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Research Article

The Role of Potent Thiols in "Empyreumatic" Flint/Struck-Match/Mineral Odours in Chardonnay Wine

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Background and Aims. A wide range of Chardonnay styles exist on the market, from fruit-forward examples to wines displaying "empyreumatic" aromas such as flint, smoky, mineral, and struck-match. The thiols 2-furylmethanethiol and phenylmethanethiol have been linked to these aromas, and this study aimed to determine the contribution of these compounds to specific sensory properties in Chardonnay wines, as well as the consumer acceptance of wine displaying "empyreumatic" aromas. *Methods and Results*. Twenty-four Australian and New Zealand Chardonnay wines were selected for volatile analysis and quantitative sensory descriptive analysis. Consumer liking of a subset of six wines was also determined, and a further sensory study involving additions of the thiols to a base wine was conducted. Partial least squares regression showed that flint/struck-match/mineral aromas were related to 2-furylmethanethiol concentration with phenylmethanethiol less well associated. The odorant addition study confirmed that 2-furylmethanethiol directed flint/struck-match/mineral aromas and exerted strong suppression of other aromas while phenylmethanethiol played a lesser role. Consumer acceptance ($n = 92$) was overall lower for wines displaying high flint/struck-match/mineral aromas, although cluster analysis of the liking scores identified a sizeable consumer group (33%) who preferred wines with this attribute. *Conclusions*. The potent thiol 2-furylmethanethiol was indicated to be the primary contributor to flint/struck-match/mineral aromas in Chardonnay wines, with phenylmethanethiol playing a subordinate role. *Significance of the Study*. Increased concentration of 2-furylmethanethiol and the conferred "empyreumatic" odours should be carefully considered when producing wine styles to appeal to consumers.

1. Introduction

Australian wines made from the Chardonnay cultivar represent a high proportion of domestic and exported white wines. They are produced in most regions with varied winemaking techniques and can display a wide range of sensory properties. Of these styles, so-called "empyreumatic" aromas are common for some Chardonnay styles, particularly for barrel-aged or barrel-fermented Chardonnay. The odour category of empyreumatic was introduced to the fragrance lexicon by Dutch scientist H. Zwaardemaker to describe smoky and burnt odours [1]. Although this category is not commonly used in wine science literature outside of Europe, it has been used to describe wines, often oaked white wines, which display aromas reminiscent of smoke, gunpowder/gun flint, minerals, roasted coffee, toast, brioche, or the smoky/sulfidic odour of a struck-match [2, 3].

Links between Chardonnay chemical composition and specific sensory properties have been reported for several compounds: thiols with tropical aromas [4]; acetate esters and terpenes with fruity and floral notes; volatile phenols with oaky nuances [5]; pyrroles and pyrrolemethanethiols with hazelnut-like aromas [6]; fatty acid ethyl and acetate esters as well as lactones with stone fruit aromas [7, 8]; higher alcohols and floral notes [9]; aldehydes and oxidation related flavour deterioration [10]. Although these studies have shown evidence of relationships using winemaking experiments, correlation tests, or regression approaches, wine compounds are in many cases ubiquitous and co-correlated, and these associations do not definitively imply chemical cause and sensory effect.

Regarding "empyreumatic" odours, two polyfunctional thiols have been linked to this aroma category, phenylmethanethiol (benzenemethanethiol and benzyl mercaptan) [2] and 2-furylmethanethiol (furan-2-ylmethanethiol, 2-furanmethanethiol, or furfuryl thiol) [3], in both still Chardonnay table wines and Champagne wines.

Sensory detection threshold testing of compounds likely to be important to Chardonnay has been conducted with 2-furylmethanethiol [11] and phenylmethanethiol [2], as well as for 4-methyl-4-sulfanylpentan-2-one [12], stereoisomers of oak lactone [13], 1-methylpyrrole-2-methanethiol, and 1-ethylpyrrole-2-methanethiol [6] which allow for the comparison of the potency of aroma compounds. Knowledge of the sensory detection threshold of a particular compound has been invaluable to gauge if a compound is likely to contribute to wine aroma and flavour at the concentration range found naturally, but again these values lack the ability to characterise the particular odour quality or intensity in a wine. Beyond "impact odorants," the demonstration of causation is further complicated by complex interactions which may occur at the chemical, sensory receptor, and cognitive levels to suppress, augment, or otherwise alter sensory perception.

Other approaches exploring perceptual interactions by capturing changes in sensory quality and intensity are robust sensory methods such as quantitative descriptive analysis (QDA) [14] coupled with tests such as reconstitution/omission and odorant spiking experiments [15].

The steps of analytical identification, correlation of chemical concentration with sensory properties followed by causally qualifying sensory effects, together contribute in explaining observed phenomena within a product such as wine. These types of experimentation, however, cannot determine if a particular sensory character, directed by a particular compound, might be important in influencing consumer acceptance or purchase behaviour. Often this question is left unresolved; however, wine is a consumer product and presumably, a wine's aroma, taste, and mouthfeel contribute strongly to acceptance and purchase behaviour, alongside important marketing cues such as price, packaging, labelling, and advertising. Consumer blind testing can involve investigation of the influence of a particular compound, such as "consumer rejection threshold" methodology reported for 2,4,6-trichloroanisole (TCA) [16], 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (1,8-cineole) [17], and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) [18]. Consumer acceptance testing, however, can also evaluate the wine styles on the market such as the previous study by Saliba et al. [19] which identified an overall preference of Australian consumers for fruity Chardonnay styles with negligible oak influence. In contrast to "empyreumatic" wine styles, these more fruit-forward Chardonnay styles, preferred by consumers, were found to display "peach" aromas with some sweetness. Using odour addition studies, the ethyl esters conferring "peach" aroma in Chardonnay have only recently been demonstrated [20], while the source of "sweetness" in sugar dry white wines is not well understood. In dry red wines, the residual amino acid proline has been shown to increase "sweetness," "viscosity," and fruit flavours [21].

Only a few studies have investigated the consumer response to wines displaying empyreumatic aromas such as those reminiscent of "smoky," "struck-match," or "flint." Smoke-related compounds such as guaiacol, cresols, and their glycoconjugates have been demonstrated to cause the "smoky" aroma and flavour of wines affected by bushfires [22]. Of these, guaiacol at high and low concentration has been reported to detract from overall consumer acceptance scores, but some consumer segmentation in preference was also reported [23]. Regarding "struck-match" or "flint" aromas, Capone et al. [4] found "flint" aroma was most strongly associated with phenylmethanethiol and weakly negatively related to liking; however, these were unoaked wines produced with standardised winemaking. No study to date has assessed the contribution of 2-furylmethanethiol to consumer acceptance of white wine. Anecdotally, empyreumatic aromas are observed to be more common with barrel-fermented white wines, particularly Chardonnay.

The concept of "minerality" in wines has been found to be ill-defined among experts [24]; however, it has been associated with empyreumatic aromas such as flint, match smoke, kerosene, slate, granite, limestone, tar, charcoal, graphite, rock dust, wet stones, metallic, steel, and ferrous [25]. In the same study relating chemical composition to "mineral" aroma and flavour ratings, phenylmethanethiol and tartaric acid were found to have the highest correlation coefficients. A sensory study investigating "minerality" by comparing wine industry professional's projective maps with flavour profiles for a trained QDA panel found minerality to be positively correlated with reduced, chalky, and grassy aromas and bitter taste [14]. Malic acid, tartaric acid, and the titratable acidity of the wines were highly associated with minerality. In this study, a series of experiments were used to better understand "empyreumatic" odours sometimes found in Chardonnay wines. A survey of the occurrence of the thiols phenylmethanethiol (PMT) and 2-furylmethanethiol (2FMT) was conducted in commercially produced Chardonnay wines from Australia, New Zealand, and France, followed by detailed chemical and formal sensory evaluations of a subset of 24 wines. Consumer testing was then completed on six wines. Finally, a follow-up odorant addition study was conducted to understand the causal effects of candidate compounds associated with wines displaying "empyreumatic" aroma nuances.

The main aim of this work was to assess the sensory significance of PMT and 2FMT in commercially produced Chardonnay wines. We hypothesised that PMT, as identified by Tominaga et al. [2] and Capone et al. [4], would play the major role in directing these smoky/mineral-like aromas and would contribute positively to consumer acceptance. A secondary objective was to assess the relative importance of ester compounds and residual proline concentration identified by Espinase Nandorfy et al. [20] and Espinase Nandorfy et al. [21], respectively, in conferring peach/stone fruit aroma, sweetness, and viscosity in Chardonnay.

2. Materials and Methods

2.1. Wine Samples. A convenience sample of 71 commercially produced white wines (2016–2020 vintages, $11–150), described as having a flint-like and/or stone fruit-like aromas by the winery or wine critic review, were purchased. The Australian wines ($n = 61$) were sourced from a wide range of regions including South-Eastern Australian blends, while eight were from New Zealand (across five regions) and two from France (Chablis, Burgundy). Each wine underwent preliminary informal tasting by AWRI staff from the sensory and research teams, and was subjected to analytical testing for thiol concentrations. 24 Chardonnay wines were selected for the commercial wine QDA, regional and basic chemical composition, and oak usage information available in Table 1, and of these, six wines were further used for consumer testing.

2.2. QDA Panels. Two sensory panels were convened to complete the formal QDA studies of this work. The first panel of eleven assessors (ten females) with an average age of 51 years (SD = 8.4) evaluated the commercial Chardonnay wines. The second panel consisting of eleven assessors (ten females, average age of 53 years, SD = 6.9), including six of the original participants, was then convened to assess the aroma of the odorant spiking samples. All panellists were part of the external AWRI trained descriptive analysis panel and had extensive experience in wine sensory descriptive analysis. All assessors provided informed consent to participate, and this work was conducted in accordance with Deakin University's ethics policy (HEAG-H 169_2019) with the evaluations conducted at the AWRI in Adelaide, South Australia.

2.3. Sensory Evaluation of Commercial Chardonnay Wines. A series of four preliminary sensory evaluations by a panel ($n = 12$) of expert technical wine assessors (four females) with an average age of 40 years (SD = 10.9), were conducted to select 24 wines, from the 71 wines surveyed. Wines were selected that displayed a range of intensities of "empyreumetic" and stone fruit aromas, excluding wines with dominant off-flavours or winemaking artefacts. After the QDA of the commercial wine set, this panel was reconvened to confirm a subselection of wines deemed appropriate for further consumer testing and the odorant addition QDA.

Wines were formally evaluated using the generic QDA method as described in Heymann et al. [14]. To evaluate the 24 commercial wines, assessors attended six two-hour training sessions to determine appropriate descriptors for rating in the formal sessions. All the wines from the study were progressively used during training sessions to generate and refine appropriate descriptive attributes and definitions through a consensus-based approach.

