quality of the volatile (Goldstein & Brockmole, 2017). The recognition threshold is the point a stimulus takes on a particular identity, which is generally higher than the detection threshold (Lawless & Heymann, 2010). A simple example of this is with salt in water. Around the detection threshold of NaCl in water, the solution is not described as salty, making the recognition threshold for this attribute at a higher concentration (Bartoshuk *et al*., 1978).

Perception, and therefore recognition, of odours occurs as patterns as opposed to individual features like other sensory modalities (Engen, 1982). When we think of an orchestra, we can pick out the various instruments while listening, while when we smell a strawberry, there are several compounds present, yet they elicit only one trait. This indicates the importance of understanding the aroma and flavour impact of blends of aromatic compounds, as their individual perception may not be representative of a multi-flavour system. In wine, there are additional complexities with flavour recognition. Wine is a complex matrix with both volatile and non-volatile components that influence the perceived flavour profile (Villamor & Ross, 2013). Interactions within wine are found both within the chemical matrix along with perceptual interaction at consumption. Volatile compounds can take on distinct characteristics depending on concentration and matrix, as seen with terpenes in Gewurztraminer wine (Chigo-Hernandez & Tomasino, 2023). McKay (2020) found that ashy flavour in wine was only produced by binary mixtures of volatile compounds. Tomasino (2023) further found that while volatile phenols indicated a greater chemical flavour attribute and thiophenols a vegetal character, the mixture of these odorant groups produced a greater ashy flavour in wine. This indicates the importance of not only understanding the

level of detection of the individual compounds but also the point at which a wine takes on a particular descriptor based on the interactions between other volatile and non-volatile components.

Several methods have been used to determine different sensory thresholds. Constant stimulus or fixed psychophysical methods are common, utilising repeated presentations at different stimulus intensities to be able to determine an entire psychrometric function (Rose *et al*., 1970). Adaptive methods have been used to be able to remove redundant presentations above and below the threshold, allowing for responses to determine the next stimulus level, which then tends towards the threshold (Rose *et al*., 1970). This helps improve limitations to studying the olfactory system, since olfaction has strong sensory adaptation and saturation to stimuli (Hoffmann-Hensel *et al*., 2017). Additionally, with wildfire-affected wines, there has been a noticeable carryover of the smoky and ashy flavours (Fryer *et al*., 2021). Due to this, there is a recommended 90-second separation of samples with a rinse procedure (Fryer & Tomasino, 2022). This leads to a lengthy time between sample evaluation, therefore requiring a method that would not depend on sample comparison, leading to bias from memory fatigue.

The number of participants, or threshold observations, varies greatly depending on the modality being studied, the product, and the method being used. While even with proper consideration based on methodology, threshold determinations are highly variable due to the variability of human response to stimuli (Mainland *et al*., 2014). These methods are many times used to estimate the population threshold. While testing the entire population produces the most accurate findings, this is impractical, inefficient, and generally impossible (Kang, 2021). A key consideration when determining sample size is the resource availability, where there is often a trade-off between resources and statistical power. When considering wine research, a large resource constraint is the available wine volume. As each vintage is going to be different, sample sizes for methodologies need to be selected within the constraints of the wine from a single vintage, as making another lot of wine would be its own distinct sample (Tomasino *et al*., 2013). Participant size estimates for sensory testing are dependent on the context in which products are consumed, along with the intrinsic differences of the products (Mammasse & Schlich, 2014). For adaptive threshold procedures, sample sizes have ranged from 19 to 60 individuals looking at both detection and recognition thresholds in various products (Wise *et al*., 2008; Yoshino *et al*., 2021). In this work, we aimed to determine the optimal sample size for the staircase methodology, the target attribute, and the context of the experimentation, all to answer the question "When would a consumer consider a wine to be ashy?".

The purpose of this work was to identify the recognition threshold level of "ashiness" in Pinot noir wines, as this attribute has been identified as a key differentiator of wildfire-affected wine and is a detractor to liking (Fryer *et al*., 2025). This indicates the importance of relating the concentration of phenols and thiophenols to this recognition point to assess the risk a consumer will consider a wine to take on this attribute. This work uses a staircase procedure to identify the concentration of a blend of thiophenol compounds present in a wine containing various concentrations of smoke-related volatile phenols. Three different styles of Pinot noir were used, including a low-extraction style, an extended maceration style, and one produced with an alternative yeast to determine how different wine production methods alter the threshold level. Additionally, the number of participants required using this methodology was determined with a bootstrap method. This work demonstrates that both thiophenols and volatile phenols are influential in ashy flavour perception in smoke-affected wines. Threshold-based risk guidelines are proposed to inform mitigation strategies across wine styles, providing a framework for understanding sensory impacts associated with wildfire exposure from analytical measures.

MATERIALS AND METHODS

1. Winemaking

Three different styles of Pinot noir wines were used in this study: low-extract, extended maceration, and alternative yeast (AMH). Wines were all produced in a similar manner, with the specific winemaking unique to each wine described in further sections. Wines were all produced from Pinot noir grapes (*V. vinifera*) harvested from Oregon State University's Woodhall III Vineyard (Monroe, OR, USA). A general Pinot noir winemaking protocol was used, as described below. To achieve the different styles, slight alterations were made to this protocol (sections 1.1–1.3).

After destemming, approximately 120 kg of grapes were portioned into two jacketed tanks (AAA Metal Fabrication, Dalles, OR, USA). Fermaid K (Lallemand Oenology, Montreal, Canada) was added at a rate of 0.4 g/L, along with 50 mg/L SO_2, and mixed. After 20 minutes, *Saccharomyces cerevisiae* Lalvin RC212 was added at a rate of 0.25 g/L.

Alcoholic fermentation was conducted at 27 °C, monitored by Brix measurements using a digital densitometer (Anton Paar, Santner Foundation, Graz, Austria) with twice-daily punch downs. Once fermentation was completed, treatments were pressed at 0.1 MPa for 5 minutes and transferred back into the jacket tank. *Oenococcus oeni* Lalavin VP41 (Lallemand Oenology, Montreal, Canada) was inoculated at a rate of 0.01 g/L. After malolactic fermentation was completed, defined as malic acid concentration less than 100 mg/L as measured by enzymatic assay (Neogen, Lansig, Michigan), 50 mg/L of SO_2 was added.

Tanks were then settled at 4 °C, and SO_2 was monitored to remain at a constant rate of 30 mg/L free SO_2 until bottling. At bottling, wines were racked and filtered first through a plate and frame filter fitted with 20 cm × 20 cm Beco K-1 3.0 µm nominal filter sheets (Langenlonsheim, Germany). Then, the wine was filtered using 1 µm and 0.45 µm cartridge filters, in sequence, and bottled into 750 mL clean bottles and sealed with aluminium screw cap closures (Stelvin, Amcor,

Australia). The wine was stored under refrigerated conditions (~4 °C) until use.

1.1. Low-extraction Pinot noir

Grapes were harvested in September 2021. To produce a low-extract style of Pinot noir wine, the wine was fermented at a lower temperature (16 °C) than previously detailed in section 1.

