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ORIGINAL RESEARCH ARTICLE

Enhancing the sensory properties and consumer acceptance of warm climate red wine through blending

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ABSTRACT

Proline has recently been found to direct several sensory attributes in red wine, including viscosity, fruit flavour and sweetness. We sought to investigate whether a red wine, deemed 'flavour deficient' by a producer, from a warm inland region could be improved by blending with a high proline wine from the same region, compared to a high colour and flavour wine, linking consumer acceptance with sensory properties and chemical composition. Three dry red wines (two Cabernet-Sauvignon wines from a warm region and one Lagrein wine from a cooler region) were blended in a constrained mixture design. Several blends were uncovered with improved sensory properties and consumer liking scores. Increased liking scores were related to heightened perceived Viscosity (unrelated to physical viscosity), Sweetness and Berry flavours, connected to proline-rich wines with small proportions of Lagrein. PLS-R models relating blend chemical composition, sensory properties and consumer acceptance associated Astringency and Bitterness to polyphenolics and organic acids and lower liking scores. Vegetal and Leather aromas in blends also reduced consumer acceptance and were related to the concentration of the thiols 3SH, 3SHA, PMT, 2FMT and MeSH, as well as guaiacol and isobutyl methoxypyrazine. Multiple blends successfully improved consumer acceptance of the 'flavour deficient' wine, particularly those with an increased proportion of the proline-rich wine. Non-linear effects resulting from blending were also assessed, with most variables modelled best by linear averaging. This study demonstrates the practical application of a design of experiment approach using sensory properties, proline and polyphenolic concentrations to guide wine blending and improve wine flavour and acceptability.

KEYWORDS: amino acids, sensory, taste, flavour, mouthfeel, sweetness, viscosity

INTRODUCTION

Wine blending has been commonly practised throughout history. Often, the goal of wine blending is to achieve targeted flavour properties or appease regulatory requirements related to a region or style (e.g., Bordeaux or Australian Cabernet Shiraz blends), increase 'complexity', and to correct 'flavour deficiencies' caused by vintage, vineyard, clonal and even barrel to barrel variation. The practice has been colloquially described to be more of an art rather than a scientific undertaking (Beckett, 2023). Despite some form of blending being ubiquitous and routine in almost every winery in the world, few scientific investigations of wine blending have been conducted.

One of the first investigations of blending wines was published by Singleton and Ough (1962), who compared the quality scores of 34 blends (50:50) to their individual components. Seven of the blends scored higher than each individual component, and no blends scored lower than the lowest individual wine. The increased 'complexity' of the wines was suggested as the major contributing factor, although dilution of winemaking faults, likely more common 60 years ago, may have also contributed.

Further, than simple dilution, a few blending examinations have reported some non-linear effects (Ferrier and Block, 2001; Lawless and Heymann, 2010) beyond simple averaging of the sensory properties; for example, the score of sensory attributes of the blended wine was higher or lower than its individual wine components. Others found that linear averaging was common for some sensory attributes, basic wine composition and anthocyanin composition but not for most volatile compounds (Hopfer *et al*., 2012).

While blended wines are common, single-origin and 'terroir'-specific wines are also produced, and climate change is projected to make consistent winemaking from single regions more challenging (Gambetta and Kurtural, 2021; Mozell and Thach, 2014). Climate change effects have, or are predicted to influence, vine phenology and development, grape and wine composition, and typicity (van Leeuwen and Darriet, 2016). Wines produced from warm inland irrigated regions of Australia can be prone to flavour deficiencies, particularly related to high temperatures post-veraison, which accelerates sugar concentration but negatively affects anthocyanins (Kliewer and Torres, 1972) and other important secondary metabolites related to grape and wine acidity and flavour. Drought stress can also affect photosynthesis, plant growth, berry characteristics, and polyphenolic compounds (Gambetta *et al*., 2020). According to Australia's wine future climate atlas, a warm inland region such as the Riverland in South Australia is projected to experience a mean increase in growing season temperature of 2.8 °C, a 10 % reduction in mean growing season rainfall, and a mean increased excess heat factor of 2.6 between 2081–2100 (Remenyi *et al*., 2019). Together, these projections would make wine growing in this area even more challenging. Although new climate change-resistant plant material, training systems and other solutions have been presented (van Leeuwen and Darriet, 2016), wine blending between climactically distinct regions and the use of non-traditional cultivars more suited to Australia's changing climate, including later ripening cultivars such as Lagrein, provides an opportunity to improve wine quality in the short to medium term.

Wines made from less familiar cultivars, classified as 'alternative varieties' in Australia, can present barriers for consumers who may not accept their sometimes distinct flavour profiles compared to those to which they are accustomed, such as Shiraz, Cabernet-Sauvignon and Grenache (Mezei *et al*., 2021). One approach to using these new cultivars is blending them with complementary traditional ones. In Australia, a varietal component must be labelled if it exceeds 15 % of a blend.

Major wine compounds that can convey sweet taste properties in isolation or at high concentrations in wine, such as residual sugars and glycerol (Nurgel and Pickering, 2006), as well as those found at more trace levels, such as astilbin isomers (Fayad *et al*., 2021), yeast heat shock protein Hsp12p (Marchal *et al*., 2011a) and Quercotriterpenosides (Marchal *et al*., 2011b) are of interest to winemaking due to their contribution to balancing bitterness, sourness and astringency, particularly of dry red wines. Even below reported detection thresholds, sapid compounds can act as potent flavour modifiers when paired with congruent odorants due to cross-modal interactions (Dalton *et al*., 2000). In a recent series of sensory-guided studies (Espinase Nandorfy *et al*., 2022), the residual amino acid L-proline strongly influenced the in-mouth sensory properties of dry red wine in a controlled addition experiment, and strong associations were found relating perceived wine viscosity to proline concentration in other data sets. Causal experimentation with a Shiraz wine low in amino acid concentration demonstrated that added L-proline increased viscosity, sweetness, and fruit flavour while decreasing perceived astringency and bitterness. The upper limits of wine proline concentrations previously reported in French red wines was 0.78 g/L (Lehtonen, 1996) compared to those measured in California Cabernet-Sauvignon from warm climates such as the Santa Ynez Valley and Napa's Oakville sub-region which both surpassed 4.0g/L (Huang and Ough, 1991; Ough and Stashak, 1974). The accumulation of proline in grapes is believed to be due to environmental factors such as water stress (Costantini *et al*., 2006; Freeman and Kliewer, 1983). The taste recognition thresholds reported for L-proline in water range from 1.5 to 15 g/L, with many reported values close to 2 g/L (Van Gemert, 2011). The range of proline concentration in Australian wines remains unknown; however, one blending component used in this study exceeded these previous reports.

Considering the challenges presented by climate change and sparse scientific investigations into the effects of red wine blending, this research study aimed to determine the effects of blending a high proline component and a climate change tolerant alternative variety with a 'flavour deficient' wine on the sensory characteristics and consumer acceptance of wine blends. To test this, we obtained two Cabernet-Sauvignon wines made from grapes grown in the Riverland, SA, with

varied proline concentrations, and blended them with each other and up to 30 % Lagrein wine sourced from the cooler Limestone Coast, SA region in a constrained mixture design similar to that used by Dooley *et al*. (2012). The 100 % Lagrein wine was also included. These wines were then subjected to chemical analysis, Quantitative Descriptive Analysis (QDA), and consumer testing. A major aim of this work was to test if the findings of Espinase Nandorfy *et al*. (2022) could be applied in a practical winemaking context, acknowledging the limitations of selecting blend components which would have multiple compositional differences. Based on this previous research, we hypothesised that increasing the proportion of proline-rich wine in a blend would increase perceived fruit flavour, sweetness and viscosity while decreasing bitterness and astringency, i.e., sensory effects likely directing positive consumer acceptance. The cooler climate, Lagrein wine, a cultivar recognised to have strong colour, tannin and flavour, is particularly adapted to retain natural acidity in hot climates (Cooper and Dry, 2015). The proportion of Lagrein was constrained to test blends above and below the Australian varietal labelling threshold (15 %). A secondary aim was to determine the extent of non-linear effects due to blending.

MATERIALS AND METHODS

1. Wines

Three 2022 vintage monovarietal wines were obtained from commercial wineries: a Cabernet-Sauvignon (CAS-1) deemed 'flavour deficient' by winemaking staff and destined for the bulk wine market; a high proline Cabernet-Sauvignon (CAS-2), both from the Riverland region (SA), and a Lagrein (LGR) from the Limestone Coast region (SA). Wines were produced using standard commercial practice at the respective winery, including the completion of malolactic fermentation. The two Cabernet-Sauvignon wines had no oak treatment and were obtained from stainless steel tank storage, while the Lagrein was sourced after two weeks in old oak barrels and had a 0.014 % v/v addition of Fermotan Liquid Tannin (AEB Group spa, NSW, Australia). The wines were assessed, together with other candidate wines, by an expert panel (n = 9) of technical wine assessors (seven females) with an average age of 35.8 years (SD = 10.3). The wines were indicated to be free of taints and faults, and the selected 'flavour deficient' Cabernet-Sauvignon wine was indicated to be a suitable example of a low-grade Cabernet-Sauvignon from the region, with relatively low flavour intensity and a 'green, vegetal' aroma character. Wines were transported in stainless steel vessels to the Hickinbotham Roseworthy Wine Science Laboratory at the University of Adelaide's Waite campus for blending and bottling.