Wines were assessed by appearance, aroma, and flavour. In the third session, standards for attributes were presented and discussed and these standards were also available during subsequent training sessions, the booth practice session, and the formal assessment sessions. As a familiarisation exercise, assessors revisited these aroma and flavour standards as well as at least one "warm-up" sample from the wine set at the beginning of each formal assessment session.

Following the fourth training session, assessors participated in two practice sessions in the sensory booths under the same conditions as those for the formal sessions. After the practice sessions, any terms which needed adjustment were discussed and the final list of terms and standards were determined. For the formal sessions, this list was refined to include one appearance term, fourteen aroma terms (thirteen defined and one "other" term) and fourteen palate terms (thirteen defined and one "other" term). The final list of attributes, definitions/synonyms, and reference standards are shown in Table 2.

2.4. Evaluation. The wines were presented to assessors in 30 mL aliquots in 3-digit-coded, covered, and ISO standard wine glasses at 22–24°C in isolated booths under daylight-type fluorescent lighting. Randomised presentation order across assessors was followed except in the practice sessions when there was a constant presentation order. All samples were expectorated. In the formal booth sessions, the assessors were presented with four trays of three samples per tray, per day. The assessors were forced to have a 60-second rest between samples and were encouraged to rinse with water, and a minimum ten-minute rest between the trays. During the ten-minute break, they were requested to leave the booths. Formal evaluation was completed in six two-hour sessions on separate days. A new bottle was used for each of the presentation days. The 24 commercial wines were presented to assessors three times, in a Williams Latin Square random block design generated by using Compusense20 sensory evaluation software (Compusense Inc., Guelph, Canada). The intensity of each attribute listed in Table 2 was rated using an unstructured 15 cm line scale (numericized 0 to 10), with indented anchor points of "low" and "high" placed at 10% and 90%, respectively. Data were acquired using Compusense20 sensory evaluation software.

2.5. Sample Preparation for Odorant Addition Study. A single, fruity commercial Chardonnay wine (South Australia, 2021 vintage) was used as the base wine with compounds PMT and 2FMT (Sigma-Aldrich, Castle Hill, NSW, Australia) added in a 5^2 full factorial design (two compounds, each added at five concentrations) that generated 25 permutation samples, with each of the five concentration levels increasing by a factor of 2.5. The concentration range chosen represented the minimum-maximum measured in commercial samples previously tested. Appropriate aliquots of PMT (100.0 μg/L) and 2FMT (100.9 μg/L) solutions in ethanol (food grade–ultra premium, Tarac Technologies, Nuriootpa, SA, Australia) were added volumetrically to the homogenized volume of base wine targeting 0, 2.6, 6.3, 16.3, and 40.6 ng/L of PMT and 0, 10, 25, 62.5, and 156.5 ng/L of 2FMT as well as all design combinations. The small amount of ethanol added from the stock

Table 1: Basic compositional data and oak usage for the 24 commercial Chardonnay wine samples from Australia and New Zealand (NZ) assessed in the commercial wine QDA study.

Code	Region	Year	Alcohol (% v/v)	Glucose + fructose (g/L)	pH	Titratable acidity (g/L)	Volatile acidity (g/L)	Malic acid (g/L)	SO_2 free (mg/L)	SO_2 total (mg/L)	Oak usage
AH 1[†]	Adelaide Hills, SA	2017	12.8	1.1	3.18	6.2	0.47	<0.40	22	72	Major
AH 2	Adelaide Hills, SA	2017	12.8	1.7	3.20	6.6	0.35	2.07	22	117	Major
AH 3	Adelaide Hills, SA	2018	12.9	1.0	3.24	6.6	0.45	0.93	28	105	Major
AH 4	Adelaide Hills, SA	2019	14.0	1.2	3.24	6.3	0.28	1.41	22	116	Partial
AH 5	Adelaide Hills, SA	2019	11.9	2.4	3.05	6.7	<0.25	1.75	12	101	None
AH 6[†]	Adelaide Hills, SA	2019	12.5	1.4	3.33	6.6	0.49	1.59	33	123	Major
AH 7	Adelaide Hills, SA	2019	12.2	1.7	3.00	7.9	0.47	1.48	9	75	Major
AH 8	Adelaide Hills, SA	2019	13.4	2.3	3.20	6.4	0.41	1.35	18	112	Major
AH 9[†]	Adelaide Hills, SA	2020	12.9	1.0	3.28	7.0	0.43	1.85	21	101	Major
TAS[†]	Coal River Valley, Tas	2019	12.5	0.8	3.17	7.2	0.55	<0.40	37	112	Major
HV	Hunter Valley, NSW	2018	12.7	0.7	3.23	5.8	0.31	1.32	25	115	Major
KR	Kumeu River, NZ	2019	13.3	2.3	3.35	5.4	0.40	<0.40	24	94	Partial
MRS	Macedon Ranges Vic	2018	13.6	3.2	3.35	5.5	0.45	0.41	16	78	Major
MR 1	Margaret River, WA	2019	12.8	0.9	3.09	7.6	0.42	1.48	23	82	Major
MR 2	Margaret River, WA	2020	13.0	1.6	3.26	6.2	0.33	0.96	23	90	Major
MBH 1	Marlborough, NZ	2016	14.0	1.3	3.34	6.3	0.65	<0.40	25	155	Major
MBH 2	Marlborough, NZ	2017	13.3	0.6	3.22	7.1	0.65	<0.40	18	108	Major
MBH 3	Marlborough, NZ	2018	13.5	3.5	3.49	5.1	0.58	<0.40	22	65	Major
MV	McLaren Vale, SA	2020	12.1	0.6	3.36	5.9	0.26	1.60	30	111	None
MP[†]	Mornington Peninsula, Vic	2019	13.4	1.4	3.21	6.4	0.62	<0.40	24	66	Major
TBA 1	Tumbarumba, NSW	2017	13.1	1.3	3.36	6.2	0.42	1.28	21	93	Major
TBA 2	Tumbarumba, NSW	2019	13.4	1.3	3.46	5.3	0.62	<0.40	14	44	Major
YV 1[†]	Yarra Valley, Vic	2018	12.8	1.2	3.21	7.0	0.39	1.71	18	89	Partial
YV 2	Yarra Valley, Vic	2019	14.4	2.2	3.19	7.1	0.50	1.10	27	105	Partial

Note: [†]Samples selected for consumer test. Oak usage data were collated from the wine labels and wine company tasting notes. Major, most or all of the wine was fermented or matured in oak vessels. Partial, a portion of the wine was fermented or matured in oak vessels. None, the wine was neither fermented nor matured in oak vessels.

Table 2: Sensory attributes, synonyms/definitions, and composition of reference standards evaluated by sensory panels during the QDA studies of commercial wines and odorant addition samples.

Study	Attribute	Definition/synonyms	Standard
	Appearance		
Commercial wine study	Yellow colour intensity	The degree of colour of the sample (colour intensity)	Conceptual standard
	Aroma		
Both studies	Pungency	The intensity of the warming alcohol and tingling sensation	12% v/v of 95% food grade ethanol (Rowe Scientific) and 0.2 mg potassium metabisulfite (Sigma-Aldrich)
	Flint	Intensity of the aroma of flint, mineral, and struck-match	Standard 1. 20 μL of 1.6 mg/L phenylmethanethiol Standard 2. A chardonnay wine displaying a pronounced struck-match aroma according to the assessors
	Peach	Intensity of the aroma of peach and stone fruit	Standard 1. 3 × chopped fuzzy peach confectionary (Maynards) not in wine Standard 2. 10 mL Crème de Peche liqueur (Massenez)
	Pineapple	Intensity of the aroma of pineapple	3 × cubes of tinned pineapple (Golden Circle)
	Citrus	Intensity of the aroma of lemon and lime	10 mL lemon and 10 mL lime cordial (Bickford's)
	Toasty	The intensity of the aroma of toasted bread and brioche	Standard 1. Small portion of warmed toast and croissant not in wine Standard 2. 1 g dried yeast (Lallemand)
	Apple/pear	The intensity of the aroma of fresh pears and red or green apples	30 mL apple cordial and 1 × slice of fresh packham pear
	Passionfruit/ grapefruit	Intensity of the aroma of passionfruit, grapefruit, box hedge, cat pee, and sweat	Standard 1. 30 mL canned passionfruit pulp (John West) and 20 μL of 14.3 mg/L 3-sulfanylhexyl acetate Standard 2. 3 mL grapefruit cordial (Bickford's)
	Floral	Intensity of the aroma of orange blossom and mixed florals	1 mL each of orange blossom water (Aoun), rosewater (Aoun), and Crème de Violette (Massenez)
Commercial wine study	Natural gas	Intensity of the aroma of drain, sewage, and natural gas	5 g of wood ash (mixed fresh each day with wine to release sulfidic aroma)
	Woody/vanilla	Intensity of the aroma of oak, vanilla, toasted coconut, and coconut shavings	2 g toasted American oak chips, 1.25 mL vanilla pod paste (Queen) 10 μL of 0.97 g/L oak lactone, and pinch of toasted coconut shavings (McKenzie's)
	Tinned vegetables	Intensity of the aroma of cooked and tinned vegetables	50 μL of 0.1% dimethyl sulfide
	Cheesy	Intensity of the aroma of cheese and rancid dairy	100 μL of 10% butanoic acid
	Palate		
	Sourness	Intensity of the perceived sour and acidic taste	1 g/L L-(+)-tartaric acid (ChemSupply) in water
	Bitterness	Intensity of the perceived bitter taste	15 mg/L quinine sulfate (Sigma-Aldrich) in water
	Viscosity	Intensity of thickness and slipperiness in the mouth	1.5 g/L carboxymethylcellulose sodium salt (Sigma-Aldrich) in water
	Hotness	Intensity of the alcohol burning sensation, including aftertaste	12% v/v of 95% food grade ethanol (Tarac Technologies) in water
	Sweetness	Intensity of the sweet taste	5 g/L sucrose (Foodland SA) in water
	Astringency	Intensity of the drying sensation in the mouth	0.5 g/L aluminium sulfate (Ajax Fine Chem Supply Pty Ltd) in water
	Citrus	Intensity of the flavour of lemon and lime	Conceptual standard
	Stone fruit	Intensity of the flavour of peaches, nectarines, and apricots	Conceptual standard
	Tropical	Intensity of the flavour of pineapple, melon, and mango	Conceptual standard
	Mineral	Intensity of the flavour flint, mineral, and struck-match	Conceptual standard
	Apple/pear	Intensity of the flavour of fresh apples and pears	Conceptual standard
	Toasty	Intensity of the flavour of toast, bread, and brioche	Conceptual standard
	Woody/vanilla	Intensity of the flavour of oak, vanilla, toasted coconut, and coconut shavings	Conceptual standard

All standards were added to 100 mL of 2019 Yalumba premium selection bag-in-box unoaked Chardonnay (2L, Angaston, SA) unless otherwise noted.

solutions was equalized for all samples including the base wine control. Addition samples were prepared freshly each day. The Chardonnay wine used as the base wine for the addition experiment was approximately 18 months old, bottled (750 mL) with screw-cap closure, 13.2% v/v ethanol, pH 3.33, titratable acidity (TA) = 5.7 g/L, SO_2 (free) = 20 mg/L, and SO_2 (total) = 100 mg/L. A preliminary informal tasting by AWRI staff from the sensory and research teams assessed the base wine as having subtle oak characters but no struck-flint-like aroma. The concentrations of PMT and 2FMT in this base wine were 1.1 ng/L and 2.6 ng/L, respectively.