1.2. Extended maceration Pinot noir

Grapes for the extended maceration wine were harvested in October 2022. To produce an extended maceration style of Pinot noir wine, skins were left in the wine for 1 week after fermentation was completed. Then pressing and bottling occurred as described in section 1.

1.3. Alternative yeast Pinot noir

Grapes for the AMH wine were harvested in October 2022. For this style of wine, the only change to the detailed procedure was the use of a different Saccharomyces cerevisiae yeast. Enoferm Assmanshausen (Lallemend Oenology, Montreal, Canada) yeast was used at a rate of 0.25 g/L in place of the Lalvin RC212. This yeast was used to promote greater fruit and spice flavours, as described by the producer. Procedures described in section 1 were followed with this replacement. Wines were determined to be different based on preliminary sensory analysis (data not shown) and chemical analysis (Supplementary Table 1).

2. Sensory analysis

2.1. Participants

Participants were recruited from the Oregon Wine Research Institute wine consumer database. Inclusion criteria included being aged 21 or older, not currently pregnant or breastfeeding, no allergies to wine, not taking medications with alcohol interactions, and being a non-smoker. Those with taste deficits, oral disorders, or oral lesions were also excluded from the study. Since consumers were intended for this study, only those who indicated they consume on average one serving of red wine a week were recruited. Approval for this study was granted by the Oregon State Institutional Review Board (IRB-8781). All sessions took place in Weigand Hall on the Oregon State University campus (Corvallis, OR). A maximum of three participants were present in a session, seated at separate tabletop booths. Participants are summarised in Supplementary Table 3.

2.2. Volatile blends

Phenol and thiophenol blends were made, frozen, and thawed for each day of testing to add at specified concentrations. The phenol blend was made up of 7 major volatile phenols found in smoke-affected wine: guaiacol, methyl-guaiacol, *o*-cresol, *p*-cresol, *m*-cresol, syringol, and methylsyringol. The phenol blend was added at three concentration levels to achieve desired concentrations of total volatile phenols—low (45 μg /L), moderate (135 μg /L), and high (450 μg/L) (Table 1).

The thiophenol blend was made up of 5 volatile thiophenols identified in smoke-affected wine in equal concentrations: thioguaiacol, thiophenol, o-thiophenol, m-thiophenol, p-thiophenol. This blend was added with logarithmic step-size concentrations following the staircase procedure, described in subsequent sections (Table 2).

2.3 Low-extract Pinot noir

2.3.1. Samples

45 μg/L of the phenol blend (section 2.2.2) was added to the low-extract wine and portioned in 15 mL aliquots into 30 mL sample vials with PTFE-lined caps (Chemglass Life Sciences, Vineland, NJ) a day prior to the evaluation session. Vials were stored at 4 °C and were brought to room temperature prior to sensory analysis. During evaluation, the thiophenol blend (section 2.2) was added to the desired concentration immediately prior to evaluation (Table 1). Samples were presented to panellists in black INAO wine glasses (Lehmann glass, Kiyasa Group, New York, NY).

2.3.2. Procedure

Participants began by taking a short demographic questionnaire to capture consumption habits and wine knowledge using RedJade Sensory & Consumer Software (Version 4.1–5.1.1) (RedJade Sensory Solutions LLC, Pleasant Hill, CA). The rest of the study was conducted verbally. Participants were

TABLE 1. Concentration of volatile phenols added at low, moderate, and high levels for staircase testing.

Volatile Phenol	Concentration (mg /L)		
	Low Phenol Level	Moderate Phenol Level	High Phenol Level
Guaiacol	5	15	50
Methyl-guaiacol	5	15	50
o-cresol	5	15	50
m-cresol	5	15	50
p-cresol	5	15	50
Syringol	10	30	100
Methyl-syringol	10	30	100
Total volatile phenols	45	135	450

TABLE 2. Concentration steps of thiophenols used in the staircase procedure for both total concentration and the concentration of each volatile thiophenol (thioguaiacol, thiophenol, *o*-thiophenol, *m*-thiophenol, *p*-thiophenol).

Step	Total concentration (ng/L)	Concentration of each thiophenol (n = 5) (ng/L)
–1*[a]	0	0
0	0	0
1	0.5	0.1
2	1	0.25
3	2	0.5
4	4	0.8
5	8	1.6
6	16	3.2
7	32	6.4
8	64	12.8
9	128	25.6
10	256	51.2
11	512	102.4
12	1024	204.8
13*	2048	409.6
14*	4096	819.2
15*	8192	1638.4

*Steps only included for the extended maceration and alternative yeast Pinot noir.
[a]Blank sample; no volatile phenols or thiophenols present.

first provided with the instructions for the staircase procedure (Cornsweet, 1962), given below.

"You will be given a sample of wine and will taste using a sip, swish, and spit procedure. After approx. 15 seconds, please respond yes or no if you felt the sample was ashy. Be certain in your answer, such as if you were presented with a bunch of flavours, you would select the ashy one. Please just say "yes" or "no", there is no maybe option. Please base all responses on the flavour of the wine and not the smell. After responding, please hand me your glass, and you will have 90 seconds to rinse with the provided solution, followed by water. If you run out of water or rinse, we can provide you with more. You will then be given the next sample and will repeat this procedure until the end of the session. There will be a maximum of 20 samples. Any questions?"

Following the instructions, participants were led through a short training. First, they were asked to try the wine reference. This was the base low-extract Pinot noir without any addition of phenolic or thiol compounds to showcase a non-ashy sample. They then tried the ashy reference, prepared as described in Fryer *et al*. (Fryer & Tomasino, 2022), to be able to identify the target attribute. Following the ashy reference, participants were given a 90-second break to rinse their mouths with the provided rinse solution (4 g/L glucose solution, NOW Foods), followed by a water rinse.

The first sample was then delivered for evaluation, always being 16 ng/L thiophenols (step 6). The staircase step sizes and starting concentration were selected so that fewer than four responses would be required before a reversal (Cornsweet, 1962). The panellist would then respond verbally "yes" or "no" if the sample was ashy, which was then recorded by the experimenter. If the participant responded yes, the next sample would be one concentration step down, whereas if the participant responded no, the next sample would be one step up (Table 2). Between each sample, participants had a 90-second break to rinse their mouth with the provided glucose solution, followed by water.

This staircase procedure then continued until five reversals occurred. Five reversals were chosen as the stopping rule to decrease fatigue and habituation, so as not to lead to arbitrarily low thresholds (Running, 2015). Reversals were considered the point where participants changed their response from "yes" on the previous sample to "no", and vice versa. Reversals were only counted if they were within 3 steps of each other, as described in Wise (2008), to ensure a steady threshold was reached. Once this qualification was reached, the session would end. If a participant responded "no" at the highest concentration 3 times in a row, the session ended and was recorded as MAX to indicate the tested range did not reach a recognisable concentration. If a participant responded "yes" at the lowest concentration 3 times in a row, the session ended and was recorded as MIN to indicate no thiophenols were required for the wine to be perceived as ashy.