2. Wine preparation

Each of the three wines was homogenised, sampled for basic wine analysis and transferred to 50L stainless steel kegs for future operations. Two wines were supplemented with 96 % v/v alcohol (SVR from Tarac, Nuriootpa, SA, Australia) to standardise all wines targeting 14.9 % v/v alcohol. A sulfur dioxide addition of 30 mg/L was made to the CAS-2 wine using potassium metabisulfite (VinCru PMS, EE Muirs, Lenswood, SA, Australia) prior to filtration and blending to approximately equalise preservative concentrations. All batches of wine were filtered from their kegs using nitrogen gas (Air Liquide, Adelaide, SA). Pressurised wine (10-15psi) was forced through polypropylene membranes sourced from Blue H_2O Filtration (Oakleigh, VIC, Australia) using a staged approach of 5 µm SupaPleat 10" code 7 into 1 µm SupaPleat 10" code 7, finally through a 0.5 µm SupaPleat 10" code 7 membrane. After final additions and filtration, the three components were blended.

3. Blending

A constrained ternary mixture design (Figure 1, Supplementary Table S1) was created, constraining the proportion of LGR to up to 30 % of a blend using Minitab 20 (Minitab Inc., Sydney, NSW, Australia). Although not part of the constrained mixture design, the 100 % LGR wine was also included in testing but excluded from some subsequent statistical analyses. Fourteen blend treatments were produced in duplicate, giving 28 individual wines. Due to the relatively small volume of each wine produced (approx. 20 L), each was blended by weight using an AND FS-KL150 platform balance with a precision of 0.05 kg (FS-KL150, A&D Australasia Pty Ltd., Thebarton, SA, Australia). Each keg of the individual components (CAS-1, CAS-2, LGR) was pressurised (5 psi) using food-grade nitrogen gas and dispensed in appropriate aliquots to each bottling keg. Each bottling keg was sparged and blanketed with nitrogen gas during the blending. The blending order was randomised across each replicate. Once completed, each wine blend was homogenised and stored at 15 °C for 2 days awaiting bottling.

4. Bottling

Each blend replicate (1 and 2) was bottled on individual days, and on each day, a randomised bottling order was followed. All treatments were packaged in 375 mL 30157 Antique Green punted Claret BVS bottles and closed using Guala Closures BVS Matt Black screw caps (Vinpac International, Angaston, SA) under nitrogen gas cover using a Gai 1005S-4292 bottling line, a six-head monoblock filler and capper (Viniquip, Hastings, NZ).

5. Sampling, storage, and transport

Once bottled, the wine was stored in a temperature and humidity-controlled warehouse (17 °C) for six months before sensory and chemical analysis. Following sensory assessment, wines were shipped to a consumer testing facility using a wine-specific transport company with temperature control measures in place (Wine Works Australia, Lonsdale, SA, Australia).

6. Consumer acceptance testing

To evaluate the consumer acceptance of the wines, 126 regular red wine consumers were recruited from the Deakin University CASS Food Research Centre (School of Exercise and Nutrition Sciences) database. Consumers were eligible for participation if they matched the following criteria: drinks

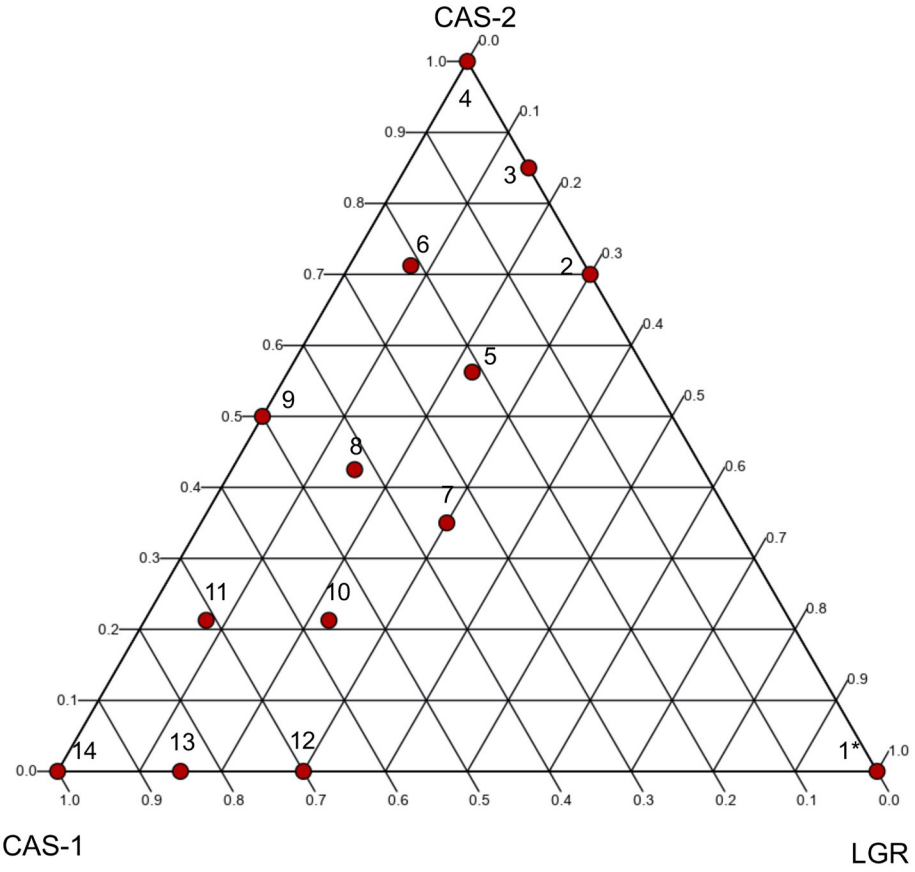


FIGURE 1. Constrained mixture design ternary plot for wine blend locations and proportions for the 14 blend treatments: three single cultivar wines, five binary blends and six ternary blends. *Wine one (100 % Lagrein: LRG) not included in the design. All wines were prepared in duplicate. Blend proportions are given in Supplementary Table 1.

red wine at least once a week; purchases bottled wine for $10–$20 or more from time to time. Consumer demographic details can be found in Supplementary Table S2. A large proportion of the recruited consumers (78 %) self-reported to drink Cabernet-Sauvignon at least once a month, with 28 % drinking that varietal wine once a week or more. A balanced proportion of participants across gender and age groups were targeted, excluding applicants below 18 and above 65 years of age. Pregnant and nursing participants were excluded from participation. Participants attended two 1 h sessions on separate days. During the first session, participants completed an entrance survey and were presented with seven samples randomly selected from the blends and balanced across blend replicates. Participants returned for the second session and were presented with the remaining seven random samples. Upon completion of the tasting, each consumer answered an exit survey describing their usage and attitudes towards wine products. Participants received an $80 gift certificate to a major national grocery store for their participation in the two sessions and were provided general information regarding the objectives and outcomes of the test.

Wines were presented to consumers monadically in a Williams Latin Square block design generated by Compusense20 sensory evaluation software (Compusense Inc., Guelph, Canada). Wines were presented in 25 mL aliquots poured into 3-digit-coded, ISO standard wine glasses (ARCOROC Viticole, France) at 22–24 °C. Consumers rated each wine for liking on a nine-point hedonic scale labelled ‘dislike extremely’ to ‘like extremely’ followed by a five-point purchase intention question on a separate test page labelled ‘would definitely not purchase’ to ‘would definitely purchase’ at a reasonable price. At the end of each consumer assessment session, participants were offered a test with an alcohol breath analyser. Upon completion of the test, ANOVA assessing replicate differences was conducted. No evidence ($P = 0.820$) of a replicate effect for liking or purchase intent ($P = 0.835$) was found, and values were aggregated for further statistical analysis.

7. Quantitative descriptive analysis

The QDA sessions were carried out over four continuous weeks. All wines were evaluated using the generic QDA method as described in Heymann *et al*. (2014). The 28 wines were progressively presented to assessors over six two-hour training sessions to generate and refine appropriate descriptive attributes, definitions/synonyms and sensory reference standards through a consensus-based approach.

TABLE 1. Sensory attributes, definitions, and composition of reference standards for the QDA study.

	Attributes	Definitions/Synonyms	Reference standard composition
Appearance	Opacity	The degree to which light cannot pass through the sample	Conceptual standard
	Purple Hue	Colour intensity	Conceptual standard
	Red Hue	Colour intensity	Conceptual standard
Aroma	Red Berries	The intensity of the aromas of red currant, strawberry, raspberry, red confectionary and red cherry.	Standard 1. 30 g sliced frozen strawberries (Welsh's), 10 g frozen raspberries (Welsh's), Morello pitted cherries (Marco Polo). Standard 2. ripe raspberries lollies (Allen's)
	Dark Berries	The intensity of the aromas of blueberry, black cherry, blackberry, and plums.	20 g frozen blackberries (Welsh's), 15 g frozen blueberries (Welsh's), 5 g canned black cherries (G-fresh), 5 g satsuma plum jam (Beerenberg)
	Cassis	The intensity of the aroma of blackcurrant and Ribena.	10 mL blackcurrant syrup (Ribena)
	Vegetal	The intensity of the aroma of fresh vegetable stalks, green capsicum, tomato vine, and leaf.	20 g fresh green bean, 5 g fresh green capsicum, 5 g fresh tomato plant stalk (Foodland)
	Cedar	The intensity of the aroma of pencil shavings and cedar.	5 g freshly shaved cedar pencil (Staedtler) not in wine
	Vanilla	The intensity of the aroma of vanilla.	5 g organic vanilla bean paste (Queen)
	Dried Fruit	The intensity of the aroma of jam, dried fruit, prunes, raisins, and sultana.	10 g of raisins (Sunbeam), 10 g of sultanas (Sunbeam), 20 g of pitted dried prunes (Angas Park)
	Leather	The intensity of the aroma of smoke, medicinal, leather and barnyard.	150 µL of a 605.3 mg/L guaiacol solution (Sigma-Aldrich), 100 µL of a 4-ethyl phenol 1.01 g/L solution (Sigma-Aldrich)
	Pungency	The intensity of the warming and burning sensation of alcohol.	15 % v/v ethanol solution (95 % ethanol, Rowe Scientific, stored in glass)
In-mouth	Sourness	The intensity of the sour and acidic taste	1 g/L L-(+)-tartaric acid (Chem-Supply) in water
	Astringency	The intensity of the drying sensation in the mouth	0.5 g/L aluminium sulfate (Ajax fine Chem Supply Pty Ltd) in water
	Bitterness	The intensity of the bitter taste	15 mg/L quinine sulfate (Sigma-Aldrich) in water
	Viscosity	The perceived thickness and 'weight' of the sample in the mouth	1.5 g/L carboxymethylcellulose sodium salt (Sigma-Aldrich) in water
	Hotness	The intensity of the alcohol burning sensation, including aftertaste	15 % v/v ethanol solution in water (95 % ethanol Rowe Scientific stored in glass)
	Sweetness	The intensity of the sweet taste	8 g/L white sugar (Black & Gold) in water
	Red Berries	The intensity of the flavour of red currant, strawberry, raspberry, red confectionary, and red cherry.	Conceptual standard
	Dark Berries	The intensity of the flavour of blueberry, black cherry, blackberry, and plums.	Conceptual standard
	Cassis	The intensity of the flavour of black currant and Ribena.	Conceptual standard
	Dried Fruit	The intensity of the flavour of jam, dried fruit, prunes, raisins, and sultana.	Conceptual standard
	Cedar	The intensity of the flavour of pencil shavings and cedar.	Conceptual standard
	Vanilla	The intensity of the flavour of vanilla	Conceptual standard