2.6. Sensory Evaluation of Confirmatory Odorant Addition Study. Very similar training and evaluation conditions as described above were used for the follow-up confirmatory study investigating the aroma contribution of PMT and 2FMT to a fruity base Chardonnay wine with the following changes. Assessors participated in three days of training where each sample was presented at least once, followed by three formal evaluation days. All samples were evaluated by aroma only with the rated attributes listed in Table 2. The 25 combinations of the experimental design were presented to assessors in triplicate, with five trays of five samples per tray presented on each of the three formal evaluation days. Assessors took 30-second breaks between each sample.

2.7. Consumer Test Participants and Evaluation Conditions. A hedonic consumer test involving 92 regular white wine drinkers took place at the AWRI sensory laboratory located in Adelaide, South Australia. The sample of consumers was screened and selected based on their drinking preferences and habits, aimed to be balanced for age and gender as practically as possible. Consumers who drink Chardonnay wine at least once or twice per year, were not pregnant and were between 18 and 65 years of age participated in the assessment. Consumer demographic details can be found in Table S1. Each consumer attended a single session to taste six Chardonnay wines selected to broadly represent the range of attributes differentiating the samples from the statistical analysis of QDA data as well as having similar basic chemical compositions. Consumers gave informed consent, completed a demographic questionnaire, and were briefed on the hedonic task which lasted less than 1 hour.

The wines were presented to respondents monadically following a Williams Latin Square random block design, presented in 3-digit-coded ISO wine tasting glasses containing 30 mL aliquots of wine at 10.5 ± 0.8°C. Tasting took place in isolated sensory booths under daylight-type lighting. A 2-minute break between the samples was enforced where participants were encouraged to drink water between the samples. Tasters could choose if they wanted to drink or expectorate the samples into the sink available in each booth. Each wine was first rated by the consumers using a nine-point hedonic scale labelled from "dislike extremely" to "like extremely" [26], then purchase intent was collected using a five-point scale labelled from "definitely would not buy" to "definitely would buy." A few questions relating to wine use and attitudes were administered after the tasting, with participants receiving a $30 gift coupon as a reward for their time.

2.8. Statistical Analysis and Interpretation. Trained panel performance was assessed using Compusense20 software and R with the SensomineR (sensominer.free.fr/) and FactomineR (factominer.free.fr/) packages. The performance assessment included analysis of variance for the effect of assessor, wine and presentation replicate and their interactions, degree of agreement with the panel mean, degree of discrimination across samples, and the residual standard deviation of each assessor by attribute. All assessors were found to be performing at an acceptable standard.

Analysis of variance (ANOVA) for the QDA data was carried out using Minitab 20 (Minitab Inc., Sydney, NSW) for the effects of wine, assessor, presentation replicate, and all their two-way interactions. A Fisher's protected least significant difference (LSD) value was calculated at a 95% confidence level using the mean sum of squares value from the assessor by treatment interaction effect. Principal component analysis (PCA) was conducted for the means of the samples of the attributes using the correlation matrix, calculated by using XLSTAT 2020 (Addinsoft, France). For the odorant addition study response surface regression modelling (RSM), ANOVA and visualisations were completed with STAT-EASE 360 (MN, USA) treating presentation replicates as blocks.

For the consumer test data, ANOVA was calculated for the effects of wine and assessor, treating consumers as a random effect. Agglomerative hierarchical clustering (AHC) of raw liking scores was then calculated, as recommended in MacFie [27],by transforming to (dis)similarity matrix and using Pearson correlation coefficient index with average linkage (unweighted pair groups), and used inspection to the level of 0.58 to truncate clusters. A Fisher's protected LSD value was calculated at a 95% confidence level for each of the consumer groups.

To explore relationships between wine chemical composition, sensory profiles, and consumer responses, partial least squares regression (PLS-R) models were generated using the NIPALS algorithm (30,000 iterations) and standardisation. Models first linked chemical composition (x) to sensory attributes (y); then, another model was generated which associated sensory attributes (x) with mean consumer liking and consumer clusters mean liking scores (y). Wine chemical compounds important to sensory attributes and sensory terms identified as important to consumer response were identified by statistical jack-knifing and considering the size of regression coefficients as recommended in [28].

Due to the relatively small sample sizes practicable in wine research, less emphasis was placed on arbitrary P value significance levels, instead attention was given to the level of statistical evidence (P value), magnitude of effect size (F

value) and absolute effect value (sample mean values) to interpret and draw conclusions about effects of sensory significance [29, 30]. Statements ascribing the level of statistical evidence in this work are as follows: $P \geq 0.10$ "virtually no evidence," $P \leq 0.10$ "weak evidence" (ǂ), $P \leq 0.05$ "evidence" (*), $P \leq 0.01$ "strong evidence" (**), and $P \leq 0.005$ "very strong evidence" (***).

2.9. Chemical Analysis. Targeted volatile compounds were quantified using previously published methods by Siebert et al. [7] and updated by Espinase Nandorfy et al. [20] that are routinely used in-house and are described briefly below. Furthermore, two new methods developed to quantify *n*-alkyl γ-lactones and benzyl compounds are described in detail. All analytical methods for volatile compounds used deuterated analogues as the internal standards, and MS in selected ion monitoring mode or MS/MS with multiple reaction monitoring except the method using a GC/sulfur chemiluminescence detector (SCD) which instead used two chemically similar compounds to the analytes.

The set of 71 survey wines, including the subset of 24 wines were analysed for polyfunctional thiols (including PMT and 2FMT) by HPLC/MS/MS after derivatisation with 4,4′-dithiodipyridine (Acros Thermo Fisher Scientific, Thebarton, SA, Australia) and SPE as described by Capone et al. [31] and Cordente et al. [32] using an Exion UHPLC coupled to a 6500 QTrap+ (Sciex, Mulgrave, Vic., Australia).

The following analyses were only conducted on the subset of 24 wines. Fermentation-derived aroma compounds were analysed by headspace (HS)-solid phase microextraction (SPME)-GC/MS as described by Siebert et al. [33] except using a polyacrylate (PA, white) 85 μm SPME fibre (Supelco, Sigma-Aldrich), a VF-624 ms ($30\,m \times 0.25\,mm \times 1.4\,\mu m$; Agilent) GC column, and an Agilent 7890A GC (Agilent Technologies Australia, Mulgrave, Vic., Australia) coupled to an Agilent 5975C MS and equipped with a Gerstel MPS2 multipurpose sampler (Lasersan Australasia, Tanunda, SA, Australia). Monoterpenes and C13-norisoprenoids were analysed according to Pisaniello et al. [34] using membrane-assisted solvent extraction (MASE)-GC/MS on an Agilent 7890B GC, coupled to an Agilent 5977B MS and equipped with a Gerstel MPS Robotic Pro (Lasersan). The MASE membrane bags were supplied by Lasersan. Oak-derived aroma compounds were quantified according to Pollnitz et al. [35]; all compounds were analysed by liquid-liquid extraction-GC/MS using an Agilent 6890 GC, coupled to an Agilent 5973 MS and equipped with a Gerstel MPS2. Volatile sulfur compounds were analysed according to Siebert et al. [36] and Cordente et al. [32] utilising static HS-GC/SCD on an Agilent 7890B GC, coupled to an Agilent 8355 SCD and equipped with a Gerstel MPS2 XL (Lasersan).

n-Alkyl γ-lactones were quantified by direct-immersion (DI)-SPME-GC-MS/MS similar to that described for (*Z*)-6-dodeceno-γ-lactone [37] using an Agilent 7000C Triple Quadrupole GC-MS/MS system (version 7.03) equipped with a Gerstel MPS2-XL (Lasersan). γ-Octa, -nona, -deca, and -dodecalactone, were purchased from Sigma-Aldrich. (*Z*)-6-Dodeceno-γ-lactone was kindly donated by Symrise (Holzminden, Germany), and γ-methyldecalactone was kindly donated by Pyrazine Specialties. (*Z*)-7-Decen-5-olide was supplied by Penta International (Livingston, NJ), and 6-pentyl-α-pyrone was supplied by Pyrazine Specialties (Ellenwood, GA). *n*-Alkyld_7-γ-lactones (C8–C12) had been synthesized in-house [38]. Stock solutions and dilutions of *n*-alkyl γ-lactones were prepared in ethanol (gradient grade for LC, Merck, Bayswater Vic, Australia). Samples were prepared by diluting wine (5 mL) with water (4 mL) and adding internal standard (25 μL) into a 10 mL vial (Agilent). Analytes were then extracted with DI-SPME using a 65 μm DVB/PDMS (blue) fibre (Supelco, Sigma-Aldrich) for 40 minutes at 30°C with agitation at 250 rpm. The fibre was then washed in a 20 mL vial containing water for 1 minute prior to desorption to decrease the amount of inlet contamination due to sugars and other nonvolatiles. Volatiles were desorbed at 260°C onto a VF-200 ms ($30\,m \times 0.25\,mm \times 0.25\,\mu m$; Agilent) which was held at a constant flow of 1 mL/min. During injection, the inlet was splitless for 2 minutes followed by inlet purging at 50 mL/min. To enable back-flushing, the analytical column was connected to a Deans switch, where, during analysis, compounds were transferred to the MS using $1.5\,m \times 0.15\,mm$ fused silica held at a constant flow of 1.2 mL/min. The temperature program for the oven was: 40°C for 1 minute, ramped to 120°C at 20°C/min, and then ramped to 180°C at 2°C/min. The analytical column was then backflushed for 5 minutes (2 column volumes) at 260°C. The MS transfer line was held constant at 240°C for the duration of the analysis. Method linearity was determined using ten calibration levels, each in duplicate, over the concentration range of 0.1–100 μg/L of all listed lactones except for (*Z*)-6-dodeceno-γ-lactone at 1–1000 ng/L, and included control wine samples without any addition of analytes. The limit of detection (LOD) was calculated as $S/N = 3$ and the limit of quantification (LOQ) was calculated as $2 \times LOD$. Method precision and recovery were determined using seven replicate samples spiked at low and high concentrations (1 and 10 μg/L for all lactones except 10 and 100 ng/L for (Z)-6-dodeceno-γ-lactone). See Table S2 for calibration and validation data. To check the accuracy of the analysis, at least one in every six wines was analysed in duplicate.

