Influence of amino acids, and their interaction with volatiles and polyphenols, on the sensory properties of red wine

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Abstract

Background and Aims: The effect of amino acids, and their interactions with volatiles and other non-volatiles, on in-mouth sensory properties of red wines is not known. This knowledge gap has been studied in a series of comprehensive sensory experiments.

Methods and Results: A solvent-assisted flavour evaporation extract of Shiraz wine volatiles, a de-aromatised polyphenolic extract and amino acids were added to model wine and wine systems. Using full factorial designs, samples were evaluated by sensory quantitative descriptive analysis. Volatiles enhanced Viscous mouthfeel ($F = 20.0$, $P < 0.001$), Sweetness ($F = 26.5$, $P < 0.001$) and Body ($F = 81.4$, $P < 0.001$), while the phenolic extract directed Astringency ($F = 170.5$, $P < 0.001$) as well as Bitterness ($F = 7.3$, $P < 0.001$) and suppressed Sweetness ($F = 16.5$, $P < 0.001$). An amino acid by volatile interaction ($F = 4.2$, $P < 0.05$) was found, and further experiments showed that L-proline enhanced Viscosity ($F = 5.0$, $P < 0.05$), Sweetness ($F = 14.4$, $P < 0.001$), Red fruit flavour ($F = 7.8$, $P < 0.001$) and suppressed Astringency ($F = 6.1$, $P < 0.05$) and Bitterness ($F = 7.0$, $P < 0.01$), while L-glutamic acid imparted an Umami taste ($F = 5.0$, $P < 0.05$) at wine-like concentration.

Conclusions: For the first time, these causal experiments showed that amino acids can influence the taste, mouthfeel and flavour of red wine.

Significance of the Study: This work provides insight into a new class of wine compounds of sensory significance that can be targeted by producers to directly influence wine flavour.

Keywords: *amino acids, flavour, mouthfeel, taste*

Introduction

The volatile and non-volatile composition of red wine is relatively well studied from a chemical and sensory point of view. Few studies, however, have examined interactive effects of the odorants, tastants and mouthfeel-related compounds, and how they contribute to flavour experienced when consuming a wine. Most sensory studies have investigated volatiles and non-volatiles in separate investigations. There is a considerable knowledge gap in understanding how volatile–non-volatile interactions change in-mouth sensory properties.

The emergence of 'flavour' in neuroscience terms is thought to result from the central integration of multiple, synchronised sensory inputs including gustatory (taste), olfactory (smell) and oral-somatosensory (touch) signals into an overall unitary perception of a food or beverage (Small and Prescott 2005). In the brain, individual taste and textural qualities localised in the mouth are signalled to the segregated, but overlapping regions in the primary and secondary cortices via the thalamus, and the signals are thought to be integrated in the orbital frontal cortex, with odour signalled directly from the olfactory cortex (Rolls and Baylis 1994, Verhagen et al. 2004, Shepherd 2006, Rolls 2015).

From a volatile perspective, many studies have endeavoured to determine and demonstrate the effect of specific odorants of wine, for example see Tominaga et al. (1998, 2000), Siebert et al. (2008, 2018), Cooke et al. (2009) and Capone et al. (2018). Francis and Newton (2005) suggested that wines of well-regarded quality or provenance often display multiple vivid characteristics and are not usually dominated by one note. Indeed, most recognisable aromas are composed of complex mixtures of volatiles, and it is thought that 3–15 key odour compounds (together with 15–40 non-volatile tastants) and their interactions, contribute the overarching flavour 'signatures' encountered in everyday life (Thomas-Danguin et al. 2014, Hofmann et al. 2018). Volatile–volatile interactions have been studied in a direct way by addition and omission of odorants to wine for example (Culleré et al. 2007, Lytra et al. 2012, 2016) and model wine (Lytra et al. 2013, de-la-Fuente-Blanco et al. 2020), but few have employed formal experimental designs to statistically assess interactions of sensory significance. Attempts have been made to classify the role of common wine volatiles (Ferreira 2010) and the effects of volatile interactions on odour intensity (Ferreira 2012a) and quality (Ferreira 2012b), but limited studies have employed detailed sensory evaluation methods such as quantitative descriptive analysis (QDA) to assess changes in both quality and intensity.

Non-volatile mouthfeel and sapid (with taste) compounds and their contribution to wine sensory properties

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are also well studied as reviewed by Cheynier and Sarni-Manchado (2010), especially phenolic compounds which have been extensively studied and reviewed (Gawel 1998, Sáenz-Navajas et al. 2012, Gawel et al. 2018). Non-volatile compounds contribute to the tastes and mouthfeel properties of wine directly, and by their interactions with other non-volatiles at the chemical, biochemical or cognitive levels. A review of taste–taste interactions suggests the emergence of perceptual phenomena, such as enhancement or suppression, tends to occur at sub and peri-threshold concentration (Keast and Breslin 2003).

Non-volatile phenolic compounds (including anthocyanins and tannins) are found at higher concentration in red wines than in white wines, and contribute strongly to in-mouth properties, particularly astringency and bitterness (Singleton and Trousdale 1992, Oberholster et al. 2009). Studies have demonstrated the ability of tannin compounds to chemically interact with volatile wine constituents through π–π stacking and hydrogen bonding (Jung et al. 2000), involving aromatic phenols (Dufour and Sauvaitre 2000), esters (Muñoz-González et al. 2020, Cameleyre et al. 2021) and methoxypyrazines (Aronson and Ebeler 2004). Although such chemical evidence exists, the extent of the effect of these chemical interactions on the sensory properties of wine is less well studied.