*All standards were added to 100 mL of 2021 bag-in-box Shiraz (2 L, Angaston, SA) unless otherwise noted.

Wines were assessed by appearance, aroma, mouthfeel, texture and flavour. From the third training session onward, reference standards were presented and discussed, and these standards were also available during subsequent training sessions, the booth practice session, and the formal assessment sessions. Aroma reference standards were prepared in 250 mL BPA-free LDPE odourless plastic squeeze bottles (Belinlen, Amazon, USA) and squeezed by assessors to release aroma. In-mouth standards were presented in ISO-standard wine glasses. As a familiarisation exercise, assessors revisited these standards as well as at least one randomly chosen 'warm-up' wine sample from the study at the beginning of each formal assessment session.

On the sixth day of training, assessors participated in one practice session in the sensory booths under the same conditions as those for the formal sessions. A reduced sample design, including each individual blend component and several blends equal across the design, was presented in duplicate during this practice booth session. After the practice session, any terms which needed adjustment were discussed, and the final list of terms and standards was determined. The list of attributes, definitions/synonyms and sensory reference standards rated in the formal evaluation are shown in Table 1.

Wines were presented to assessors in isolated sensory booths under daylight-type fluorescent lighting. A 25 mL aliquot of each wine was poured into 3-digit-coded, ISO standard wine glasses at 22–24 °C and covered with 50 mm watch glasses. The wines were presented to assessors three times in a Williams Latin Square random block design generated by Compusense20 sensory evaluation software. Sample randomisation was shuffled and non-repeating across both assessors and presentation replicates except in the practice sessions when there was no shuffling across assessors. Blend replicate 1 wines were presented on evaluation days one, three and five, while blend replicate 2 wines were presented on days two, four and six of the formal evaluation period. All samples were expectorated. In the formal booth sessions, the assessors were presented with four trays, two with three samples per tray and two with four samples per tray per day. The intensity of each attribute listed in Table 1 was rated using an unstructured 15 cm line scale (numericised 0 to 10), with indented anchor points of 'low' and 'high' placed at 10 % and 90 %, respectively. Data was acquired using Compusense20 sensory evaluation software displayed on tablet computers (Apple, Cal, USA). The sensory acquisition software enforced a 60-second rest between samples and a minimum ten-minute rest between trays. Assessors were prompted to rinse with water between samples and requested to leave the booths between trays. Formal evaluation was completed in six two-hour sessions on separate days. A new bottle of each sample was used each day of the study.

8. Ethics

All sensory assessors and consumer testing participants provided informed consent to participate, and this work was conducted in accordance with Deakin University's ethics policy (HEAG-H 169_2019).

9. Statistical analysis and interpretation

QDA 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 (ANOVA) for the effect of the assessor, sample 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.

For the chemical and physical measurement data, one-way ANOVA for blends was carried out using Minitab 20. For the sensory data, ANOVA tested the effects of the factors: blend, assessor, and the interactions assessor by blend, blend replicate nested into the blend, assessor by blend replicate nested into the blend, and presentation replicate nested into the blend and blend replicate, treating assessor as a random effect (Minitab 20). If statistical evidence supported differences across blends, then further modelling and post-hoc testing were conducted. Fisher's least significant difference (LSD) value was calculated at a 95 % confidence level using the mean sum of squares value from the assessor by blend interaction effect.

For the consumer test data, ANOVA was calculated for the effects of blend, assessor and blend replicate, assessor by blend replicate and blend by blend replicate interactions, treating consumers as a random effect. Using XLSTAT 2020, a variety of clustering methods were calculated and inspected from the raw liking scores as recommended in MacFie (2007). Agglomerative hierarchical clustering by Euclidean distance (dissimilarity) using Ward's method was chosen for final interpretation. Internal preference mapping (IMP) of the consumer groups was also conducted. A Fisher's protected LSD value was calculated at a 95 % confidence level for each of the consumer groups.

Partial Least Squares Regression (PLS-R) models were generated using Unscrambler (Aspentech, Bedford, MA, USA) to explore relationships between wine chemical composition, sensory profiles and consumer responses using the NIPALS algorithm (30,000 iterations) and standardisation. Models first linked chemical composition (x) to sensory attributes (y), and then another model was generated that associated sensory attributes (x) with mean consumer liking (y). Wine chemical compounds important to sensory attributes and sensory terms identified as important to consumer response were determined by statistical jack-knifing and considering the size of regression coefficients as recommended in Esbensen *et al*. (2002).

Variables evidenced to have varied significantly by ANOVA and indicated as important in the PLS-R were further subjected to response surface regression modelling (RSM) −excluding but visualising wine 1 (100 % LGR) to preserve a constrained mixture design −of variable means and visualisations were completed with STAT-EASE 360 (Minneapolis, MN, USA). The mean standard error of 0.6 was observed for 80 % of the Design Space. Inspection of the 3D plot, an elongated bowl shape, of the mean model standard error, had up to 0.9 for edge and corner points and 0.5 for inner points and was within the desirable range for prediction recommended by STAT-Ease 360 (Supplementary Figure S1).

Attention was given to the level of statistical evidence (P-value), the magnitude of effect size (F-value) and absolute effect value (sample mean values) to interpret and draw conclusions about the effects of chemical and sensory significance (Sullivan and Feinn, 2012; Wasserstein and Lazar, 2016; Wasserstein *et al*., 2019). 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" (***).

To identify linearity or non-linearity across the blends for the variables measured, data were inspected for statistical evidence to have, firstly, the statistical difference across blends by ANOVA, and secondly, evidence supporting the inclusion of higher-order effects from the RSM analysis beyond the linear mixture effect (STAT-EASE 360). The range of each response variable and the variability of each measure were also considered when reporting.

10. Chemical analysis

The wines were analysed for a suite of target volatile compounds utilising deuterated analogues as internal standards for stable isotope dilution assay (SIDA). Details of the methods are available in the publications cited below. Briefly, monoterpenes and C13-norisoprenoids were analysed by membrane-assisted solvent extraction (MASE) gas chromatography (GC) mass spectrometry (MS) (Pisaniello *et al*., 2022). MASE bags were supplied by Lasersan Australasia Pty Ltd. (Tanunda, SA, Australia). An Agilent 7890B GC (Agilent Australia Pty Ltd., Mulgrave, VIC, Australia) was coupled to an Agilent 5977B MS and equipped with a Gerstel MPS Robotic Pro (Lasersan Australasia). Fermentation-derived aroma compounds were analysed by headspace solid phase microextraction (HS-SPME-GC-MS) (Siebert *et al*., 2005). The samples were analysed using an Agilent 7890A GC combined with an Agilent 5975C MS and Gerstel MPS2 multipurpose sampler. The C6 alcohols and aldehydes were analysed by HS-SPME-GC-MS utilising an Agilent 6890N GC coupled to an Agilent 5973N MS and a Gerstel MPS2 autosampler (Lasersan Australasia) as reported by Capone *et al*. (2012). The resultant GC-MS data were processed using Agilent MassHunter (Version B.09.00). Volatile sulfur compounds were analysed as described in Siebert *et al*. (2010) with slight modification. Analysis was carried out using an Agilent 7890B GC coupled to a sulfur selective detector SCD 8355 equipped with a Gerstel MPS2 XL (Lasersan) autosampler. Separation was achieved using an Agilent DB Sulfur SCD column (0.75 µm, 40 m × 0.32 mm) (Agilent, Mulgrave, VIC, Australia) with 1 m of retention gap (0.53 mm) with a flow rate of 2.7 mL/min (Cordente *et al*., 2022). Polyfunctional thiols were analysed by HPLC/MS/MS using an Exion UHPLC coupled to a 6500 QTrap+ (Sciex, Mulgrave, Vic., Australia) after derivatisation with 4,4′-dithiodipyridine (Acros Thermo Fisher Scientific, Thebarton, SA, Australia) and solid phase extraction (Capone *et al*., 2015; Cordente *et al*., 2022).