Benzyl compounds were quantified by HS-SPME-GC/MS using an Agilent 7890A GC coupled to an Agilent 5975C MS and equipped with a Gerstel MPS2-XL. Benzaldehyde, benzyl alcohol, and benzyl acetate were supplied by Sigma-Aldrich, d_6-benzaldehyde by Cambridge Isotopes (Novachem, Collingwood, Vic, Australia), and d_5-benzyl acetate and d_5-benzyl alcohol by CDN Isotopes (SciVac, Hornsby, NSW). Stock solutions of benzaldehyde, benzyl alcohol, and benzyl acetate, and mixed dilutions were prepared in ethanol (LC grade, Merck). The GC was fitted with a Deans switch (Agilent) to utilise a postrun backflush program. The analytical column used was a VF-624 ms ($60\,m \times 0.25\,mm \times 1.4\,\mu m$; Agilent) and the restrictor column was deactivated fused silica $1.0\,m \times 0.10\,mm$; Agilent). The carrier gas was helium (ultrahigh purity, BOC, Adelaide, SA, Australia) in constant flow mode: analytical column

1.75 mL/min (initial pressure 273 kPa) and restrictor column 1.85 mL/min (initial pressure 137 kPa). A polyacrylate (PA, white) 85 μm SPME fibre (Supelco, Sigma-Aldrich) was exposed to the headspace (20 mins at 45°C) with agitation (250 rpm). The SPME fibre was desorbed in splitless mode and left in the injector for 10 min. The splitter, at 29:1, was opened after 2 min. The injector temperature was held at 250°C. The oven temperature was started at 50°C, held for 1 min, raised to 140°C at 20°C/min, and then further raised to 235°C at 5°C/min. Subsequently, the inlet pressure was reduced to 7 kPa, the reversed flow through the analytical column at −4.5 mL/min via the Deans switch, and the oven heated to 280°C and held for 5 min. The temperature of the transfer line was 240°C. The mass spectrometer was operated in electron (EI+) ionization mode at 70 eV and utilising simultaneous scan/SIM mode. The wine samples were prepared for HS-SPME; sampling was as follows: a 5 mL aliquot of wine, 50 μL of internal standard mixed solution d_6-benzaldehyde, d_5-benzyl acetate, and d_5-benzyl alcohol (each at 20 mg/L), a 5 mL aliquot of tartrate buffer (pH 3.20), and sodium chloride (2 g; Merck) was added to a 20 mL screw-cap vial (magnetic, Teflon lined silicone septum; Agilent). Method precision and calibration linearity were validated by a series of standard addition experiments to white wine diluted 1:1 with model wine (12% v/v ethanol, pH 3.20). Method linearity was determined using ten calibration levels, each in duplicate, over the concentration range of 2–2000 μg/L of benzaldehyde, benzyl alcohol, and benzyl acetate and included control wine samples without any addition of analytes. The LOD was calculated as S/N = 3 and the LOQ was calculated as 3 × LOD. Method precision was determined using seven replicate samples spiked at low and high concentrations (50 and 500 μg/L). See Table S3 for calibration and validation data. To check the accuracy of the analysis, at least one in every six wines was analysed in duplicate. To check the recovery, a master mix of all samples was spiked with 50 μg/L and 500 μg/L of the analytes in duplicate.

The concentration of proline was quantified using ^{1}H NMR. Analysis was performed on a Bruker Avance Neo operating at 400 MHz (Bruker, Sydney, Australia). Samples were prepared as follows: 900 μL of wine was buffered with Bruker "Buffer C," and then automatically titrated with 1.0 M HCl or 1.0 M NaOH to pH 3.10 using a microtitrator (Bruker). A 600 μL aliquot of the titrated wine was then transferred to a 5 mm tube (Duran Wheaton Kimble, Economic, ASIS Scientific, Adelaide, Australia) and submitted for acquisition. Experiments, including tuning, matching, locking, shimming, and pulse calibration, were performed automatically according to the Bruker FoodScreener module [39]. Proline was then quantified from the water and ethanol suppressed *noesygpps* spectrum (ds 4, ns 32, TD 64k, sw 20 ppm, rg 16) using an in-house workflow (Python3.9, https://github.com/AWRIMetabolomics/pronmr-quant.), where the area under the curve (AUC) of the multiplet at ~2.3 ppm was obtained and regressed against a calibration function. The ppm coordinates were identified as a range containing a clean signal specific to proline, relatively free of other compounds in wine.

The subset of 24 wines was analysed by Affinity Labs for their basic composition using a Foss WineScan FT 2 as described by the manufacturer (Foss, Hillerød, Denmark), and the free and total sulfur dioxide (F/T SO_2) were measured using a Gallery discrete analyser (Thermo Fisher Scientific, Thebarton, SA, Australia).

3. Results and Discussion

3.1. Chardonnay Survey of the Occurrence of 2FMT and PMT. A total of 71 white wines (66 Chardonnay) were analysed for the concentration of 2FMT and PMT, with the results shown in Figure 1. The range of 2FMT in the wines was approximately 0.2–164.5 ng/L, with a mean of 15.1 ng/L and median value of 3.5 ng/L. For PMT, the range was 0.2–7.8 ng/L, with a mean of 1.7 ng/L and a median value of 1.4 ng/L. 2FMT and PMT were significantly correlated ($P < 0.0001$, $r = 0.47$, $n = 71$), but differed in their distribution, with PMT following a normal distribution while 2FMT was strongly right skewed with many values in the low range and a few high concentrations. The concentration of both compounds was above the reported aroma detection thresholds (0.4 ng/L and 0.3 ng/L) for almost all wines. The concentration range of 2FMT measured here was much higher than the eight white wines reported by [40] or the nine Spanish Chardonnays reported in [41] (2–19 ng/L) but below the range reported in some aged Champagnes [3] (up to 5500 ng/L). The maximum concentration of PMT measured in this study was approximately five times lower than the maximum non-Sauvignon Blanc white wines reported in [41] (36 ng/L) and lower than the values reported in [4] (up to 40 ng/L).

3.2. Sensory Descriptive Analysis of Commercial Chardonnay Wines. The attributes rated by the trained panel were generated by a consensus-based approach during training sessions. The attributes (Table 2) consisted of one colour attribute, one nasal sensation (pungency), twelve aromas, three tastes, three mouthfeel terms, and seven flavour attributes.

From the ANOVA, very strong evidence was found that all the attributes rated by the panel differed between the 24 commercial wines except for toasty aroma (Table 3). The largest differences, indicated by the largest *F* values, between the wines were the degree of yellow colour intensity, flint aroma, and mineral/flint flavour.

As a visual overview of the sensory properties of the 24 Chardonnay samples, a PCA (Figure 2) was conducted on the mean values of the sensory attributes (Tables S4 and S5). Principal components 1 and 2 explained 63.4% of the variation in the sensory data. PCs 3, 4, and 5 were also found to have eigenvalues above 1 and explained a further 8.8%, 6.8%, and 5.2% of the variation in the data. However, these PCs are mainly related to the intensity of cheesy aroma. The horizontal separation of the wines along PC1 related to the intensity of nonfruit sensory attributes pungency, natural gas aroma, flint aroma, mineral/flint flavour, tinned vegetable aroma, and woody/vanilla aroma and flavour, which

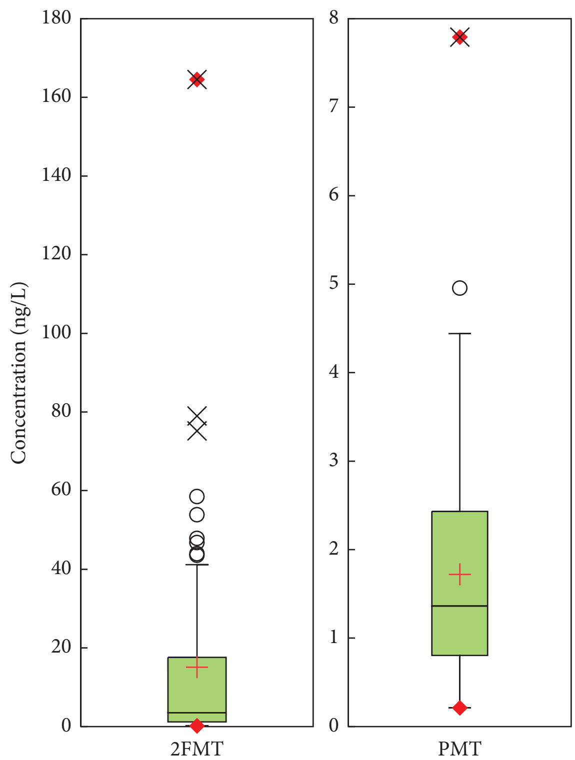


FIGURE 1: Box plots visualising the distribution of 2FMT and PMT from a survey of 71 white wines from Australia, New Zealand, and France, including 66 Chardonnay samples. The inclusive median quartile occurs within the box are shown with vertical whiskers corresponding to minimum and maximum values within a range limit and absolute maximum and minimum indicated by (◆) and mean by (+). Outlier point wines fall beyond the upper whisker with those values beyond 2.5 standard deviations marked (◯) and 3 standard deviations marked (×).

TABLE 3: F ratios, probability values[†], degrees of freedom (df), and mean square error (MSE) from the analysis of variance of QDA data.