2.4 Extended maceration and alternative yeast wines

2.4.1. Samples

Samples were prepared according to section 2.3. Along with the low phenol addition, moderate (135 μg /L) and high (450 μg/L) phenol additions were also tested for a total of six separate panels. Three additional concentration steps of thiophenols were added to the staircase method to capture more individuals with a higher threshold (based on the low extract study) and a hypothesised higher threshold in a more complex wine style. Moreover, an additional "–1" step was used to quantify those who said yes with no thiophenols or phenols present (Table 2).

2.4.2. Procedure

For these portions of the study, the verbal test from section 2.3 was translated into an online survey using RedJade Sensory & Consumer Software (Version 5.1.1) (RedJade Sensory Solutions LLC, Pleasant Hill, CA). The testing followed the same procedure as described in section 2.3, with some modifications. Participants would first be guided through the training, then complete the demographic survey. Once the threshold testing began, participants would select yes or no for the specified sample from an option list on the screen. The interstimulus protocol remained the same as in section 2.3. Number of thresholds, those above the tested range (MAX), those that did not meet the stopping criteria (incomplete), and those with a threshold at 0 ng/L are indicated in supplementary Table 3.

3. Data analysis

To determine the number of threshold estimates needed to achieve a margin of error (E) of 0.25 log units, the method proposed by Qumsiyeh (2013) was used with the 91 thresholds in the low-extract data set. This method used bootstrapping to determine the level of error associated with various sample sizes. In total, 1000 random samples were drawn from the log-transformed dataset of calculated thresholds, with replacement, and the mean margin of error of the sample was calculated. This process was repeated 500 times to obtain a 95 % confidence interval of the 500 average E values. This process was repeated for various sample sizes (90, 80, 70, 60, 50, 40, 39) to determine at what level the desired E falls within the confidence interval.

Thresholds were determined by taking the geometric mean of the concentration of the last 4 reversals for each individual. If a reversal occurred at a concentration of 0 ng/L (Step 0), the arithmetic mean was used. The best estimate thresholds were then determined from the geometric mean of each individual threshold. In this procedure, the calculated threshold estimates the 50 % point on a psychometric function, assuming a stable function exists (Rose *et al*., 1970).

To explore the distribution of thresholds further, the quickpsy package in RStudio was used (Linares & López-Moliner, 2016). This function fits the psychometric curve based on direct maximisation of the likelihood and was set to use 1000 bootstrapped samples to estimate the plot parameters. This plot was based on each individual threshold measurement, eliminating inconclusive, minimum, and maximum observations. Using this function, the 0.5 proportion point was plotted to indicate the estimated ashy recognition threshold for 50 % of individuals. Additional proportions (0.4, 0.3, 0.2) were calculated to consider different risk levels.

RESULTS

1. Low-extract wine

1.1. Sample size calculations

The number of individual thresholds needed to accurately estimate the group threshold within 0.25 log units was determined to determine how many participants would be needed in further threshold testing with this method. Bootstrapping samples of various sizes showed that 40 or greater threshold measurements kept the 0.25 log units within the 95 % confidence interval of "E" (Table 3). As expected, as the sample size increased, the margin of error decreased. This indicates that while a larger number of thresholds will reduce the margin of error in the group threshold, 40 is an acceptable number to produce this estimation.

TABLE 3. Estimated "E" and 95 % confidence intervals of bootstrapped data sets from the low-extract Pinot noir threshold estimates.

Number of sample sets	"E" Estimate	95 % confidence interval of the "E" estimate
90	0.176	[0.166, 0.186]
80	0.187	[0.176, 0.198]
70	0.200	[0.188, 0.212]
60	0.216	[0.203, 0.229]
50	0.236	[0.222, 0.251]
40	0.263	[0.248, 0.278]
39	0.267	[0.253, 0.293]

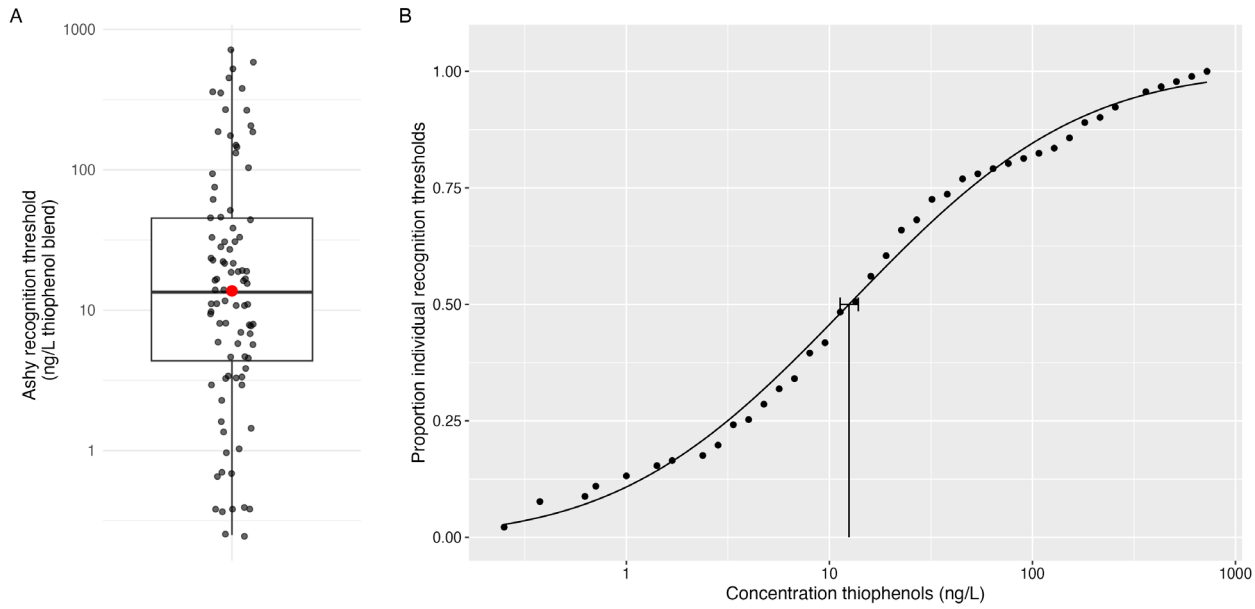


FIGURE 1. Threshold visualisation for low-extract Pinot noir with low concentration of phenol blend (45 mg/L) A). Boxplot of individual ashy recognition thresholds. Black points represent individual threshold estimates (n = 91), and the red point indicates the group threshold level; B). Psychometric curve based on the individual thresholds for the low-extract Pinot noir with a low concentration of phenol blend. Vertical lines indicate the estimated 50 % point and error bars indicate a 95 % confidence interval.

1.2. Thiophenol ashy recognition threshold at low phenol concentration

The group threshold calculated from the 91 individual thresholds was found to be 13.7 ng/L, with a geometric standard deviation of 7.25 ng/L, and the recorded thresholds ranged from 0.250 ng/L to 724 ng/L (Figure 1A). For 11 % of the 120 participants, the range of tested thiophenol concentrations was not high enough for ashy flavour to be recognised.

Based on the fitted psychometric curve, the estimated recognition threshold concentration for 50 % of individuals is 12.5 ng/L with a 95 % confidence interval from 11.3 ng/L to 13.9 ng/L (Figure 1B).