10.1 Chemicals

All the chemicals and solvents used for chemical analysis were of analytical reagent grade, and water was prepared from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Dimethyl disulfide, dimethyl sulfide, ethanethiol, ethyl methyl sulfide, sodium sulfide nonahydrate (for hydrogen sulfide), sodium thiomethoxide (for methanethiol), ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, hexyl acetate, 2-phenylethyl acetate, 2-methylpropanol, butanol, 2-methylbutanol, 3-methylbutanol, hexanol, 2-phenylethanol, propanoic acid, butanoic acid, 2-methylpropanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, hexanoic acid, octanoic acid, decanoic acid, limonene, 1,8-cineole, terpinolene, linalool, α-terpineol, ß-citronellol, nerol, geraniol, (-)-rose oxide, ß-damascenone, ß-ionone, α-ionone, (*E*)-2-hexenal, (*E*)-2-hexenol and (Z)-3-hexenol were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). *cis*-Vitispirane was supplied by Firmenich (Balgowlah, NSW, Australia), and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) was donated by Hochschule Geisenheim University, Germany). Methyl thioacetate, ethyl thioacetate, and propyl thioacetate were obtained from Lancaster Synthesis (Jomar Bioscience, Kensington, SA, Australia). The deuterated analogues used for stable isotope dilution analysis (SIDA): d_8-ethyl acetate, d_{10}-butanol, d_{13}-hexanol, d_3-acetic acid, d_5-propanoic acid, d_7-butanoic acid, d_{11}-hexanoic acid, d_{15}-octanoic acid, and d_{19}-decanoic acid were purchased from Sigma-Aldrich; and d_5-ethyl propanoate, d_5-ethyl 2-methylpropanoate, d_5-ethyl butanoate, d_5-ethyl 2-methylbutanoate, d_5-ethyl 3-methylbutanoate, d_9-2-methylpropyl acetate, d_9-3-methylbutyl acetate, d_9-3-methylbutanol, d_5-ethyl hexanoate, d_{13}-hexyl acetate, d_5-ethyl decanoate, d_6-1,8-cineole, d_6-α-terpineol, d_7-geraniol, d_6-linalool, d_2-ß-citronellol, d_4-ß-damascenone, d_3-α-ionone, d_3-ß-ionone, d_8-naphthalene, d_9-(*E*)-2-hexenal, d_4-(*E*)-2-hexenol, d_{10}-3-sulfanylhexanol, d_5-3-sulfanylhexyl acetate, d_{10}-4-methyl-4-sulfanylpentan-2-one, d_5-2-furylmethanethiol, d_5-phenylmethanethiol were synthesised in-house.

10.2. Tannin characterisation

Tannin concentration and composition were determined according to (Kassara and Kennedy, 2011). A 25 µL aliquot of wine was mixed with 300 µL of 0.04 % (w/v) methylcellulose (Sigma Aldrich, Castle, NSW, Australia) solution (treatment) or water (control) in a 96-well plate (1 mL volume), shaken thoroughly, and left to stand for 3 min. Saturated ammonium sulfate (Sigma Aldrich) was then added (200 µL), followed by water (475 µL). Plates were shaken well and left to stand for 10 min, then centrifuged. The 280 nm absorbance of a 300 µL aliquot of both the treatment and control samples was then measured using a microplate reader. The difference in the 280 nm absorbance between the control and treatment samples was used for tannin quantification using a quantitative standard of (−)-epicatechin (Sigma Aldrich). Standardisation of the tannin analysis was monitored by using a purified commercial seed extract (Tarac Technologies, Nuriootpa, SA, Australia) in each 96-well plate assayed. Solid phase extraction on Oasis HLB (3 mL, 60 mg, 30 µm) cartridges (Waters, Rydalmere, NSW, Australia) was used to purify wine tannin for compositional analysis according to the published approach (Kassara and Kennedy, 2011). Tannin isolates were reconstituted in methanol and analysed by phloroglucinolysis

with the modifications described previously (Kassara *et al.*, 2011).

10.3. Amino acid quantification by HPLC

Amino acid quantification was performed according to (Kassara *et al.*, 2022). An aliquot of wine was dried under nitrogen, resuspended in 100 µL coupling buffer (aceto-nitrile/ethanol/triethanolamine/water, 10:5:2:3) and evaporated to dryness under vacuum. A further 100 µL aliquot of coupling buffer and 5 µL phenylisothiocyanate (Merck Pty. Ltd., Bayswater VIC, Australia) were added, thoroughly mixed, and allowed to stand for 20 min. The samples were evaporated to dryness, resuspended in 50 mM ammonium acetate, pH 6.5 (200 µL, Solvent A) and centrifuged at 13,400 g for 5 min prior to injection to an Agilent 1260 UHPLC with a UV detector and equipped with a Trajan C18 column (120 Å, 5 µm, 250 mm × 4.6 mm) (Trajan, Ringwood, VIC, Australia). Separation was achieved at a column temperature of 50 °C with solvents A (50 mM ammonium acetate, pH 6.5) and B (100 mM ammonium acetate/acetonitrile, pH 6.5) at a flow rate of 2 mL/min. A linear gradient was applied from 100 % solvent A (0 min) to 70 % solvent B (45 min) and then to 100 % solvent B (48 min). Conditions were then returned to 100 % solvent A for 60 min to re-equilibrate the column. Amino acids were quantified at 254 nm with separate calibration curves generated for each amino acid, ranging from 1.6 µM to 100 µM.

11. Physical viscosity measurements

Physical wine viscosity was measured by a capillary viscometer (Cannon–Fenske routine viscometer 9721-B53, Cannon Instrument Company) in a water bath at 25 °C as described in (Wang *et al.*, 2023). The viscometer has an expanded uncertainty (k = 2) = 0.156 %; kinematic viscosity range = 0.8–4 mm^2/s; viscometer constant C_0 = 0.003812, B = 88 × 10^{-6}/°C; Charge = 6.7 mL; driving fluid head = 8.6 cm; working diameter of lower reservoir = 3.0 cm. Let t be outflow time, ρ be the density of the sample and viscosity (η) was calculated as

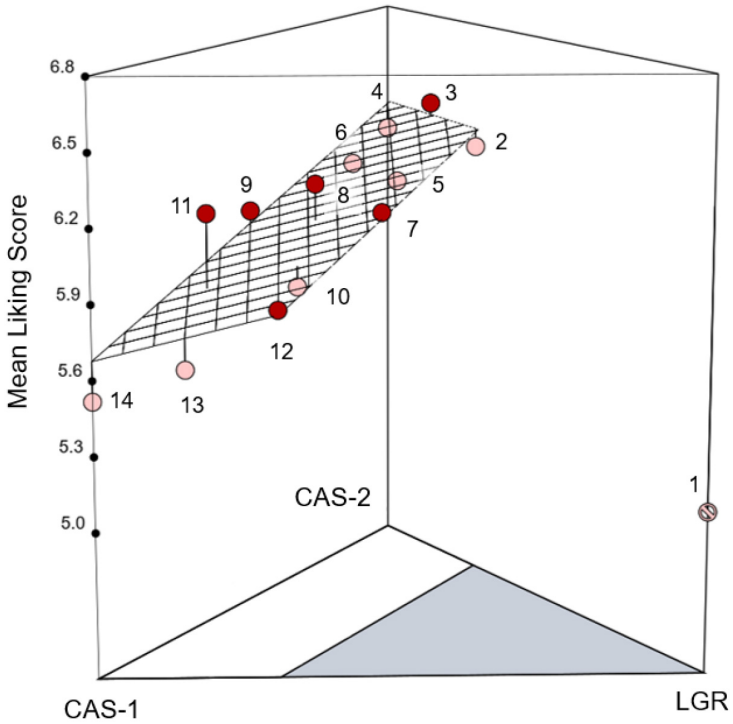
$$\eta = \frac{C_0 \cdot t}{\rho}$$

The viscosity for each blend's replicate was measured twice, making a total of 56 measurements.

RESULTS AND DISCUSSION

1. Consumer acceptance of wine blends

A Cabernet-Sauvignon (CAS-1) from a warm inland region with a relatively low proline concentration (3.0 g/L) and destined for the bulk wine market was blended with a



Legend: individual blend mean values above (red) and below (pink); the response surface indicated (pink) is not included in the model.

FIGURE 2. 3D response surface plot of the consumer liking scores for the 13 wines from the constrained mixture design, together with the excluded LRG (Blend 1) sample.

proportion of a higher proline wine (CAS-2, 4.8 g/L proline concentration) from the same region, and compared to blends with the alternative variety Lagrein from a cooler region (LGR, proline concentration 2.1 g/L). 14 dry red blends, produced using a formal ternary experimental design, were presented to 126 regular red wine consumers. The blend components CAS-1, CAS-2 and LGR had glucose and fructose concentrations of 0.5, 0.7 and 1.2 g/L, respectively. The range of glycerol concentrations for CAS-1 (9.8 g/L), CAS-2 (9.5 g/L) and LGR (9.8 g/L) was similar, and alcohol equalised (14.85 % ± 0.15).

Very strong evidence was found supporting differences in liking across wine blends ($F = 5.04$, $P < 0.0001$), and there was no statistical evidence of differences in liking between blend replicates ($F = 0.05$, $P = 0.820$). All mean consumer scores for the blends are given in Supplementary Table S3. Variation among the consumers tested was explored, with cluster analysis indicating three similar consumer segments across several clustering methods tested. Clusters 1, 2 and 3 represented 39 %, 40 % and 21 % of the total consumer participants. Clusters 1 and 2 mean liking scores were found to be highly correlated with the total mean consumer response, significantly ($P \leq 0.004$) discriminating between the wines, while mean scores from cluster 3 were not significantly different ($P = 0.16$), with all wines well-liked. Cluster 3 had significantly more individuals with high self-reported wine knowledge ($P = 0.013$) and slightly lower income ($P = 0.015$) than the other clusters. Clusters 1 and 2 gave high liking scores for all the wines except for the 100 % LGR (Blend 1, mean liking score = 4.5) and rated Blend 3 the highest (85 % CAS-2 and 15 % LGR, mean liking score = 7.1).

TABLE 2. F-ratios, probability values†, degrees of freedom (df) and mean square error (MSE) from the analysis of variance of QDA data.