Attribute	Wine (W)	Assessor (A)	Rep (R)	W ∗ A	R ∗ W	R ∗ A	MSE
Yellow colour	22.12***	104.79***	0.50	1.83***	1.12	5.27***	0.182
Pungency A	3.99***	68.80***	0.45	1.19	2.08***	4.18***	0.285
Flint A	19.4***	8.37***	0.89	1.74***	1.16	1.51	2.268
Peach A	3.74***	30.82***	2.29	1.89***	1.27	0.78	1.600
Passionfruit/grapefruit A	2.84***	8.73***	0.01	1.63***	0.94	2.56***	1.819
Pineapple A	4.91***	20.15***	2.71	1.95***	1.35	1.72*	1.488
Citrus A	2.18***	40.73***	1.82	1.28*	1.22	6.90***	0.436
Natural gas A	4.75***	5.68***	1.16	1.62***	1.36	1.99**	0.453
Woody/vanilla A	4.79***	24.79***	1.15	1.81***	1.46*	0.61	1.350
Toasty A	1.37	68.22***	0.19	1.33***	1.17	1.56	0.841
Apple/pear A	4.79***	15.17***	1.05	1.80***	1.30	6.60***	0.876
Tinned vegetables A	3.44***	10.86***	0.75	1.19	1.13	2.40***	1.005
Cheesy A	4.12***	5.24***	0.25	2.00***	0.75	2.19***	1.150
Floral A	5.92***	15.60***	0.59	1.70***	0.71	2.28***	1.800
Sourness T	3.98***	46.81***	0.68	1.29*	1.06	3.53***	0.468
Bitterness T	1.90**	41.34***	0.84	1.39***	1.43*	7.18***	0.422
Viscosity MF	1.65*	258.86***	1.10	1.32**	0.78	1.76*	0.313
Hotness MF	3.47***	58.89***	5.31*	0.93	0.63	3.39***	0.535
Sweetness T	3.19***	93.26***	2.47	1.09	0.99	1.25	0.766
Astringency MF	3.20***	26.61***	1.04	1.49***	0.99	9.57***	0.378
Citrus F	3.28***	113.87***	4.04*	1.27*	0.76	2.74***	0.466
Stone fruit F	2.30***	34.78***	2.23	1.71***	1.36	2.34***	0.965
Tropical F	2.59***	48.53***	5.33*	1.55***	0.97	1.35	1.186
Mineral/flint F	7.35***	15.13***	0.19	1.76***	1.26	1.16	1.325
Apple/pear F	3.58***	28.63***	0.71	1.99***	1.36	3.14***	0.823
Toasty F	1.66*	81.22***	0.54	1.39***	1.20	1.44	0.792
Woody/vanilla F	5.44***	23.17***	0.08	1.43***	0.73	2.15***	1.444
df	23	10	2	230	46	20	460

Note: A: aroma, F: flavour, T: taste, MF: mouthfeel, Rep: presentation replicate. [†]Significance levels are as follows: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; and $^{‡}P < 0.10$. df = degrees of freedom.

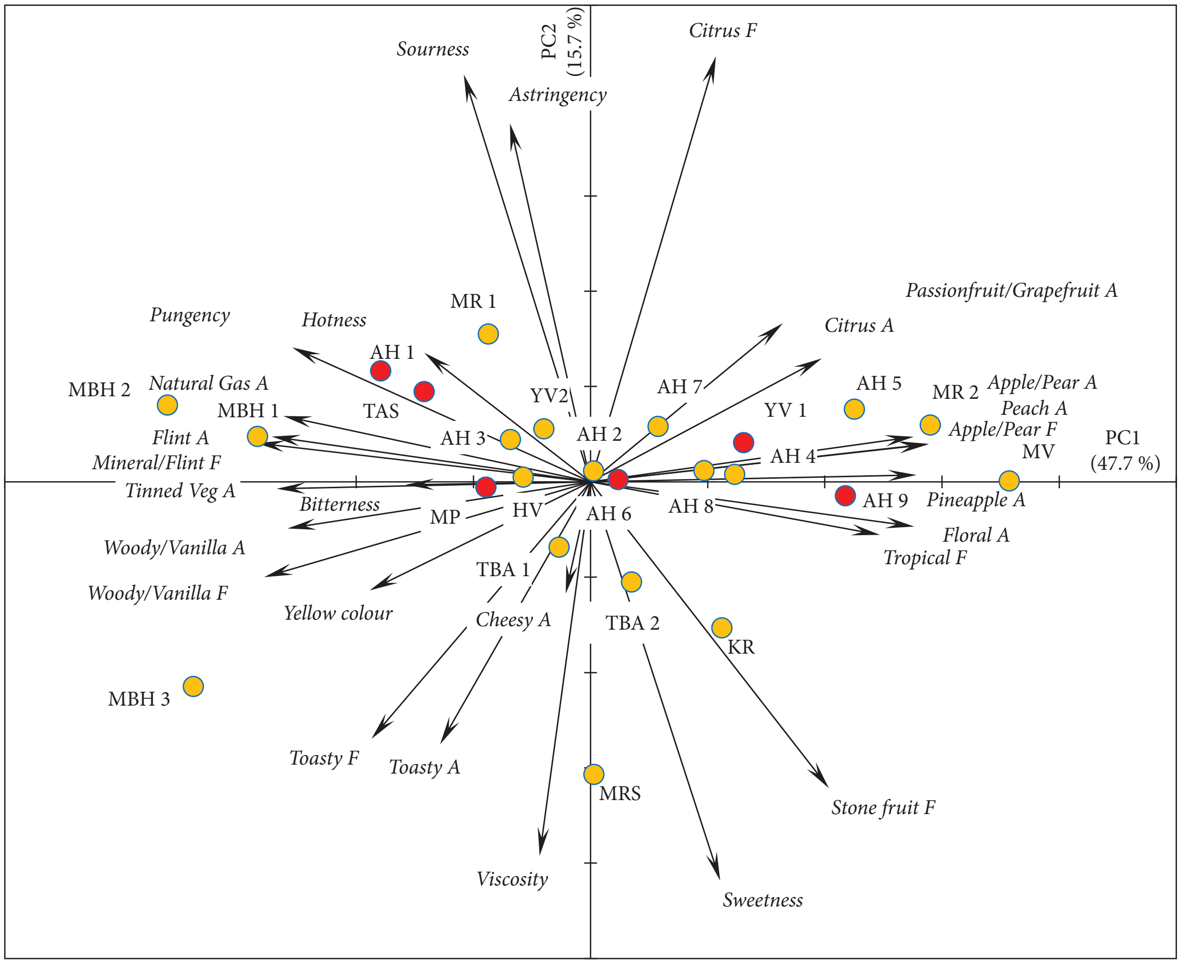


Figure 2: Biplot of principal component analysis PC1 and PC2 of all the wine sensory attributes and the scores for the 24 Chardonnay wines assessed by quantitative descriptive analysis. Wines selected for consumer testing are highlighted (●).

were heavily negatively loaded on PC1 with wines from Marlborough (MBH1, MBH2, and MBH3) as well as samples AH1 and TAS rated higher in these attributes. Conversely, fruity attributes passionfruit/grapefruit aroma, citrus aroma, apple/pear aroma and flavour, peach aroma, pineapple aroma, tropical flavour, as well as floral aroma were positively loaded on PC1 with wines MV, MR2, AH9, and AH5 rated the highest in these attributes. The vertical separation along the PC2 was driven by the ratings of sourness, astringency, citrus flavour, as well as toasty aroma (ns) and flavour, viscosity, sweetness, and stone fruit flavour. Generally, the retail price of the wines was higher for those to the left of Figure 2, with price positively and significantly ($P < 0.05$) correlated with flint aroma ($r = 0.45$), woody/vanilla aroma ($r = 0.55$) and flavour ($r = 0.52$) and pungency ($r = 0.42$). Overall, the wines selected showed a range of "empyreumatic" aroma and flavour intensities.

3.3. Assessing the Association of PMT and 2FMT with Smoky/Burnt Sensory Characteristics Using PLS-R. Basic chemical composition for the 24 wines is shown in Table 1, and Table S6 lists the volatile compounds quantified in the wines, together with their CAS numbers; abbreviation codes; published aroma detection thresholds; and mean, minimum, and maximum concentrations.

To link chemical composition and sensory response using PLS-R, a five-factor model was used that explained 72% of the total sensory response variance from the chemical compositional data. Visualisation of the scores and loadings for factors 1 and 5 from this model can be seen in Figure S1. Chemical compounds (X's) and sensory attributes (Y's) located together in Figure S1 are generally positively associated, and those towards the outside of the plots are considered well modelled. Compounds of sensory significance were identified by considering both the size of their regression coefficients (Figure 3) and statistical importance as determined by a jack-knife resampling test. Significant compounds most strongly and positively associated with flint aroma were 2FMT, acetic acid, the oak compounds 4-methyl guaiacol, eugenol, guaiacol, and *trans*-oak lactone, while β-damascenone was significantly negatively associated with this attribute and positively related to several of the fruity attributes. In addition to those identified as significant, the compounds PMT, ethyl thioacetate, and ethanethiol had relatively high and positive regression coefficients for flint aroma intensity. The model was strong (R^2 calibration predicted vs measured = 0.91 and R^2 validation predicted vs. measured = 0.70). Similar compounds were also identified as important to mineral/flint flavour, and β-citronellol and 3-sulfanylhexanol were additionally implicated, again with a strong model. Linear regression tests of PMT ($R^2 = 0.102$, $P = 0.128$) and 2FMT ($R^2 = 0.589$, $P < 0.0001$) with flint/struck-match/mineral aroma intensity confirmed 2FMT was much more strongly correlated than PMT. Previous studies implicated PMT with "flint" aroma in research Chardonnay wines [4] and other white wines [2]; however, the stronger association uncovered for 2FMT was unexpected.

As a secondary objective of this work, we sought to determine the role of ethyl esters which had been identified in a recent association study and multistep screening reconstitution study as conferring peach aroma to model wine samples [20]. For the peach aroma attribute in the present study, 3-sulfanylhexyl acetate, 2-methylbutyl acetate, β-damascenone, 3-methylbutyl acetate, and 2-phenylethyl acetate were all identified as significant and had high positive regression coefficients (Figure 3). Ethyl octanoate and ethyl hexanoate had small negative regression coefficients for the five-factor model, with a small positive regression coefficient for a one-factor model. Ethyl octanoate was present in a much narrower concentration range in these wines (933–1560 μg/L, Table S6) than previously tested with reconstitution experiments (0–1500 μg/L). The importance of ethyl esters of fatty acids to fruity aromas was thus not confirmed here, likely due to masking effects on fruity odorants making a statistical association difficult to be uncovered. Even though monoterpenes were found at relatively low concentrations, as is commonly reported for Chardonnay wines [42], linalool (0–5 μg/L) was also significant and positive to peach aroma, while α-terpineol (3–12 μg/L) had a high positive regression coefficient for stone fruit flavour. Monoterpenes were also found to be linked to fruity attributes in an earlier study [4].

Compounds understood to be related to odours resulting from reductive fermentations and oak maturation were generally strongly and negatively associated with fruity attributes, likely due to masking effects. For natural gas aroma, an attribute denoting reductive off-odour, compounds methanethiol and ethanethiol had the highest regression coefficients along with 2FMT and PMT; however, none were significant by the jack-knifing test. For the oak-related aroma attribute woody/vanilla, 4-methyl guaiacol, *cis*- and *trans*-oak lactone, eugenol, guaiacol, and vanillin all had significantly high regression coefficients, with PMT and 2FMT relatively high but nonsignificant, while passionfruit/grapefruit aroma was most significantly and positively associated with 3-sulfanylhexyl acetate, linalool, 2-phenylethyl acetate, hexyl acetate, and 2- and 3-methylbutyl acetate (Figure 3).