2. Extended maceration Pinot noir

2.1. Thiophenol ashy recognition threshold at low phenol concentration

The group threshold calculated from the 53 individual thresholds was found to be 39.6 ng/L, with a geometric standard deviation of 7.32 ng/L. The recorded thresholds ranged from 0.750 ng/L to 4871 ng/L (Figure 2). For 18 % of the 76 participants, the range of tested thiophenol concentrations was not high enough for ashy flavour to be recognised.

Based on the fitted psychometric curve, the estimated recognition threshold concentration for 50 % of individuals is 32.2 ng/L with a 95 % confidence interval from 28.0 ng/L to 37.4 ng/L (Figure 3). Looking at more conservative percentages of the possibility of individuals recognising ashy flavour, Table 4 indicates the estimated concentration to capture 40 %, 30 %, and 20 % of individuals' recognition thresholds.

2.2. Thiophenol ashy recognition threshold at moderate phenol concentration.

The group threshold calculated from the 51 individual thresholds was found to be 23.0 ng/L, with a geometric standard deviation of 10.2 ng/L. The recorded thresholds ranged from 0.275 ng/L to 3,444 ng/L (Figure 2). For 15 % of the 73 participants, the range of tested thiophenol concentrations was not high enough for ashy flavour to be recognised.

Based on the fitted psychometric curve, the estimated recognition threshold concentration for 50 % of individuals is 18.8 ng/L with a 95 % confidence interval from 15.28 ng/L to 22.64 ng/L (Figure 3). Looking at more conservative percentages of the possibility of individuals recognising ashy flavour, Table 4 indicates the estimated concentration to capture 40 %, 30 %, and 20 % of individuals' recognition thresholds.

2.3. Thiophenol ashy recognition threshold at high phenol concentration

The group threshold calculated from the 40 individual thresholds was found to be 14.1 ng/L, with a geometric standard deviation of 7.67 ng/L. The recorded thresholds

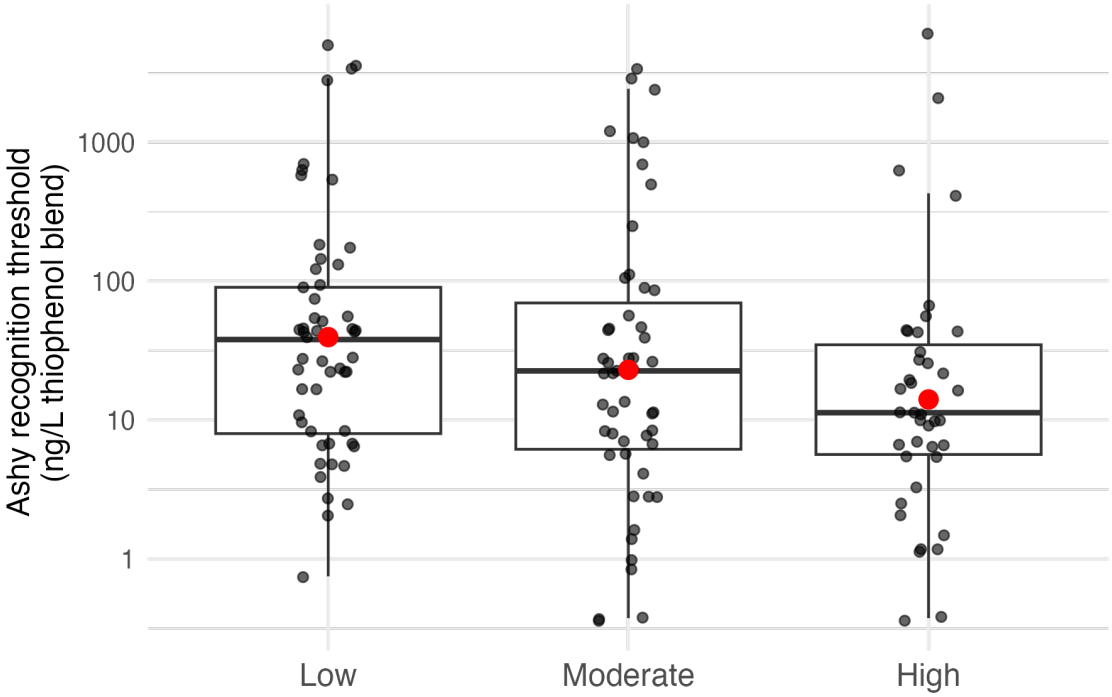


FIGURE 2. Boxplot of ashy recognition thresholds for extended maceration Pinot noir with low (45 µg/L), moderate (135 µg/L), and high (450 µg/L) concentrations of phenol blend. The black point represents the individual threshold estimate, and the red point indicates the group threshold level within each phenol level.

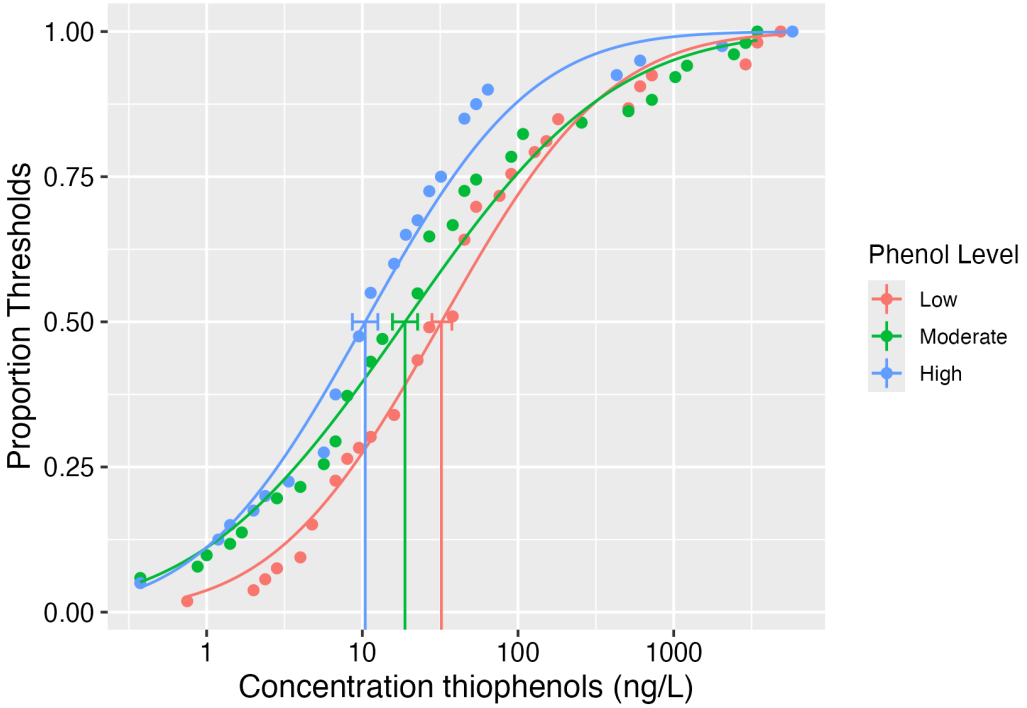


FIGURE 3. Psychometric curve based on the individual thresholds for the extended maceration Pinot noir with low (45 µg/L), moderate (135 µg/L), and high (450 µg/L) concentrations of the phenol blend. Vertical lines indicate the estimated 50 % point, and error bars indicate a 95 % confidence interval.

ranged from 0.375 ng/L to 5,792 ng/L (Figure 2). For 7 % of the 58 participants, the range of tested thiophenol concentrations was not high enough for ashy flavour to be recognised. For 3 participants, their recognition threshold was 0 ng/L of thiophenols, indicating this concentration of phenols alone produced a recognizably ashy flavour in the wine.