Attribute	Blend (B)	Assessor*B	BRep(B)	Assessor*BRep(B)	PRep(B, BRep)	MSE
Opacity	48.02***	2.48***	1.79*	1.1	1.12	0.312
Purple Hue	41.04***	2.97***	0.89	1.37**	1.02	0.809
Red Hue	17.42***	4.77***	0.97	0.97	1.16	0.573
Dark Berries A	6.76***	1.74***	1.06	1.00	0.99	1.003
Red Berries A	10.94***	1.72***	0.79	1.07	1.11	1.416
Cassis A	3.64***	2.03***	1.73ǂ	0.9	1.37*	1.607
Vegetal A	3.19***	1.34ǂ	1.33	1.07	0.98	2.046
Cedar A	1.3	1.72***	0.57	0.87	1.24	1.573
Vanilla A	2.15*	1.37*	1.76ǂ	0.89	0.99	0.758
Dried Fruit A	9.00***	1.03	0.88	1.25*	1.26	1.653
Leather A	11.75***	2.53***	3.23***	0.67	1.00	2.655
Pungency A	2.62***	1.5*	0.96	0.94	1.49*	0.276
Sourness T	4.69***	2.98***	1.02	0.79	1.23	0.480
Sweetness T	9.40***	1.78***	1.31	1.02	1.04	1.123
Viscosity MF	4.56***	1.99***	1.27	0.50	1.00	0.366
Astringency MF	20.79***	1.49*	0.19	1.23ǂ	0.95	0.728
Bitterness T	3.28***	1.36*	0.78	1.20ǂ	0.75	0.577
Hotness MF	1.06	0.73	0.44	1.23ǂ	1.15	0.972
Dark Berries F	5.93***	1.68***	0.95	1.22ǂ	1.27ǂ	0.792
Red Berries F	12.61***	1.98***	0.71	1.13	1.37*	1.078
Cassis F	3.00***	1.89***	1.23	1.02	1.41*	1.409
Dried Fruit F	5.27***	1.28ǂ	1.11	1.24ǂ	0.57	1.527
Cedar F	1.77ǂ	1.79***	0.99	0.89	0.97	1.490
Vanilla F	2.24*	1.40*	0.89	0.95	1.08	0.727
df	13	117	14	126	56	504

A: Aroma, F: Flavour, T: Taste, MF: Mouthfeel. BRep: Blend replicate, PRep: Presentation replicate. †Significance levels are as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; ǂ $P < 0.10$. df = degrees of freedom. Assessor (A) effects were significant for all attributes.

In modelling the consumer liking scores for the constrained mixture design, i.e. not including the 100 % LRG wine, a linear model (P = 0.0008, F = 15.84) was reasonably well fitted (Adjusted $R^2 = 0.71$, Predicted $R^2 = 0.58$).

A response surface of the mean consumer liking for the different blends is shown in Figure 2. The lowest liking score was for the wine composed of 100 % LGR (Blend 1, mean score 5.1), with the 100 % CAS-1 wine (Blend 14) also not well accepted (mean score 5.5), confirming the categorisation of this wine as 'flavour deficient'. An optimal set of well-liked blends included 2, 3, 4, 5, 6, 7, 8 and 11.

Increasing the proportion of CAS-2 in the CAS-1 wine resulted in a largely linear response, with progressively greater liking, indicating that the proline of CAS-2 may have had an effect. Conversely, blending the LGR wine, selected as a high colour, flavour and tannin blending component, resulted in increased liking of up to 30 % LGR in the blend, but this wine was not well-liked individually. The most liked blend overall, Blend 3 (85 % CAS-2 and 15 % LGR), had only a slightly higher liking score (mean score of 6.4) than the 100 % CAS-2 (Blend 4, mean score of 6.3). Importantly, of wines which contained a substantial proportion of 'flavour deficient' CAS-1, Blend 11 (71.3 % CAS-1, 21.3 % CAS-2, LGR 7.5 %) was scored highly (mean liking score = 6.2), suggesting some synergistic localised optimum of sensory properties. Mean consumer liking scores are shown in Supplementary Table S4.

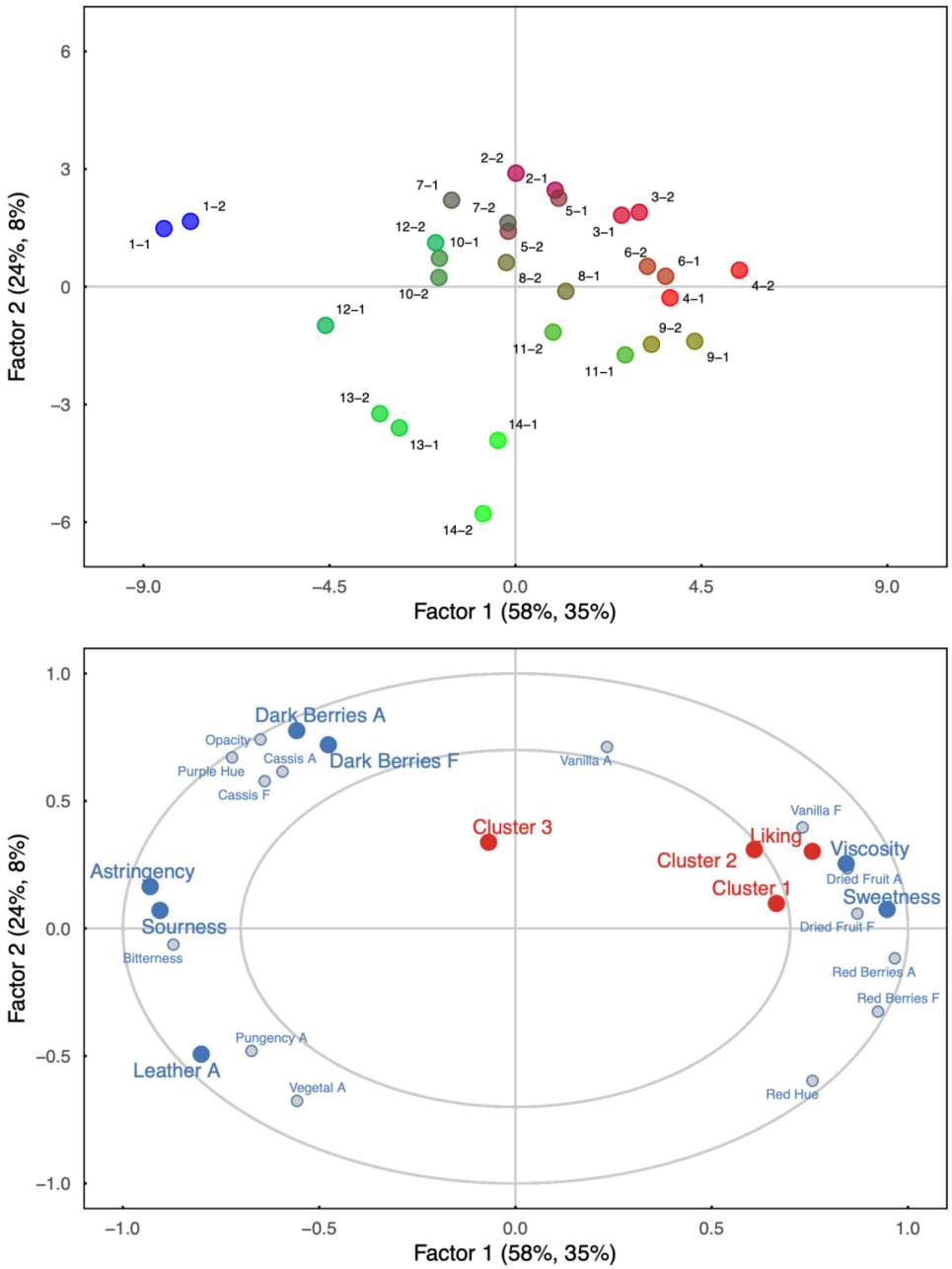
2. Sensory properties related to consumer acceptance

The trained QDA panel rated 24 attributes for the 14 wine blends (28 wines). ANOVA (Table 2) revealed very strong statistical evidence that the wine blends differed in all sensory attributes rated, except for Cedar aroma and Hotness. Weaker evidence suggested differences among the wines for Cedar flavour, Vanilla aroma and Vanilla flavour; however, the range of scores of these attributes were low, 1.2−2.2, 0.1−0.7 and 0.1−0.8, respectively. Statistical evidence was found for blend replicate variation only for the attributes Opacity and Leather aroma. Cassis aroma and Vanilla aroma may have also differed across the blend replicates. Mean wine scores sensory properties are available in Supplementary Tables S4 and S5.

PLS-R was used to identify sensory attributes that related to the consumer liking scores. A three-factor model calculated from the 28 blend replicates, including the 100 % LGR wines, was found to be optimal, explaining 50.6 % of the consumer liking variance, with the calibration predicted vs. measured $R^2 = 0.76$ and the validation predicted vs. measured $R^2 = 0.57$. A visualisation of the blends that were well-liked and sensory attributes that were associated with consumer liking responses is shown in Figure 3 from factors 1 and 2 of the PLS-R model. Consumer clusters 1 (calibration $R^2 = 0.62$, validation $R^2 = 0.32$) and 2 (calibration $R^2 = 0.51$, validation $R^2 = 0.34$) were also relatively well modelled, while cluster 3 (calibration $R^2 = 0.13$) was not modelled well enough to validate. Attributes that were significant and had large positive regression coefficients in the model, strongly positively associated with mean liking, were Dark Berries aroma (β = 0.157), Sweetness (β = 0.115), and Dark Berries flavour (β = 0.238), while conversely attributes with significant large negative coefficients included Leather aroma (β = –0.208), Sourness (β = –0.164), and Astringency (β = –0.179), and were associated with lower consumer acceptance. Viscosity (β = 0.122), Pungency (β = 0.101), Purple hue (β = 0.070), Opacity (β = 0.064) and Red berries flavour (β = 0.063) also had positive and moderately high regression coefficients, while Cassis flavour (β = –0.134) and aroma (β = –0.112), as well as Bitterness (β = –0.091) and Vegetal aroma (β = –0.059), had negative values. Those wines that were highest in Astringency, Sourness, Bitterness, Leather A, Pungency A and Vegetal A included Blend 1 (100 % LGR) and those with higher proportions of the LGR wine. A further two-factor PLS-R model excluding Blend 1 (100 % LRG) found similar attributes to be important to consumer response; however, Sourness (β = –0.04), Bitterness (β = –0.05) and Astringency (β = –0.04) became less influential (data not shown).