Recently, Espinase Nandorfy et al. [21] demonstrated the sensory influence of the residual amino acid proline in dry red wine, which increased perceived sweetness, fruit flavour, and viscous mouthfeel, while diminishing bitterness and astringency. Although the proline concentration is reported to be lower in white wines (0.025–1.4 g/L) compared to red wines (0.018–4.4 g/L) as reviewed by Gutiérrez-Gamboa et al. [43], the range can span across the sensory detection threshold reported in water of approximately 2 g/L [44] and has been linked to wine "body" previously [45]. In this study, the range of proline measured was 0.6–1.4 g/L and had modest, but not significant regression coefficients from the PLS model, relating positively to viscosity (0.0346) and negatively to astringency (−0.0298). Stronger and significant associations, however, with taste and mouthfeel terms were found for pH and titratable acidity (TA) in agreement with the previous findings [46].

3.4. Aroma QDA of a Chardonnay Wine with Added PMT and 2FMT. From the sensory evaluation of the 25 wines created by adding PMT and 2FMT to a fruity and lightly oaked base Chardonnay wine in a full factorial design, nine aroma attributes were generated to describe their sensory properties by a consensus-based approach. Nearly identical definitions and standards as those used during the QDA of the commercial Chardonnay wines were agreed to by the sensory panel.

From the response surface regression models summary presented in Table 4, very strong evidence was found that wines differed in their aroma intensity of all attributes with the addition of PMT and 2FMT. The largest linear effect observed was for 2FMT to increase the intensity of flint aroma while added PMT also imparted this aroma, albeit with an effect size 22 times smaller. It is noteworthy that the maximum concentration of 2FMT was nearly four-fold higher than that of PMT in the addition samples, which was aimed to better represent the observed maximum found naturally in Chardonnay wines. Therefore, it is feasible, that PMT could be as potent as 2FMT in white wine if ever found at similar concentrations. From a practical perspective, the concentration of 2FMT was found to be 21-fold higher than that of PMT in this survey, suggesting the influence of PMT is naturally limited. Weak evidence ($P = 0.052$) of an interactive effect between PMT and 2FMT was also found to result in slight mutual suppression for this attribute. These effects on flint aroma can be visualised in Figure 4. The other attributes (Table 4) were all found to be suppressed by the two compounds, with 2FMT exerting stronger suppression on peach, apple/pear, and floral attributes than PMT, as indicated by the effect size values.

Overall, the results of this study have clarified the role of 2FMT and PMT in "empyreumatic" aromas, including smoky, gun smoke, flint, or struck-match characters, in Chardonnay wines. From the commercial wines QDA, flint/struck-match/mineral aroma was only modestly related to PMT, as previously reported by Tominaga et al. [2, 3] with more evidence found supporting a link to 2FMT. Although 2FMT is reported to contribute a roasted coffee aroma to certain wines [11], no evidence of this was found during either sensory studies conducted here. Both sensory panels described and rated wines high in 2FMT (and PMT) as high in flint/struck-match/mineral aroma rather than any roasted coffee-related attribute, suggesting the context set by other red or white wine volatiles

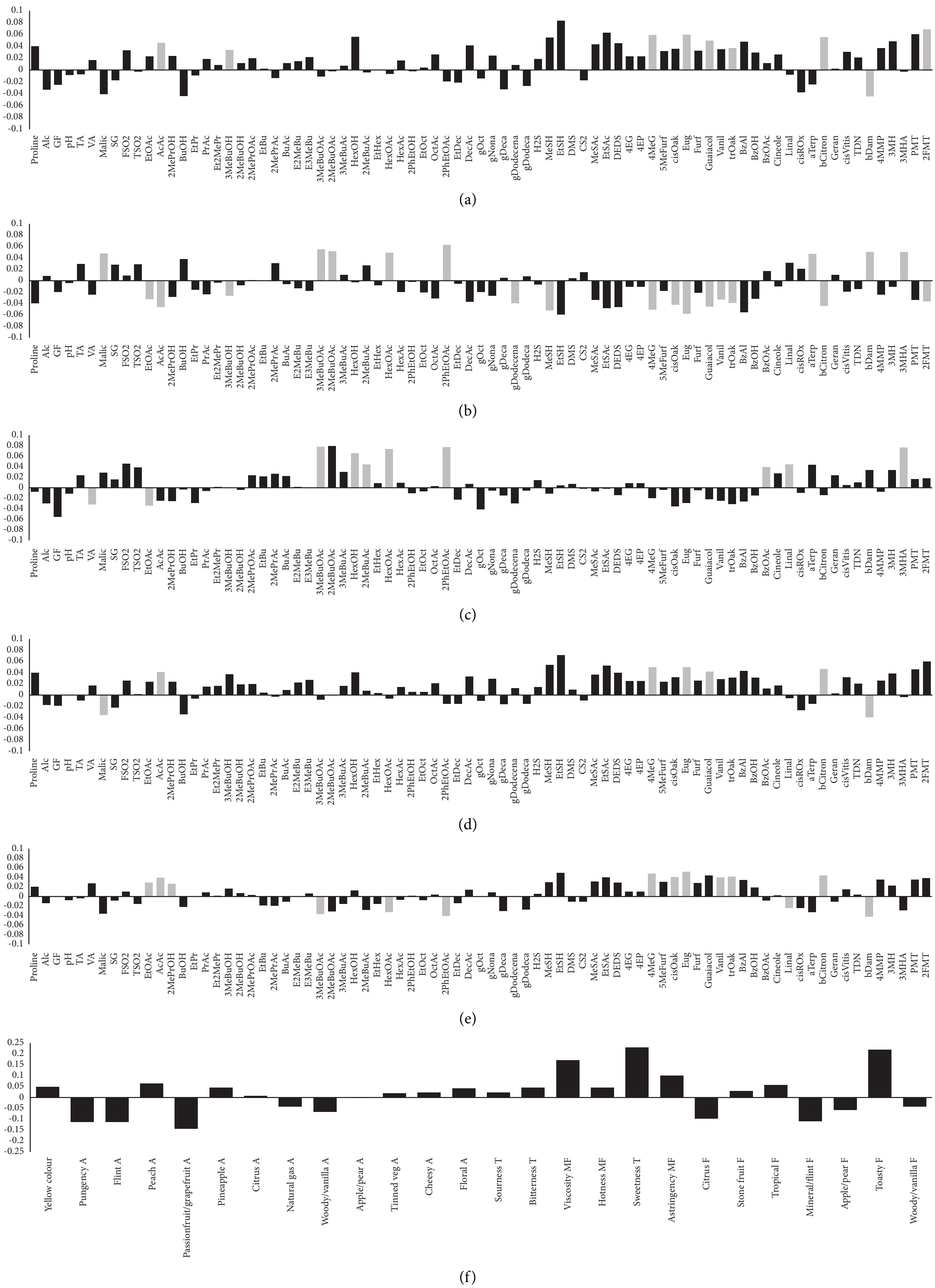
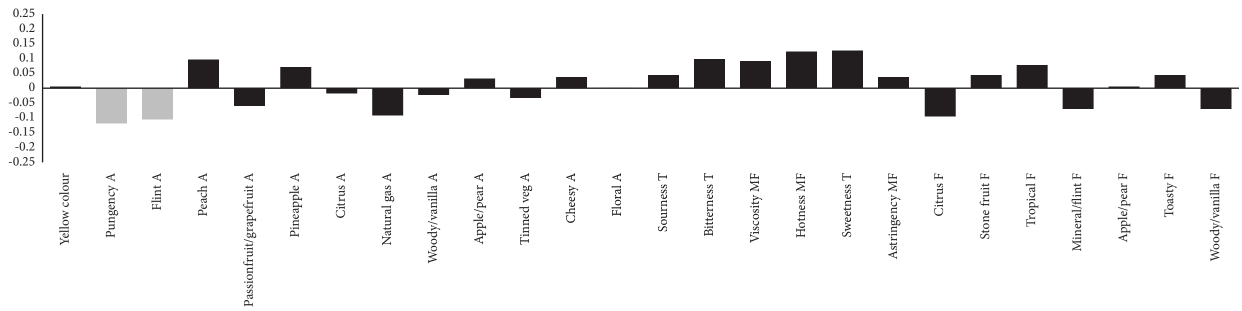


Figure 3: Continued.



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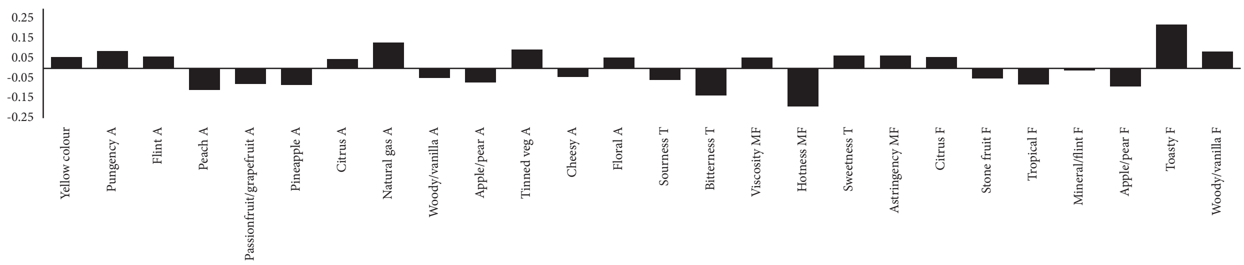

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Figure 3: Regression coefficients from partial least squares models generated to relate the chemical composition of the 24 commercial Chardonnay wines with aromas (a) flint, (b) peach, (c) passionfruit/grapefruit, (d) natural gas, (e) woody/vanilla, as well as sensory attributes related to (f) overall consumer liking ($n = 92$), (g) consumer cluster 1 ($n = 62$), and (h) consumer cluster 2 ($n = 30$) liking scores. Significant variables shaded grey (■), not significant shaded in black (■). Abbreviations of chemical compounds can be found in Table S6.

Table 4: F ratios, probability values†, degrees of freedom (df), and mean square error (MSE) from the response surface model of quantitative descriptive analysis aroma data from the individual PMT and 2FMT addition samples blocked by presentation replicate.