TABLE 4. Thiophenol level for ashy recognition threshold for extended maceration of Pinot noir at various percentages of individuals based on the fitted psychometric curve. Values in brackets indicate the 95 % confidence interval.

Percentage of recognition thresholds	Low Phenol	Moderate Phenol	High Phenol
50 %	32.2 ng/L [27.4, 37.7]	18.8 ng/L [15.5, 22.7]	10.4 ng/L [8.66, 12.7]
40 %	19.7 ng/L [16.8, 23.0]	10.2 ng/L [8.35, 12.3]	6.41 ng/L [5.21, 7.98]
30 %	11.6 ng/L [9.73, 13.8]	5.33 ng/L [4.27, 6.65]	3.80 ng/L [2.99, 4.71]
20 %	6.24 ng/L [5.09, 7.61]	2.49 ng/L [1.90, 3.19]	2.06 ng/L [1.54, 2.74]

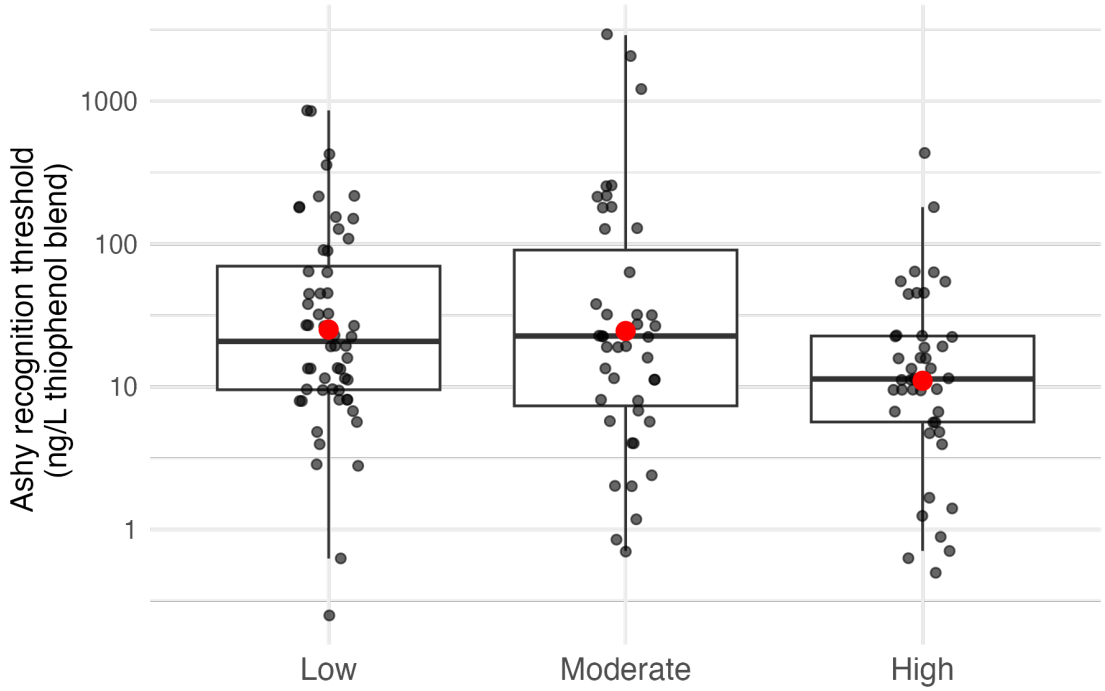


FIGURE 4. Boxplot of ashy recognition thresholds for the alternative yeast Pinot noir with low (45 μg/L), moderate (135 μg/L), and high (450 μg/L) concentrations of phenol blend. The black point represents the individual threshold estimate, and the red point indicates the group threshold level within each phenol level.

Based on the fitted psychometric curve, the estimated recognition threshold concentration for 50 % of individuals is 10.43 ng/L with a 95 % confidence interval from 8.60 ng/L to 12.57 ng/L (Figure 3). Looking at more conservative percentages of the possibility of individuals recognising ashy flavour, Table 4 indicates the estimated concentration to capture 40 %, 30 %, and 20 % of individuals' recognition thresholds.

3. Alternative yeast Pinot noir

3.1. Thiophenol ashy recognition threshold at low phenol concentration

The group threshold calculated from the 56 individual thresholds was found to be 25.1 ng/L, with a geometric standard deviation of 5.03 ng/L. The recorded thresholds ranged from 0.250 ng/L to 861 ng/L (Figure 4). For 11 % of the 75 participants, the range of tested thiophenol concentrations was not high enough for ashy flavour to be recognised.

Based on the fitted psychometric curve, the estimated recognition threshold concentration for 50 % of individuals is 22.6 ng/L with a 95 % confidence interval from 20.1 ng/L to 25.5 ng/L (Figure 5). Looking at more conservative percentages of the possibility of individuals recognising ashy flavour, Table 5 indicates the estimated concentration to capture 40 %, 30 %, and 20 % of individuals' recognition thresholds.

3.2. Thiophenol ashy recognition threshold at moderate phenol concentration

The group threshold calculated from the 43 individual thresholds was found to be 24.5 ng/L, with a geometric