Figure 4 and 5 shows the response surfaces for those attributes that were most related to consumer acceptance. Regarding the sensory properties of the three individual wines used for the blending study, CAS-1 100 % (Blend 14) was relatively low in Purple hue, Viscosity, Cassis aroma and flavour and Sweetness; it was especially low in Dark berries aroma and flavour (Figures 4 and 5). This wine was also rated highly for Pungency, Red berries flavour, Vegetal aroma and Leather aroma. The CAS-2 100 % (Blend 4) was highest in Viscosity and Sweetness and lowest in Pungency, Bitterness, Cassis aroma and flavour and Astringency. The LGR 100 % wine (blend 1) was particularly high in Opacity, Purple hue, Bitterness, Cassis aroma and flavour, Dark berries aroma and flavour, Sourness, Astringency and Leather aroma, and low in Red berries flavour, Sweetness and Viscosity. Considering the response curves more specifically, wines with high Dark berries aroma and flavour—the strongest drivers of liking from the PLS model—were those with low or no CAS-1 in the blend (Figure 3) and with an increasing proportion of LGR up to 30 %, declining thereafter. Those blends with high consumer liking namely blends 2 to 8, are grouped together in the response surfaces. Viscosity was indicated to have a local minimum in the response surface, with Blend 5 having lower perceived Viscosity than blends 2, 3, 4, 7 and 8.

Comparing the top two most liked wines, the most liked wine, Blend 3, was higher in Opacity, Purple hue, Dark berries aroma and flavour than Blend 4 (100 % CAS-2) and was lower in Vegetal aroma. Blend 11, with a high proportion of CAS-1—indicated to have a higher liking score than expected from the liking response surface model—was lower in Vegetal and Leather aromas than other blends with high CAS-1 proportions, indicating the importance of these attributes in consumer liking responses. Most of these attributes have previously been shown to be influential in consumer liking for red wines (Francis and Williamson, 2015).

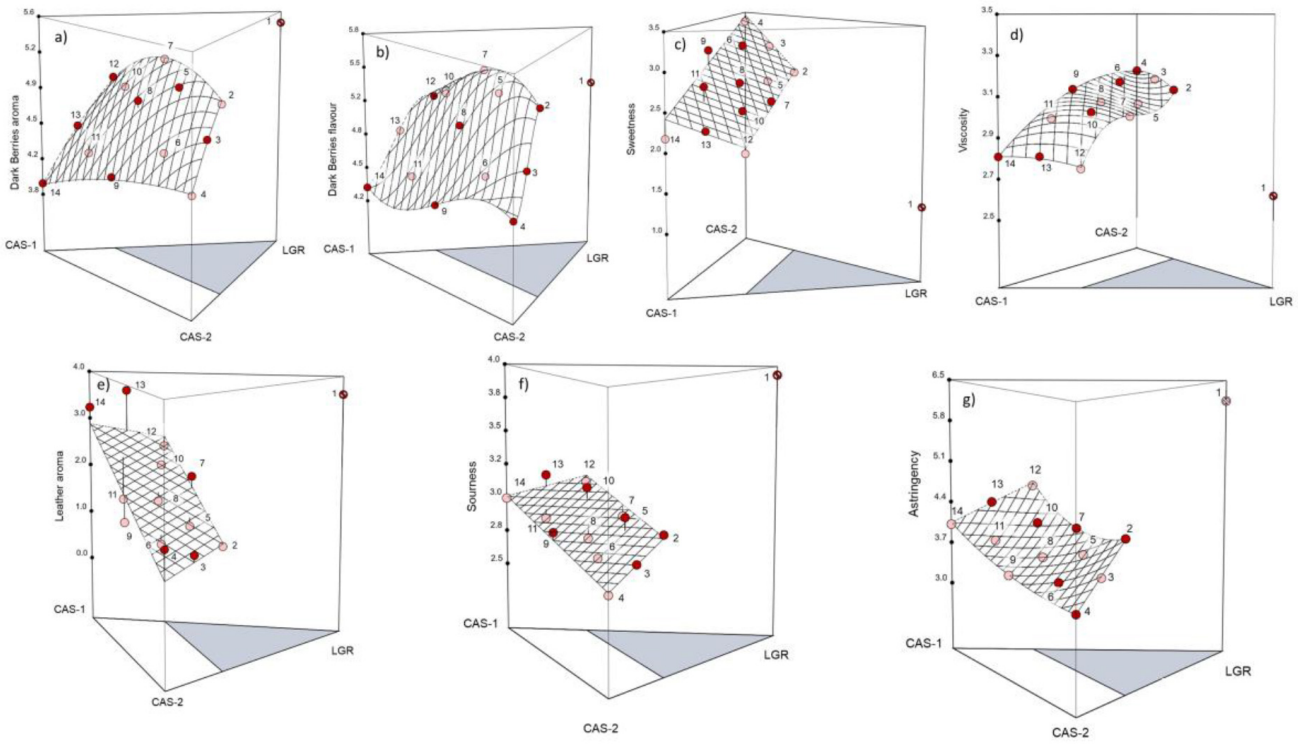


Legend:
x-variables
models statistically significant
y-variable
Cas-2 (), Cas-1 () and LRG ()

FIGURE 3. PLS regression models for the first two factors of a) scores and b) correlation loadings plots of sensory properties (x-variables), and those statistically significant, relating to consumer mean cluster and mean liking scores (y-variable) of the 28 samples composing 14 blending treatments. Blend replicates coloured by proportion, represented by the RGB index with Cas-2, Cas-1 and LRG.

3. Chemical composition related to wine blend sensory properties

Another PLS-R model was produced to understand which chemical compounds in the blends were most related to the sensory attribute ratings, particularly those most important to consumer acceptance. A four-factor model (Figure 6, Factors 1 and 2 shown) was optimal, with the chemical measures explaining 82.8 % of the sensory data variance. The appearance attributes Opacity and Purple hue were both well-modelled (predicted vs. measured validation $R^2 = 0.89$ and 0.90), with titratable acidity (TA) and tannin measures most strongly associated with these attributes. The aroma attributes Leather

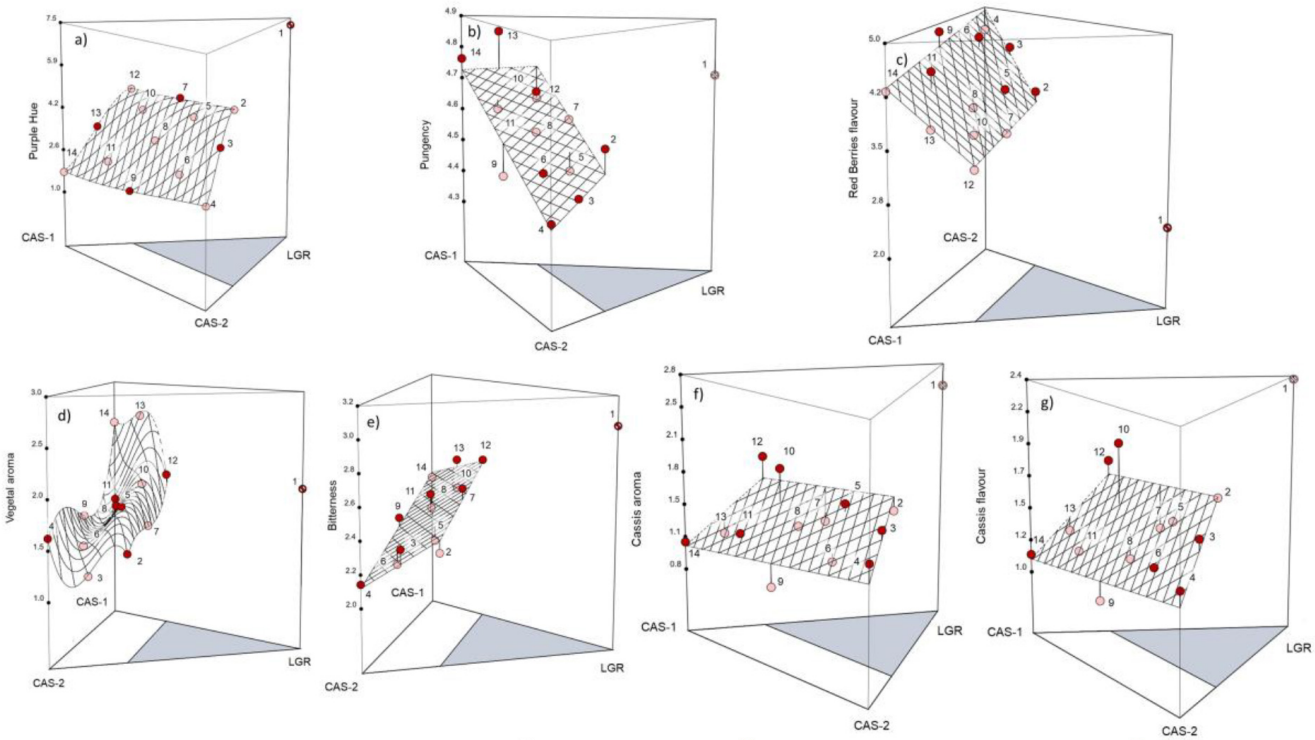


Legend: Individual blend mean values above (red ●) and below (pink ○); the response surfaces indicated (⊘, ⊘) are not included in the surface model.