Attribute	Factors and interaction				
	Model	PMT	2FMT	PMT ∗ 2FMT	MSE
Citrus	6.08***	1.53	13.90***	0.02	0.085
Peach	21.01***	8.18**	39.81***	0.14	0.225
Flint	78.11***	7.01**	152.70***	3.90‡	0.309
Apple/pear	21.35***	0.083	41.63***	2.09	0.108
Pineapple	8.25***	3.51‡	14.65***	0.13	0.147
Floral	15.63***	3.69‡	34.90***	0.01	0.223
Toasty	2.51‡	3.25‡	5.48*	1.53	0.219
Sweaty	4.62***	1.36	10.33***	0.02	0.202
Pungency	3.82*	2.65	0.2	7.83**	0.024
DF	3	1	1	1	69

Note: †Significance levels are as follows: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; and $^‡P < 0.10$. df = degrees of freedom.

may affect the odour percept conferred. Ferreira [47] stated PMT together with 2FMT can impart empyreumatic aromas to some aged wines including Chardonnay, but also young wines, based on the studies of Mateo-Vivaracho et al. [41] and Tominaga et al. [2, 3], which is in some degree of agreement with the results presented here for high concentration ranges, although our results provide evidence for a greater role of 2FMT. The addition studies reported by Mateo-Vivaracho et al. [41] indicated that the low concentrations of 2FMT were described (by free choice notes) as increasing fruitiness and pineapple character, while contributing toasty and coffee nuances above 5.3 ng/L. Our study did not find any evidence that 2FMT at low levels contributed fruity nuances; however, the first addition step of 10 ng/L was higher than their study. Conversely, for PMT, very low concentrations were reported in the same study to impart toasty, burnt, and empyreumatic notes at levels of 0.7 and 1.4 ng/L. Our concentration range of PMT (2.6–40.6 ng/L) was again higher than that of Mateo-Vivaracho et al. [41] and the effect was less pronounced compared to 2FMT. These differences may also be explained by the use of a dearomatised wine in the 2010 report rather than a wine with all other aroma compounds still present.

3.5. Consumer Acceptance and Associations with Sensory Properties. A selection of six of the wines from the QDA was assessed for consumer liking. The wines were selected to represent the range of sensory properties heavily loaded on PC1 from the QDA (Figure 2), particularly targeting the range of flint aroma intensity while attempting to have basic chemical composition measures such as alcohol and

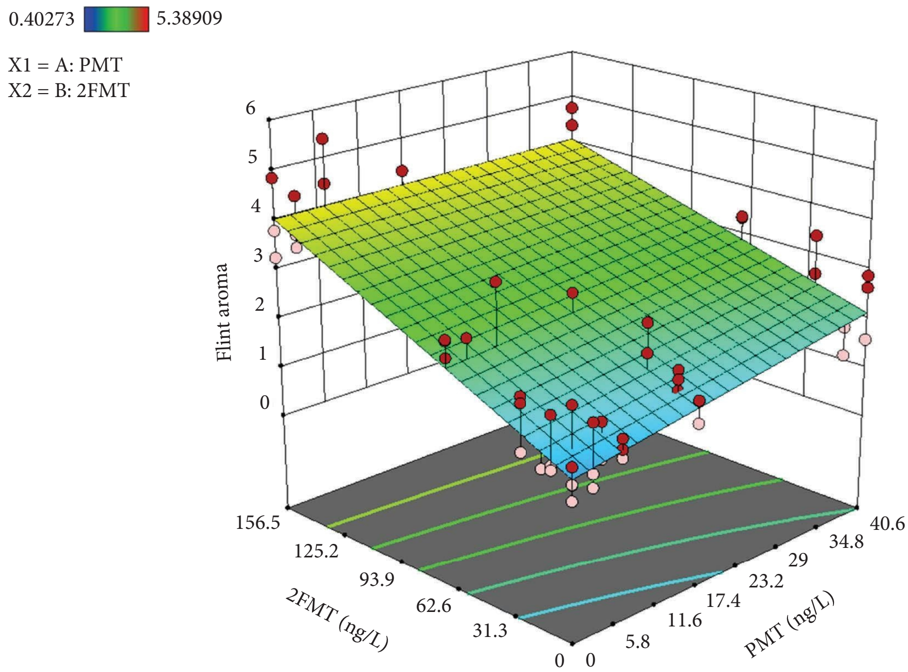

Figure 4: Response surface relating the intensity of flint aroma to the concentration of added 2-furylmethanethiol (2FMT) and phenylmethanethiol (PMT) in Chardonnay base wine from the confirmatory odorant addition quantitative descriptive analysis. Presentation replicated mean values of design points are displayed with those above (●) and below (○) the response surface indicated.

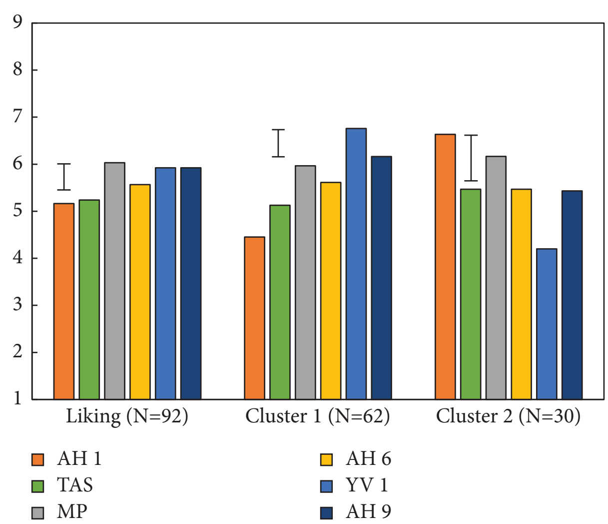

Figure 5: Mean liking scores for the total consumer sample as well as for two clusters of consumers for the six Australian Chardonnay wines, AH1 (■), TAS (■), MP (■), AH6 (■), YV1 (■), and AH9 (■) with varied intensity of flint/struck-match/mineral aroma. Fisher's LSD value for each consumer group (black bar).

titratable acidity as similar as practicable. Wines with other characters such as overt bitterness or sweetness were not included in the consumer test.

From the ANOVA of the consumer liking scores, strong evidence was found ($P = 0.002$, $F = 3.78$) supporting a difference across the wines. The mean liking scores ($n = 92$) are shown in Figure 5. Wines AH1 and TAS were high in flint aroma intensity (mean values of 4.4 and 3.6), MP and AH6 were scored moderately (mean values of 2.4), while wines YV1 and AH 9 were the lowest (mean values of 1.1 and 1.0). The wines MP, YV1, and AH9 were most well liked, with the high flint wines AH1 and TAS liked the least. From the PLS-R (Figure S2), mean liking scores were positively related to sweetness, viscosity, and toasty flavour, with the model having a high calibration predicted versus measured R^2 value of 0.98, although the validation R^2 was relatively low (0.41) and the MP wine was especially poorly predicted.

There was evidence for two clusters of consumers based on the liking scores (Figures 5 and S2). For cluster 1 (67% of the consumers) the pattern of liking scores was similar to that of the total sample of consumers, with the two high flint wines (AH1 and TAS) not well liked. For cluster 2 (33% of the consumers), the AH1 wine was the most well liked, while the lowest flint intensity wine YV1 was liked the least, and the PLS-R indicated that the toasty flavour was most strongly and positively associated with liking for this cluster, flint aroma being only moderately associated, while hotness was strongly negatively associated.

This finding expands on the consumer test conducted by Capone et al. [4] with unwooded research wines which also found a consumer group who responded negatively to samples with higher flint aroma. Overall wines with low to moderate flint aroma were well accepted, while those with high flint intensity could be considered polarizing to consumers. There was no difference found in demographics or usage and attitudes between the two clusters.

4. Conclusion

This study showed that the potent thiols, 2FMT and PMT, are at concentrations of sensory significance in most commercially produced Chardonnay wines from Australia. PMT and 2FMT were confirmed to be associated with "empyreumatic" nuances with 2FMT most strongly related to flint/struck-match/mineral aroma. Challenging our original hypothesis, the role of 2FMT is newly highlighted as a major contributor to this character in Chardonnay, rather than roasted coffee as suggested by previous reports [11]. The flint/struck-match/mineral note was found to be polarizing to consumer acceptance, with the largest proportion of consumers responding negatively to wines high in this character. Further work should assess the winemaking practices responsible for the occurrence of PMT and 2FMT. The odorant addition study demonstrated that different volatile compounds can, when present in a complex natural mixture such as wine, contribute to the same odour quality, even if in isolation (such as assessed in water or on a smelling strip) they are aromatically distinct. These findings emphasise the importance of pairing analytical quantification with robust sensory evaluation such as QDA and the need for confirmatory experiments when attempting to draw conclusions from associational tests with commercial samples. The inclusion of formal consumer testing can also provide an extra layer of practical insight into the flavour research.

Data Availability

The data used in this study are included in the manuscript or supporting information attached.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Damian Espinase Nandorfy and Tracey Siebert contributed equally to this original research article.

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Supplementary Materials

Table S1: summary of the self-reported demographic data collected from all participating consumers. Table S2: white wine calibration and validation data quantifying for *n*-alkyl γ-lactones using direct-immersion (DI)-SPME-GC-MSMS method, as determined in duplicate in commercially available Chardonnay wine (13.0% alcohol). Table S3: calibration and validation data for the quantification of benzyl compounds in white wine using HS-SPME-GC/MS in SIM mode. Table S4: mean sensory scores for the appearance and aroma attributes of the 24 commercial Chardonnay wines. Table S5: mean sensory scores for the taste, mouthfeel, and flavour attributes of the 24 commercial Chardonnay wines. Table S6: summary of the aroma compounds quantified in the 24 commercial Chardonnay wines. Figure S1: factors 1 and 5 of the scores and loadings plots from the PLS regression model. Plots were generated using sensory terms (Y variables) and compositional compounds (X variables) supported by statistical evidence ($P < 0.05$) to have differed among the wines. Figure S2: factors 1 and 3 of scores and loadings plots from the PLS regression model. Plots were generated using consumer response (Y variables) and sensory attributes (X variables) supported by statistical evidence ($P < 0.05$) to have differed among the wines. (*Supplementary Materials*)

References

[1] H. Zwaardemaker, A. J. von Langegg, and F. A. J. von Langegg, *Die physiologie des geruchs*, W. Engelmann, Germany, 1895.

[2] T. Tominaga, G. Guimbertau, and D. Dubourdieu, "Contribution of benzenemethanethiol to smoky aroma of certain Vitis vinifera L. wines," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 5, pp. 1373–1376, 2003.