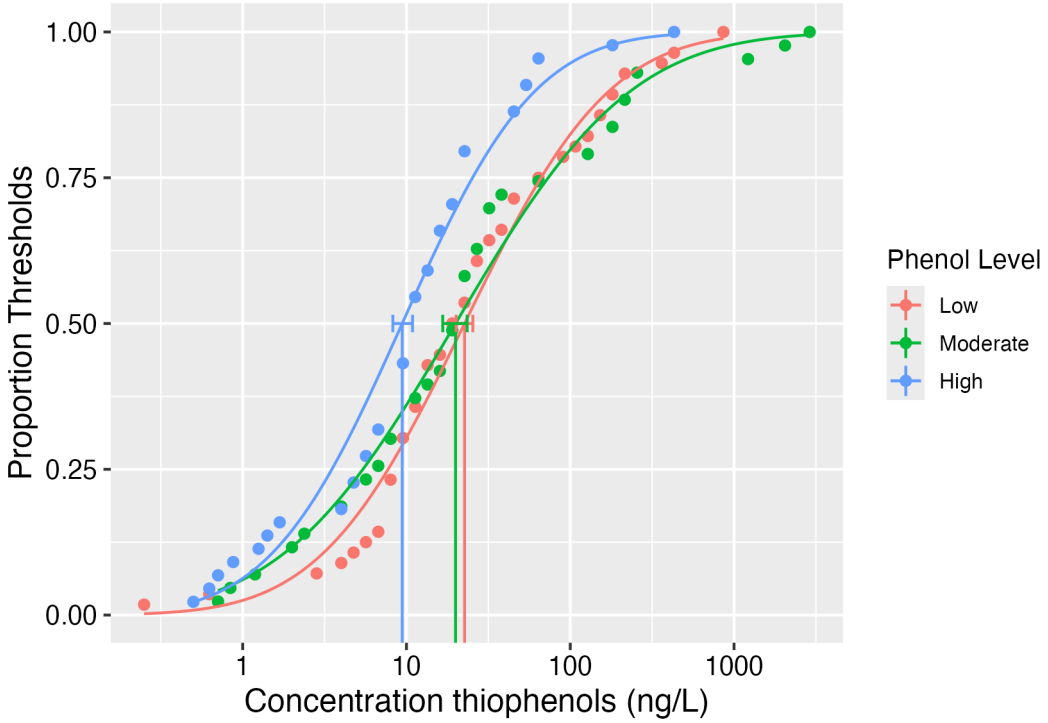


FIGURE 5. Psychometric curve based on the individual thresholds for the alternative yeast Pinot noir with low (45 µg/L), moderate (135 µg/L), and high (450 µg/L) concentrations of the phenol blend. Vertical lines indicate the estimated 50 % point, and error bars indicate a 95 % confidence interval.

TABLE 5. Thiophenol level for ashy recognition threshold for the alternative yeast Pinot noir at various percentages of individuals based on the fitted psychometric curve. Values in brackets indicate the 95 % confidence interval.

Percentage of recognition thresholds	Low Phenol	Moderate Phenol	High Phenol
50 %	22.6 [20.1, 25.4]	19.9 [16.5, 23.4]	9.42 [8.15, 11.0]
40 %	15.1 ng/L [13.2, 17.1]	12.2 ng/L [10.1, 14.6]	6.50 ng/L [5.65, 7.53]
30 %	9.81 ng/L [8.47, 11.3]	7.24 ng/L [5.92, 8.89]	4.37 ng/L [3.73, 5.13]
20 %	5.92 ng/L [5.03, 7.00]	3.92 ng/L [3.04, 4.96]	2.74 ng/L [2.27, 3.28]

standard deviation of 7.11 ng/L. The recorded thresholds ranged from 0.707 ng/L to 2,896 ng/L (Figure 4). For 11 % of the 70 participants, the range of tested thiophenol concentrations was not high enough for ashy flavour to be recognised. For 3 participants, their recognition threshold was 0 ng/L of thiophenols, indicating this concentration of phenols alone produced a recognizably ashy flavour in the wine.

Based on the fitted psychometric curve, the estimated recognition threshold concentration for 50 % of individuals is 19.9 ng/L with a 95 % confidence interval from 16.7 ng/L to 23.8 ng/L (Figure 5). Looking at more conservative percentages of the possibility of individuals recognising ashy flavour, Table 5 indicates the estimated concentration to capture 40 %, 30 %, and 20 % of individuals' recognition thresholds.

3.3. Thiophenol ashy recognition threshold at high phenol concentration

The group threshold calculated from the 44 individual thresholds was found to be 11.0 ng/L, with a geometric standard deviation of 4.24 ng/L. The recorded thresholds ranged from 0.5 ng/L to 431 ng/L (Figure 4). For 15 % of the 68 participants, the range of tested thiophenol concentrations was not high enough for ashy flavour to be recognised. For 2 participants, their recognition threshold was 0 ng/L of thiophenols, indicating this concentration of phenols alone produced a recognizably ashy flavour in the wine.

Based on the fitted psychometric curve, the estimated recognition threshold concentration for 50 % of individuals is 9.42 ng/L with a 95 % confidence interval from 8.21 ng/L to 10.85 ng/L (Figure 5). Looking at more conservative percentages of the possibility of individuals recognising ashy flavour, Table 5 indicates the estimated concentration to capture 40 %, 30 %, and 20 % of individuals' recognition thresholds.

4. Comparing threshold estimates

Using the estimated psychometric functions, the threshold concentration of thiophenols was significantly different for all phenol concentrations at the 50 % estimation point for the extended maceration wine. The recognition threshold concentration of thiophenols is lowest at the high phenol concentration, followed by the moderate phenol concentration, and highest at the low phenol concentration. Looking at the more conservative percentages, as the percentage increases, the high phenol and moderate phenol wines no longer are significantly different at the 30 % and 20 % points. For the alternative yeast wine, at 50 %, 40 %, and 30 % the recognition threshold estimations of the high phenol concentration had significantly lower estimates than the low and moderate phenol wines. For the 20 % point, this relationship changes to the low phenol concentration being significantly higher than the moderate and high.

In comparing the wine styles at each of the phenol concentrations, the alternative yeast wine had significantly lower recognition threshold concentrations of thiophenols across all percentages for the low phenol wine. For the moderate phenol concentration, there are no significant differences in the estimates at the different percentage levels between the extended maceration and alternative yeast wines.

DISCUSSION

1. Number of thresholds

Using the proposed method by Qumsiyeh (2013) and controlling the margin of error at 0.25 log units, 40 or greater ashy recognition thresholds were found to be acceptable to provide an estimate within these constraints. As expected, with larger sample sizes, the margin of error decreases (Gacula & Singh, 1984). While in an ideal world, there would be unlimited resources to minimise error as much as possible, there are limits that need to be taken into consideration. Eckerman *et al*. (2010) define the value of information as the balance of the cost of collecting additional datapoints against the increase in information the data provides (Lakens, 2022). While more data points lead to a lower margin of error, the cost may not outweigh the increased information. In this study, the value of information was controlled largely based on the wine volume available. As mentioned, each wine vintage can provide different chemical and sensory properties, making them distinct samples. Therefore, the sample size needed to be optimised for this method for wine research to ensure the number of participants was within resource constraints.

To further evaluate the sample size estimate given by this method, G*Power software was used to confirm this size. This has been used in previous recognition threshold work with staircase procedures (Yoshino *et al*., 2021). Using the G*Power software (Faul *et al*., 2007) for a one-sample case, the sample needed to obtain a power of 0.8 with an alpha level set to 0.05 and an effect size of 0.5, was 26.138 thresholds. With the 40 samples proposed using the previously described method, this achieves a power of 92.5 % in estimating the population mean.

2. Relationship of phenol concentration and thiophenol concentration on ashy recognition

Wine is a complex matrix that impacts all flavour expression, from both a physiological and psychological perspective. When considering the expression of volatile compounds in a wine, the interaction with other components within the wine matrix is an important consideration, which is impacted by grape varietal and winemaking strategy (Villamor & Ross, 2013). These matrix components include alcohol content, polyphenolic compounds, polysaccharides and other carbohydrates, and the other aroma compounds present within the wine. These can all impact the sensory expression of volatile compounds via several different mechanisms, including presence in the headspace and release upon consumption (Villamor & Ross, 2013). Perceptual experience of odorants is also a combinatorial experience, indicating how the mixture of volatile compounds within a wine influences both the identity and intensity of the perceived flavour (Malnic *et al*., 1999). Additionally, flavour perception can be dependent on the valence of the flavour attributes, with less pleasant flavours being able to suppress the perception of pleasant ones (Ferreira *et al*., 2021).