FIGURE 4. Response surfaces relating the rating of the intensity ratings of significant sensory attributes to blend proportion for (a) Dark berries aroma, (b) Dark berries flavour, (c) Sweetness and (d) Viscosity, positively associated with consumer acceptance from the PLS-R model, as well as those negatively associated: (d) Leather aroma, (e) Sourness and (f) Astringency.

and Vegetal that negatively related to consumer liking were also well modelled from the chemical measures (calibration $R^2 = 0.87$ and 0.73, validation $R^2 = 0.79$ and 0.55), with similar volatiles found to be positively associated: the sulfur compounds methane thiol (MeSH), 2-furfurylmethanethiol (2FMT), phenylmethanethiol (PMT), 3-sulfanylhexan-1-ol (3SH), 3-sulfanylhexyl acetate (3SHA), and 2-methoxy-3-(2-methylpropyl)pyrazine (IBMP). IBMP is well known to confer 'green' flavour to Cabernet-Sauvignon wines and was at the highest concentration in Blend 14 (6 ng/L, 100 % CAS-1). Regarding the in-mouth attributes found to be positively related to consumer acceptance, Sweetness (calibration $R^2 = 0.89$, validation $R^2 = 0.87$), Viscosity (calibration $R^2 = 0.81$, validation $R^2 = 0.74$), Dark berries flavour (calibration $R^2 = 0.84$, validation $R^2 = 0.60$) and Red Berries flavour (calibration $R^2 = 0.93$, validation $R^2 = 0.86$) were all well modelled.

In agreement with the previous findings of Espinase Nandorfy *et al*. (2022), proline was significantly and positively related to both Sweetness ($\beta = 0.026$) and Viscosity ($\beta = 0.029$) and significantly negatively associated with Astringency (calibration $R^2 = 0.98$, validation $R^2 = 0.97$) and Bitterness (calibration $R^2 = 0.80$, validation $R^2 = 0.73$). The physical viscosity values for the wine blends measured were all around 1.57 (± 0.1) cP and did not show any statistical significance by ANOVA ($P = 0.470$). This finding, in addition to those of Danner *et al*. (2019) and Wang *et al*. (2021), supports the concept that wine ethanol concentration is the major driver of differences in physical viscosity. For blends with equalised alcohol concentration, their physical viscosity values remained narrow. This also showed that the physically measured viscosity does not explain the differences in perceived Viscosity. The work done by Wang *et al*. (2021), Wang *et al*. (2023) and Gawel *et al*. (2013) suggested that lowering matrix pH or increasing tannin level decreased perceived wine fullness which is thought to be perceptually similar to viscosity and body. Espinase Nandorfy *et al*. (2022) also found that the addition of a red wine polyphenolic extract decreased sweetness and viscosity but had no effect on wine body, instead imparting astringency and bitterness in agreement with Arnold *et al*. (1980); Fischer and Noble (1994) and Noble (1994). One possible explanation could be that lowered wine pH reduces saliva viscosity through organising salivary protein micelles (NordbÖ *et al*., 1984; Veerman *et al*., 1989; Zanchi *et al*., 2008), and high tannin cross-linkage might precipitate salivary protein (Halsam and Lilley, 1988; Ma *et al*., 2016; Watrelot *et al*., 2016; Yan *et al*., 2009). Consequently, the viscosity-reduced



Legend: individual blend mean values above (red ●) and below (pink ●), the response surface indicated (○, ⊘) are not included in the surface model.

FIGURE 5. Response surfaces relating the intensity ratings of sensory attributes to blend proportion: (a) Purple colour, (c) Viscosity and (c) Red Berries flavour, positively associated with consumer acceptance from PLS-R models, as well as those negatively related: (d) Vegetal aroma, (e) Bitterness, (f) Cassis aroma and (g) Cassis flavour.

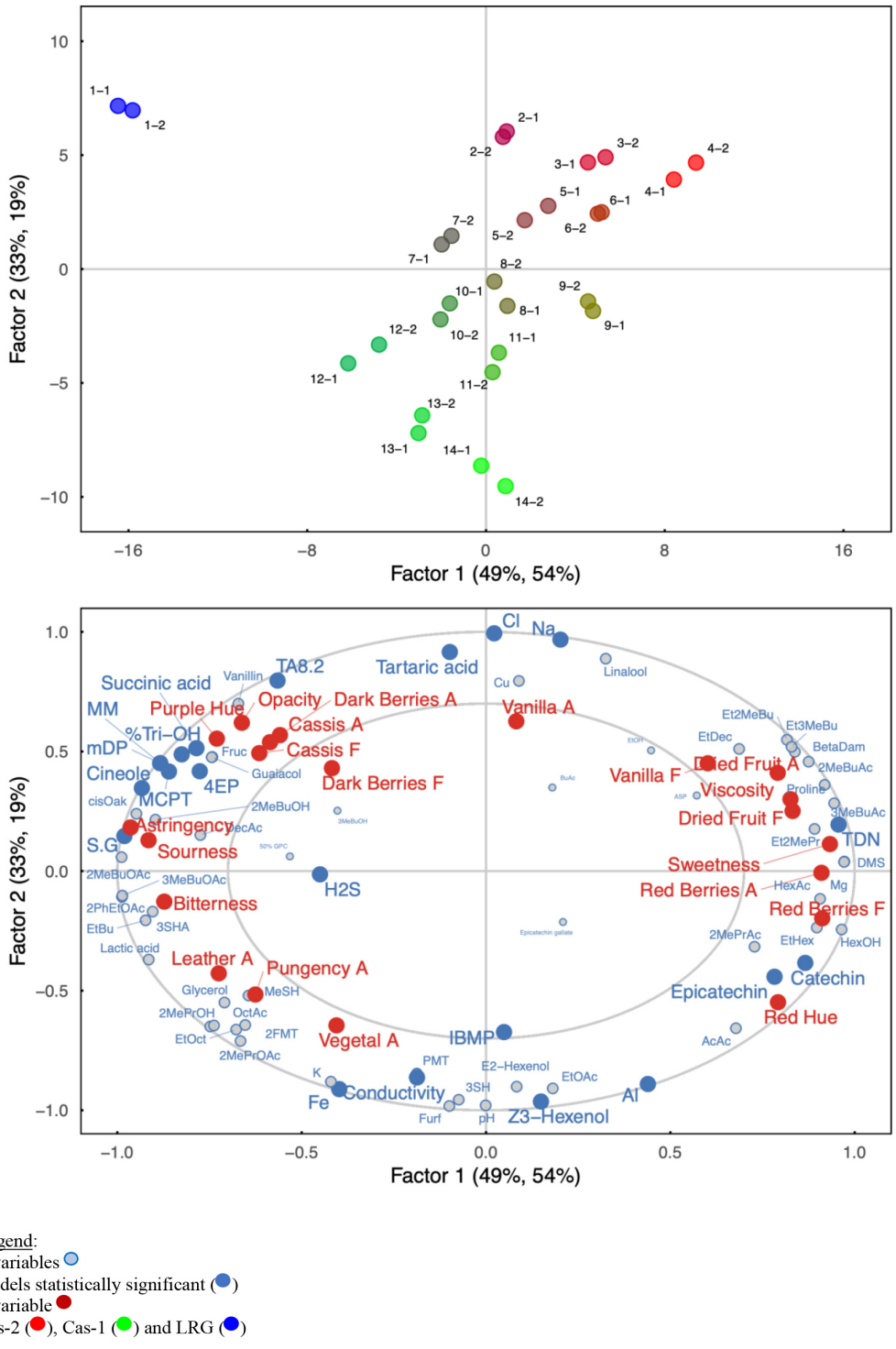
saliva-wine mixture could be perceived as low in viscosity, which is irrelevant to the physically measured viscosity of the wine itself.

Proline was also positively associated with Dark berries aroma and flavour (β = 0.033, 0.054), together with β-damascenone and hydrogen sulfide (H_2S), and were most strongly negatively related to IBMP (β = –0.152, –0.190), 4-ethylphenol (4-EP) (β = –0.171, –0.256) and guaiacol (β = –0.088, –0.130). Previously, proline was found to increase red fruit flavour when added to Shiraz wine (Espinase Nandorfy *et al*. 2022). Although dark berry and red fruit have distinct, congruent odours (fruit/berry), taste (sweetness) enhancement is likely at play for increased perceived flavour intensity (Dalton *et al*., 2000; Small and Prescott, 2005). The polyphenolic compound measures methyl cellulose precipitable tannin (MCPT) (β = 0.027), mean degree of polymerisation (mDP) (β = 0.028), molar mass (MM) (β = 0.028), percent of trihydroxylated subunits (%Tri-OH) (β = 0.02) and the organic acid succinic acid (β = 0.0320) were significantly positively associated with Astringency, while pH was significantly negatively related to this attribute (β = –0.015). Similar measurements were related to Bitterness, such as MCPT (β = 0.034), mDP (β = 0.018), MM (β = 0.018), epicatechin gallate (β = 0.029), succinic acid (β = 0.030) and %tri-OH (β = 0.023) although only mDP and MM were significant according to the uncertainty test. The compositional range of measured variables is available in Supplementary Table S6.

4. Linear and non-linear models of wine blends

Non-linear effects of wine blending on chemical composition have been suggested to be responsible for enhanced quality but might be limited to volatile compounds and their influence on sensory properties (Ferrier and Block, 2001; Lawless and Heymann, 2010). It could be hypothesised that blending may cause rapid chemical changes, releases or equilibrium shifts among flavour active compounds, resulting in measurable chemical and sensory differences. To probe for evidence of non-linear effects, models of the constrained mixture design were conducted, excluding wine 1 (100 % LRG), which was not part of the design and was a highly influential sample. Here, we define non-linearity as those variables which discuss statistical evidence by RSM Supplementary Table S7 beyond simple averaging of the blend components (linear) such as significant higher order terms including quadratic, cubic, special cubic and special quadratic terms.