[3] T. Tominaga, G. Guimbertau, and D. Dubourdieu, “Role of certain volatile thiols in the bouquet of aged Champagne wines,” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 4, pp. 1016–1020, 2003.
[4] D. L. Capone, A. Barker, P. O. Williamson, and I. L. Francis, “The role of potent thiols in Chardonnay wine aroma,” *Australian Journal of Grape and Wine Research*, vol. 24, no. 1, pp. 38–50, 2018.
[5] S. J. Lee and A. C. Noble, “Characterization of odor-active compounds in Californian Chardonnay wines using GC-olfactometry and GC-mass spectrometry,” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 27, pp. 8036–8044, 2003.
[6] J. Gros, V. Lavigne, F. Thibaud et al., “Toward a molecular understanding of the typicality of Chardonnay wines: identification of powerful aromatic compounds reminiscent of hazelnut,” *Journal of Agricultural and Food Chemistry*, vol. 65, no. 5, pp. 1058–1069, 2017.
[7] T. E. Siebert, A. Barker, W. Pearson et al., “Volatile compounds related to ’stone fruit’ aroma attributes in Viognier and Chardonnay wines,” *Journal of Agricultural and Food Chemistry*, vol. 66, no. 11, pp. 2838–2850, 2018b.
[8] T. E. Siebert, S. R. Barter, M. A. de Barros Lopes, M. J. Herderich, and I. L. Francis, “Investigation of ’stone fruit’ aroma in Chardonnay, Viognier and botrytis Semillon wines,” *Food Chemistry*, vol. 256, pp. 286–296, 2018c.
[9] A. G. Cordente, D. Espinase Nandorfy, M. Solomon et al., “Aromatic higher alcohols in wine: implication on aroma and palate attributes during Chardonnay aging,” *Molecules*, vol. 26, no. 16, p. 4979, 2021.
[10] L. Culleré, J. Cacho, and V. Ferreira, “An assessment of the role played by some oxidation-related aldehydes in wine aroma,” *Journal of Agricultural and Food Chemistry*, vol. 55, no. 3, pp. 876–881, 2007.
[11] T. Tominaga, L. Blanchard, P. Darriet, and D. Dubourdieu, “A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several Vitis vinifera grape varieties,” *Journal of Agricultural and Food Chemistry*, vol. 48, no. 5, pp. 1799–1802, 2000.
[12] P. Darriet, T. Tominaga, V. Lavigne, J.-N. Boidron, and D. Dubourdieu, “Identification of a powerful aromatic component of Vitis vinifera L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one,” *Flavour and Fragrance Journal*, vol. 10, no. 6, pp. 385–392, 1995.
[13] R. C. Brown, M. A. Sefton, D. K. Taylor, and G. M. Elsey, “An odour detection threshold determination of all four possible stereoisomers of oak lactone in a white and a red wine,” *Australian Journal of Grape and Wine Research*, vol. 12, no. 2, pp. 115–118, 2006.
[14] H. Heymann, H. Hopfer, and D. Bershaw, “An exploration of the perception of minerality in white wines by projective mapping and descriptive analysis,” *Journal of Sensory Studies*, vol. 29, pp. 1–13, 2014.
[15] H. Guth, “Quantitation and sensory studies of character impact odorants of different white wine varieties,” *Journal of Agricultural and Food Chemistry*, vol. 45, no. 8, pp. 3027–3032, 1997.
[16] J. Prescott, L. Norris, M. Kunst, and S. Kim, “Estimating a “consumer rejection threshold” for cork taint in white wine,” *Food Quality and Preference*, vol. 16, no. 4, pp. 345–349, 2005.
[17] A. J. Saliba, J. Bullock, and W. J. Hardie, “Consumer rejection threshold for 1,8-cineole (eucalyptol) in Australian red wine,” *Food Quality and Preference*, vol. 20, no. 7, pp. 500–504, 2009.
[18] A. Tarasov, N. Giuliani, A. Dobrydnev et al., “1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) sensory thresholds in Riesling wine,” *Foods*, vol. 9, no. 5, p. 606, 2020.
[19] A. J. Saliba, H. Heymann, J. W. Blackman, and J. B. MacDonald, “Consumer-sensory evaluation of Australian Chardonnay [online],” *Wine & Viticulture Journal*, vol. 28, pp. 64–66, 2013.
[20] D. E. Nandorfy, T. Siebert, F. Watson, R. Keast, and I. L. Francis, “Understanding the interactive effects of volatile compounds contributing to ‘stone fruit’ aroma nuances in white wines,” *Australian Journal of Grape and Wine Research*, vol. 28, no. 3, pp. 424–438, 2022.
[21] D. E. Nandorfy, F. Watson, D. Likos et al., “Influence of amino acids, and their interaction with volatiles and polyphenols, on the sensory properties of red wine,” *Australian Journal of Grape and Wine Research*, vol. 28, no. 4, pp. 621–637, 2022.
[22] M. Parker, P. Osidacz, G. A. Baldock et al., “Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine,” *Journal of Agricultural and Food Chemistry*, vol. 60, no. 10, pp. 2629–2637, 2012.
[23] M. J. Herderich, T. E. Siebert, M. Parker et al., “Spice up your life: analysis of key aroma compounds in Shiraz,” in *Flavor Chemistry of Wine and Other Alcoholic Beverages*, pp 3–13, American Chemical Society, Washington, DC, USA, 2012.
[24] J. Ballester, M. Mihnea, D. Peyron, and D. Valentin, “Exploring minerality of Burgundy Chardonnay wines: a sensory approach with wine experts and trained panellists,” *Australian Journal of Grape and Wine Research*, vol. 19, no. 2, pp. 140–152, 2013.
[25] E. Zaldívar Santamaría, D. Molina Dagá, and A. T. Palacios García, “Statistical modelization of the descriptor “minerality” based on the sensory properties and chemical composition of wine,” *Beverages*, vol. 5, no. 4, p. 66, 2019.
[26] D. R. Peryam and F. J. Pilgrim, “Hedonic scale method of measuring food preferences,” *Food Technology*, vol. 11, 1957.
[27] H. MacFie, “23- Preference mapping and food product development,” in *Consumer-Led Food Product Development*, H. MacFie, Ed., pp. 551–592, Woodhead Publishing, Cambridge, UK, 2007.
[28] K. H. Esbensen, D. Guyot, F. Westad, and L. P. Houmoller, *Multivariate Data Analysis: In Practice: An Introduction to Multivariate Data Analysis and Experimental Design*, Multivariate Data Analysis, Oslo, Norway, 2002.
[29] G. M. Sullivan and R. Feinn, “Using effect size—or why the P value is not enough,” *Journal of Graduate Medical Education*, vol. 4, no. 3, pp. 279–282, 2012.
[30] R. L. Wasserstein, A. L. Schirm, and N. A. Lazar, “Moving to a world beyond “p < 0.05”,” *The American Statistician*, vol. 73, no. 1, pp. 1–19, 2019.
[31] D. L. Capone, R. Ristic, K. H. Pardon, and D. W. Jeffery, “Simple quantitative determination of potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis,” *Analytical Chemistry*, vol. 87, no. 2, pp. 1226–1231, 2015.
[32] A. G. Cordente, C. D. Curtin, M. Solomon et al., “Modulation of volatile thiol release during fermentation of red musts by wine yeast,” *Processes*, vol. 10, no. 3, p. 502, 2022.
[33] T. E. Siebert, H. Smyth, D. Capone et al., “Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS,” *Analytical and Bioanalytical Chemistry*, vol. 381, no. 4, pp. 937–947, 2005.
[34] L. Pisaniello, F. Watson, T. Siebert, L. Francis, and J. L. Hixson, “The varietal influence of flavour precursors from

grape marc on monoterpene and C13-norisoprenoid profiles in wine as determined by membrane-assisted solvent extraction (MASE) GC-MS," *Molecules*, vol. 27, no. 7, p. 2046, 2022.

[35] A. P. Pollnitz, K. H. Pardon, M. Sykes, and M. A. Sefton, "The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 11, pp. 3244–3252, 2004.

[36] T. E. Siebert, M. R. Solomon, A. P. Pollnitz, and D. W. Jeffery, "Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 17, pp. 9454–9462, 2010.

[37] T. E. Siebert, A. Barker, S. R. Barter, M. A. de Barros Lopes, M. J. Herderich, and I. L. Francis, "Analysis, potency and occurrence of (Z)-6-dodeceno-γ-lactone in white wine," *Food Chemistry*, vol. 256, pp. 85–90, 2018a.

[38] R. C. Cooke, D. L. Capone, K. A. van Leeuwen, G. M. Elsey, and M. A. Sefton, "Quantification of several 4-alkyl substituted γ-lactones in Australian wines," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 2, pp. 348–352, 2009.

[39] M. Spraul, M. Link, H. Schaefer, F. Fang, and B. Schuetz, "Wine analysis to check quality and authenticity by fully-automated 1H-NMR," *BIO Web of Conferences*, vol. 5, p. 2022, 2015.

[40] T. Tominaga and D. Dubourdieu, "A novel method for quantification of 2-methyl-3-furanthiol and 2-furanmethanethiol in wines made from Vitis vinifera grape varieties," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 1, pp. 29–33, 2006.

[41] L. Mateo-Vivaracho, J. Zapata, J. Cacho, and V. Ferreira, "Analysis, occurrence, and potential sensory significance of five polyfunctional mercaptans in white wines," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 18, pp. 10184–10194, 2010.

[42] C. A. Black, M. Parker, T. E. Siebert, D. L. Capone, and I. L. Francis, "Terpenoids and their role in wine flavour: recent advances," *Australian Journal of Grape and Wine Research*, vol. 21, pp. 582–600, 2015.

[43] G. Gutiérrez-Gamboa, T. Garde-Cerdán, Y. Moreno-Simunovic, and E. P. Pérez-Álvarez, "10- Amino acid composition of grape juice and wine: principal factors that determine its content and contribution to the human diet," in *Nutrients in Beverages*, A. M. Grumezescu and A. M. Holban, Eds., pp. 369–391, Academic Press, Cambridge, MA, USA, 2019.

[44] L. J. Van Gemert, *Flavour Thresholds. Compilations of Flavour Threshold Values in Water and Other media*, Oliemans Punter and Partner, Utrecht, Netherlands, 2nd edition, 2011.

[45] K. Skogerson, R. Runnebaum, G. Wohlgemuth, J. de Ropp, H. Heymann, and O. Fiehn, "Comparison of gas chromatography-coupled time-of-flight mass spectrometry and 1H nuclear magnetic resonance spectroscopy metabolite identification in white wines from a sensory study investigating wine body," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 15, pp. 6899–6907, 2009.

[46] R. Gawel, P. A. Smith, S. Cicerale, and R. Keast, "The mouthfeel of white wine," *Critical Reviews in Food Science and Nutrition*, vol. 58, no. 17, pp. 2939–2956, 2018.

[47] V. Ferreira, "1- Volatile aroma compounds and wine sensory attributes," in *Managing Wine Quality*, A. G. Reynolds, Ed., pp. 3–28, Woodhead Publishing, Cambridge, UK, 2010.