The retronasal detection threshold of guaiacol, a key contributor to smoke-related flavours, has been found to be 27 μg/L in red wine (Parker *et al*., 2012). While this compound is found in suprathreshold levels in smoke-impacted wine, it is also found above this threshold in oak-aged wines as well (Parker *et al*., 2024a). The concentration of this compound alone, therefore, does not predict the off flavours associated with smoke taint, as in oak-aged wines, it does not take on the flavour properties indicative of smoke-impacted wine. This indicates the importance of understanding the combinatorial experience of volatile compounds in smoke-impacted wine, along with when a wine is taking on the perceptible ashy quality. In this current study, concentrations of guaiacol in the phenol mixture ranged from sub (5 μg/L and 15 μg/L) to supra-threshold levels (50 μg/L), indicating further how the detection of each of these individual compounds does not necessarily indicate the presence or absence of ashy flavour. At all phenol concentrations, the group ashy recognition threshold was found within the tested range for the panel as a whole. While this group thiophenol threshold was found, 8 individual thresholds were found at 0 ng/L of thiophenols between the moderate and high phenol concentrations (Supplementary Table 3). This effect indicates that, overall, both volatile types were required for the recognition of ashy flavour; however, some individuals recognised this flavour

with only the phenol blend present at the moderate and high levels. This highlights the individual nature of flavour recognition and how consumers are not going to respond the same way, leading to the importance of understanding thresholds as levels of risk as opposed to absolute measures.

As seen in this work, there is an inverse relationship between the concentration of total smoke-related volatile phenols and the ashy recognition threshold concentration of total thiophenols. This further supports the importance of both these compounds in the negative ashy description found in smoke-impacted wine and the combinatorial relationship between them. This creative interaction occurs with different aroma vectors at specific ratios; in this case, the thiophenols and phenol mixtures lead to the recognition of an attribute with different properties of the original components (Ferreira *et al*., 2021; Tomasino *et al*., 2023). This relationship was seen across both wine styles, with the calculated thresholds being highest in the low phenol wine, followed by the moderate, and the lowest concentration with the high phenol wine.

The calculated recognition threshold of thiophenols in these wines, based on the calculated group threshold, ranged from 13.7 ng/L to 39.6 ng/L. These concentrations are most likely above those found in non-smoke-impacted wine, but it is not possible to determine the relationship of these thresholds to smoke-impacted or tainted wines to date, as a quantitative method to measure thiophenols does not currently exist. It is known from previous studies using qualitative analysis that thiophenols are found at greater levels in smoke-impacted areas than in non-smoke-impacted areas, but the actual concentration is currently unknown (Tomasino *et al*., 2023). When looking at these values, the total concentration of thiophenols is considered, containing five different individual compounds. This makes the individual contribution of each of these compounds, as they were in equal proportions, range from 2.74 ng/L to 7.92 ng/L. These concentrations agree with what is known about other smoky-related thiols in wine, namely the threshold of phenylmethanethiol (PMT) in model hydroalcoholic solutions (Tominaga *et al*., 2003). This further indicates the high potency of thiol compounds when present in wine, the small concentration required to elicit this ashy off-flavour, and the impact a small difference in concentration can have in different wine systems.

This relationship between total phenol concentration and concentration of thiophenols required for ashy flavour is important to understand, as volatile phenol concentration in a wine can increase throughout the winemaking process. Therefore, a wine made from smoke-exposed grapes that were deemed to be fine may not be dependent on the winemaking practices. As previously mentioned, oak is a contributor to total phenols within a wine system, with the concentration being dependent on oak type and level of toasting (Parker *et al*., 2024a). Oak ageing is a typical practice in the production of red wine, and the use of oak alternatives is considered a strategy to mitigate smoke-taint (Mirabelli-Montan *et al*., 2021). However, if the oak is contributing more smoke-related volatile phenols to the system containing a set concentration of thiophenols, this may be able to push a wine identified to be without ashy properties into the range at risk of being perceived as ashy.

Additionally, smoke-impacted wines contain non-volatile phenols as well. These glycosylated non-volatile phenols can be released into aromatic free phenols throughout the winemaking process, therefore increasing the amount of perceptible volatile phenols in the resulting wine. In total, 20 mg/L of phenolic glycosides has the potential to release over 100 μg/L of volatile phenols during winemaking and storage (Hayasaka *et al*., 2010). This can additionally push a wine not at risk to potentially having a perceptible ashy flavour. As the mild acidic conditions favour this reaction, the release of these compounds post-bottling needs to be better understood to simulate a wine sitting in a consumer's home before consumption and therefore impacting the perceived quality (Merrell *et al*., 2021). These bound phenols are additionally released rapidly in the oral cavity upon consumption. This release in the mouth and the interaction with the thiophenols upon consumption need to be better understood, with this rapid release contributing to the perception and lasting nature of this ashy flavour. However, adding to consumer variability, the release from person to person can be very different, with Mayr *et al*. (2014) noting a range of 12 % to 63 % release among a small panel.

3. Threshold in different wine styles

The wine styles in this work were selected to alter the wine matrix in different ways to see the style's impact on the threshold for recognizable ashy flavour in Pinot noir. Thresholds are dependent on the matrix the compounds are present within, as seen with the detection threshold of guaiacol in water (0.48 μg/L), white wine (95 μg/L), and red wine (23 μg/L) (Eisele & Semon, 2006; Ferreira *et al*., 2000; Parker *et al*., 2012). This underpins the importance of understanding the dependence of threshold-based production decisions.

In the low-extract wine, the lower fermentation temperature is less favourable for the extraction of polyphenolic compounds, which contribute to the mouthfeel and colour of a wine (Amerine & Ough, 1957). To contrast, the extended maceration wine leaves the wine in contact with the skins for an extended period of time to increase the extraction of polyphenolic compounds (Brossard *et al*., 2016). For the alternative yeast wine, yeast selection is an effective tool in altering the properties of the wine matrix, introducing different aroma and flavour properties (Takush & Osborne, 2011). It has been found that smoke-impacted wines benefit from having a greater sensorial complexity, with additional attributes being able to distract from the more unfavourable smoke-related attributes (Mirabelli-Montan *et al*., 2021). Additionally, fruity aromatics have been known to mask wood-related aromatics (Atanasova *et al*., 2005).

Between these differing styles of Pinot noir wine, it was found that there were differences in the observed ashy recognition thresholds. Focusing on the low phenol concentration, as this was the only level tested with the low-extract wine, the low-

extract required the lowest concentration of the thiophenol blend to be perceptually ashy, followed by the alternative yeast, then the extended maceration. This shows that as the style of the wine is altered, there are changes to the threshold level of thiophenols required to produce an ashy flavour. For the extended maceration wine, there is an increase in phenolic content with the use of this wine-making strategy, which can change the release of aroma compounds (Jung & Ebeler, 2003). The hydrophobicity of aroma compounds is considered to be the main contributor to the interaction between volatiles and polyphenols (Dufour & Bayonove, 1999). Aroma compounds that contain a benzene ring, which several smoke-related compounds do, specifically may be impacted by the π–π interaction with the polyphenols (Jung *et al*., 2000). This is additionally coupled with the perceptual interactions, which are universal in the wine drinking process (Arvisenet *et al*., 2016). Increasing the complexity of the stimuli by increasing the amount of provided sensory information, there can be decreased attention on one specific area and therefore lessening perception of a single attribute (Sáenz-Navajas *et al*., 2020). This effect can additionally explain the change in threshold found with the alternative yeast. The alteration of the aromatic profile can introduce different aroma compounds and therefore perceived flavours that can distract from the ashy flavour. Additionally, aroma-aroma interactions can alter both the quality and intensity of the perceived flavour (Polášková *et al*., 2008).