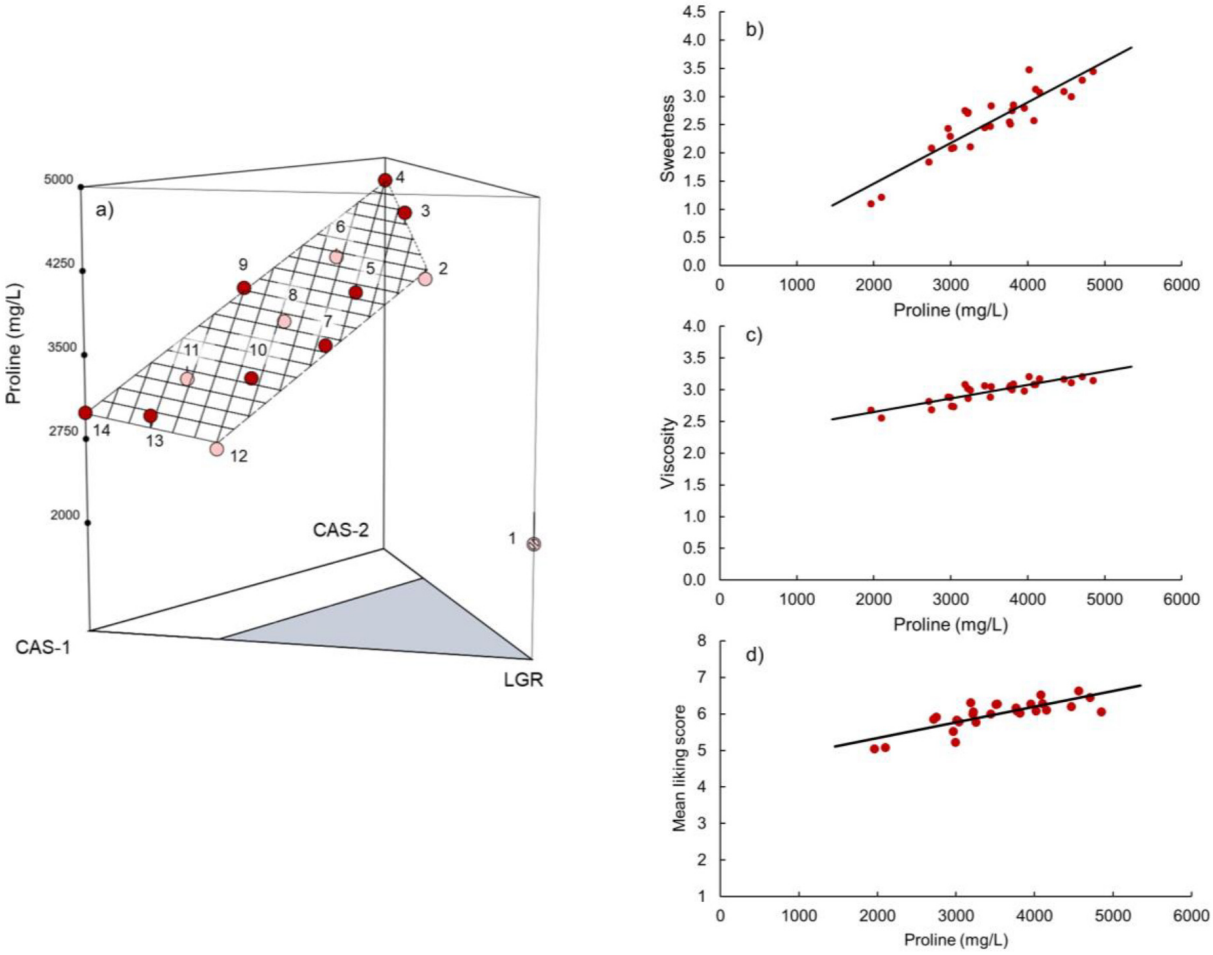
The overall visualisations of the scores plots (Figure 6a), which position blend replicates based on chemical measures (x-variables), shows nearly proportional sample positions compared to the original experimental design when



Legend:
x-variables
models statistically significant ( )
y-variable
Cas-2 ( ), Cas-1 ( ) and LRG ( )

FIGURE 6. PLS regression models for the first two factors of a) scores and b) correlation loadings plots of chemical composition (x-variables), and those statistically significant, relating to significant sensory properties (y-variable) of the 28 samples composing 14 blending treatments. Blend replicates coloured by proportion, represented by the RGB index with CAS-2, CAS-1 and LRG.

based on chemical composition, suggesting largely linear effects. From the RSM, 11 volatile aroma compounds: guaiacol, (*E*)2-hexenol, ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, butanoic acid, hexanoic acid, ethyl decanoate, vitispiranes, β-damascenone, 2FMT and acetic acid showed significant higher order effects beyond linear averaging. Of the non-volatile compounds and compositional measures, glutamate, serine, arginine, leucine, lysine, epicatechin, pH, nickel and zinc also showed some suggested higher-order terms, but the value range was generally



Legend: individual blend mean values above (red) and below (pink), the response surfaces indicated (,) are not included in the surface model.

FIGURE 7. Relationships of proline concentration to a) blend proportion response surface, b) Sweetness R2 = 0.81, c) Viscosity R2 = 0.76, and d) Consumer liking R2 = 0.76 (n = 28).

small. However, for all of these measurements, the linear effect sizes were 1 to 4 orders of magnitude larger than any suggested non-linear terms, rendering the resulting response surfaces virtually planar. In many cases, the concentration range detected was low, narrow, and likely of no sensory significance.

Opposed to the chemical data, overall visualisations of the scores plot (Figure 3a), which positions the blend replicates based on sensory attribute ratings show a large deviation from the experimental design pattern, suggesting non-linear sensory effects at play. Sensory attributes Opacity, Purple hue, Red hue, Dark Berries aroma and flavour, Vegetal aroma, Cedar aroma, Vanilla aroma and flavour, perceived Viscosity and Astringency showed statistical evidence of non-linear effects in modelling (Supplementary Table S7). Of the odour qualities which have not previously been linked to direct 'impact' compounds, non-linear effects could result from multiple compounds and their familiar proportions, a phenomenon known as configural odour processing (Thomas-Danguin *et al*., 2014).

The taste and smell responses stimulated by concentrations of specific compounds are known to be non-linear from the psychophysical literature, particularly at the low and high range of sensitivity (McBurney, 1976; Stevens, 1957). Dilution by blending aroma compounds at peri-threshold concentrations such as IBMP may explain the emergence of non-linear models of both aromas directed by these compounds as well as those released from suppression by these trace compounds. In addition to this non-linearity of single chemical stimuli, interactions between compounds through receptor and cognitive effects resulting in enhancement and suppression beyond simple averaging are well known (Keast *et al*., 2007; Keast and Breslin, 2003). Surprisingly, within the constrained design space, most sensory attributes and even consumer liking were well-modelled by linear model terms, which had much larger effect sizes.

5. Practical application

A major limitation of this study was the involvement of only three distinct red wines, with a large number of compositional

differences and some basic matrix differences, meaning that the results obtained may not be generalisable. While PLS-R, as a modelling approach, guards against multicollinearity issues and provides good evidence regarding analytes that may be causative, they are nonetheless only associations and require confirmatory experiments. However, this study supported the conclusions of previous addition studies and highlighted the correlation of specific key measures with consumer response and sensory properties. Overall, from these results, it would be feasible in the future for a winemaker to determine a relatively small number of chemical compositional measures and calculate the linear (averaging) effects of blends on their concentration and estimate the resultant sensory properties of the blended wines, followed by sensory evaluation to confirm the blended wines were on target. The measures determined to be most important from the PLS-R for consumer acceptance, such as MCPT, proline, H_2S, MeSH, guaiacol, TA, 4-EP and IBMP concentration, could be used as guides to attempt to maximise fruit/berry flavour, viscosity, and sweetness in dry red wine blends while minimising sourness, astringency, vegetal and leather characters. Indeed, a further PLS-R model only including these compounds provided reasonable predictions for the key sensory attributes that drove consumer liking (data not shown). However, caution should be exercised when attempting to apply statistical findings from this study alone; more research and in-house pilot studies should be conducted to evaluate the local effects. Proline, which ranged from 2.0–4.8 g/L and was evidenced to be linear by RSM (Figure 7a), also had very strong evidence ($P < 0.005$) of positive linear relationships with Sweetness ($R^2 = 0.81$, Figure 7b), Viscosity ($R^2 = 0.76$, Figure 7c) and consumer liking ($R^2 = 0.63$, Figure 7d).

CONCLUSION

This study has highlighted an example of a straightforward approach to producing wines with optimal sensory properties for consumers. This work provides practical evidence supporting the hypothesis that adding a component with elevated proline would have a strong positive effect on consumer liking by enhancing sweetness and viscosity and diminishing undesirable bitterness and astringency, building on previous addition experiments and statistical associations (Espinase Nandorfy *et al*., 2022). Surprisingly, the effect of blending a high proline wine on consumer preference was stronger than that of a high colour, tannin and flavour blend component, highlighting the potential for wine producers to target proline in grapes and wine as a non-carbohydrate sweetener, viscosity agent and flavour enhancer. However, formal standardised sensory thresholds for proline and other sweet-tasting wine compounds should be conducted in various wines to allow for comparison of potency. The majority of chemical compound concentration changes and consumer scores in blends followed linear averaging. Higher-order non-linear effects found were relatively small, indicating that simple averaging of compound concentration in the prediction of blending outcomes was adequate in this study. Some key sensory properties, however, were best modelled with non-linear effects, including highlighting the importance of formal sensory evaluation in wine research and production. Red wine proline concentration provides a new industry measure directing positive sensory properties associated with increased consumer acceptance.

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