In looking at the moderate and high concentrations of the phenol blends in the extended maceration and the alternative yeast wines, the significant differences are eliminated between the styles. This suggests that at a higher level of smoke-related phenols, a more consistent concentration of thiophenols to produce a recognisable ashy flavour may exist, regardless of style. As smoke-affected wines are found to have concentrations more similar to the moderate and high concentrations of volatile phenols, this indicates that wine makers may be able to use a single value to assess the recognisable "ashy" potential of their wines.

4. Limitations

As seen in the boxplots, there is a high level of variability within the measured thresholds under each condition. This high amount of variability is not uncommon in threshold testing, with individual differences found to span about 2–3 orders of magnitude up to over 10 (Wise *et al*., 2008). Even among highly trained individuals, the distribution of individual thresholds in wine can span over 3 log units (Tempere *et al*., 2011). This variation can be due to a wide variety of reasons, including wine expertise and experience with the flavour, along with various other cognitive factors and personal preferences (Malfeito-Ferreira, 2023). Additionally, genetically specific anosmias can influence recognition, especially when discussing blends of compounds, and could be the reason for the individuals whose recognition threshold was not within the tested range (Mainland *et al*., 2014). The ability to recognise an odour is highly dependent on learning and previous experiences, which relates to a person's ability to retrieve an odour from memory (Goldstein & Brockmole, 2017). While training can improve this, training individuals to be able to recognise a flavour can decrease the observed threshold by as much as 1,000-fold, therefore not being representative of the true recognizability of consumers.

In this work, an additional source of variability is found in the participants' ability to set their own criterion in the yes-no procedure. While a potential area of variability between participants, when a consumer is tasting wine, during each tasting scenario, they are setting their own criterion (Höchenberger & Ohla, 2019). This criterion is based on both the intrinsic properties of the wine but additionally the extrinsic properties, like bottle label, awards, and several other areas (Running, 2015). This is not only dependent on the wine properties, but additionally, each person's sensitivity, the context of consumption, and the participants' desire not to be wrong. In sensory studies, the goal is to remove external distractors, but when tasting in an ecologically valid scenario, these distractors can exist, which can alter flavour perception and recognition due to the cognitive effects of perception. While this adds variability to our understanding of human perception, it is important to understand quality markers and consumer experience with the wine.

Regarding the wines, the smoke-related compounds were added to the wine as opposed to occurring due to the smoke impact. With this, the sensory profile of these wines is going to differ from wines that contain these compounds from smoke exposure. Each smoke event is going to produce smoke clouds with a unique compositional breakdown of the major volatile phenols. This, in turn, alters what is absorbed by the grape and found in the resulting wine, which is also influenced by the density of smoke, which varies by distance along with production practices (Parker *et al*., 2024b). In this work, a simple breakdown of the phenols was included to focus on the total volatile composition for analysis, but it needs to be understood that this breakdown may not always align with what is observed in nature. Additionally, as these wines were only exhibiting ashy flavour due to the volatile additions, this did not include the phenolic glycosides known to be present within smoke-impacted wines, which are influential in the lasting nature of ashy flavour upon consumption. While this is an important factor in ashy flavour perception, there is also some individual variation in the in-mouth release of the volatile from the bound state; this work aimed to remove this level of variability (Mayr *et al*., 2014). Future work should focus on this particular area, understanding how this in-mouth release within individuals influences their recognition of ashy flavour.

Using the yes-no method with the staircase procedure, the threshold estimates indicate the point at which a participant would identify a wine as ashy 50 % of the time. While this percentage may be considered low, tasting wine often requires multiple sips, so this needs to be taken into consideration. At the first sip, there is a 50 % chance of recognition. With the second sip, there is a 75 % chance the flavour was recognised on at least one of the sips. At 3 sips, there is an 88 % chance, and with 4, there is a 94 % chance. This also

ignores the flavour carry-over potential from non-controlled consumption scenarios that will alter this probability of recognition, along with external influences on recognition. Further work is required to understand how the multi-sip experience impacts the recognition threshold of ashy flavours in these wines.

5. Future work

While this work gives key insights into understanding the recognition of ashy flavour in different Pinot noir wine systems, there are additional areas to expand on. First are rejection thresholds, which are an important area for better understanding the opinion of these wines from consumers. While this work explored how ashy flavours might be recognised in wines, which is not necessarily desired from winemakers, it does not mean it will be rejected by consumers. A recognisable property in wine and a wine fault are important to differentiate between (Parker *et al*., 2024b). Some works have indicated that ashy flavour in a wine is a negative contributor to liking, while others have shown that some consumers are less bothered by the smoke-related attribute (Fryer *et al*., 2025). This indicates that the level of recognition and rejection may show a similar variability within the population.

An additional area is looking at different ratios and wine varietals. The ratios within the phenol and thiophenol blends were simple breakdowns of the major components previously identified. As these can exist in different ratios in wildfire-affected wines, therefore could have an impact on the ashy recognition threshold. Additionally, there may be a varietal dependence for the recognition thresholds, with it being known that Pinot noir has a greater susceptibility to exhibit smoke taint than other varietals (Ristic & Wilkinson, 2013). Different varietals have different chemical compositions and sensory attributes; therefore, they may have different ashy recognition thresholds and tolerance for these flavours due to the different compositions and typical attributes (Bilogrevic, *et al*., 2023).

CONCLUSION

This work shows the importance of both thiophenols and phenols present in wildfire-affected wines on the perception of ashy flavour. At the level of total volatile phenols increased in this work, there was a decrease in the concentration of total thiophenols required to produce a recognisable ashy flavour. As no significant differences were found between the styles at the higher phenol concentrations, overall, at a moderate concentration of total volatile phenols (~135 μg/L), it is recommended that thiophenols are below 20 ng/L to limit ashy flavour recognition by consumers in Pinot noir. At a high concentration of total volatile phenols (~450 μg/L), total thiophenols should be below 10 ng/L. These recommendations are based on 50 % of individuals' ashy recognition point, with more conservative levels of risk of ashy flavour perception discussed. Additional considerations need to be taken based on the introduction of volatile phenols from other parts of wine making and from glycosidic breakdown that can alter the recognition level. This work also determined the minimal number of thresholds to produce reliable estimates to ensure proper allocation of resources using this adaptive staircase method for smoke-affected wine research. Further work is needed to understand how this level and relationship between volatile phenols and thiophenols change in other wine varietals and styles, along with various ratios of these compounds within the mixture.

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