Nitrogenous compounds in wine grapes are routinely measured in relation to yeast fermentation metabolism. They have been studied as precursors for yeast-derived volatile aroma compounds, but there has been scarce study of these compounds from a sensory perspective. Skogerson et al. (2009) reported that a range of nitrogenous compounds, especially proline, was positively associated with increased white wine 'body' and 'viscosity'. Proline has been reported to taste sweet (Van Gemert 2011) and is not normally consumed by yeast during fermentation conditions (Ingledew et al. 1987). In a study of taste-active compounds in a Dornfelder red wine by Hufnagel and Hofmann (2008), L-proline was shown to be a significant contributor. In early viticultural studies, L-proline was found to greatly vary in wines vinified from different red cultivars (Ough and Stashak 1974).

Taste-active amino acids include L-tryptophan, L-tyrosine, L-valine, L-phenylalanine, L-lysine, L-leucine, L-isoleucine and L-histidine, which are described to taste bitter; L-alanine, L-glycine, L-methionine, L-serine and L-proline which can taste sweet; and L-glutamic acid (glutamate) and L-aspartic acid (aspartate) which are responsible for umami taste when deprotonated in some product types (Delompré et al. 2019). Except for L-proline, yeast consumes the vast majority of grape-derived amino acids during fermentation, converting some of them into volatile aroma compounds (Huang and Ough 1991, Bell and Henschke 2005). L-glutamic acid concentration has also been reported in wine (Lehtonen 1996, Gutiérrez-Gamboa et al. 2019) higher than reported sensory threshold values (Van Gemert 2011). Peptides have been less studied in wine, with recent investigations into glutathione (GSH) showing that there can be a large variation in concentration in finished wines (Kritzinger 2012, Kritzinger et al. 2013). Glutathione is known to be a taste enhancer in other products (Dunkel et al. 2007, Miyaki et al. 2015), adding 'kokumi' (mouthfullness), and has been indicated as affecting the aroma of foods such as beef broth (Hong et al. 2010). To the best of our knowledge, the specific sensory contribution of amino acids and GSH to red wine has not been studied.

The importance of cross-modal interactions has been recognised in wine textbooks, for example, Peynaud and Blouin (1996) state, '… the majority of wine's qualities are not a result of a unique constituent present at a particular level, but of a harmony of all its constituents and of certain relative concentrations'. Flavour compound interactions can occur at three levels, chemically between compounds for example, contributing to red wine astringency (Singleton and Trousdale 1992), between wine odorants and biological receptors matching woody and fruity aroma enhancing sensory effects (Chaput et al. 2012), and even at the cognitive level understanding how the brain generates a unitary food and beverage flavour percept from multiple sensory modalities (Small 2008, 2012). Although understanding these interactions is important to explaining wine flavours, few wine sensory studies have been aimed at unravelling multimodal effects in wine, instead research to date has largely focused on identification of 'impact' compounds (Guth 1997a,b, Frank et al. 2011, Benkwitz et al. 2012, Mayr et al. 2014, Rutan et al. 2014). Some studies targeting cross-modal interactions of colour–aroma (Morrot et al. 2001), taste–aroma (Arvisenet et al. 2016), aroma–taste–mouthfeel (Sáenz-Navajas et al. 2010, 2020, Pittari et al. 2020) have been conducted. Only a few, however, have used highly trained QDA panels (de-la-Fuente-Blanco et al. 2017) or have employed formal experimental design approaches providing adequate statistical power to detect interactions of commercial importance; for exceptions see Jones et al. (2008) and Frost et al. (2017).

In the present study, a series of statistically designed QDA experiments was conducted with a sensory-directed approach, with the aim of determining the relative importance of amino acids, volatiles and polyphenolics on in-mouth sensory properties in a red wine system, and to assess the effect of their interactions. Three QDA studies were conducted, summarised in Table 1, to test whether specific amino acids influence red wine sensory properties, either by direct or by an interaction effect with volatiles and other non-volatile components. Discrimination testing was also deployed to gauge the level of the amino acid, proline, detectable in wine. Findings from the studies were further explored by examining experimental and commercial Shiraz wine sets where detailed chemical composition and QDA sensory data were available.

Materials and methods

Shiraz wines

A 2018 vintage commercially produced Shiraz wine was sourced from McLaren Vale, SA, Australia and used in the series of experiments. The detailed composition of the wine is provided in Table S1. A set of 14 commercial Shiraz wines from the 2017 vintage was sourced from a single winery from different vineyards in the Barossa Valley, SA, Australia. Wines were barrel-aged in old oak to minimise oak flavour in the wines, basic chemical composition is provided in Table S2.

Volatile extract (SAFE)

The volatile fraction of the 2018 Shiraz wine was directly distilled using the solvent-assisted flavour evaporation (SAFE) apparatus and technique, modified from Engel et al. (1999). The ethanol present in the Shiraz wine was used as an internal solvent. Room temperature wine was slowly added to the evaporation side of the SAFE apparatus which was held at 25°C. Liquid N_2 was poured into the cooling chamber and around the collection flask which was under vacuum (20–30 kPa). Each 750 mL wine bottle was

Table 1. Summary of experimental parameters and sample information for the series of the sensory tests conducted.

Study	Method	Factors	Experimental design	Factor level type	No. samples	Base medium	Evaluation conditions
1	QDA†	Polyphenolic extract, Shiraz volatile extract, several amino acids/glutathione at concentration matching the Shiraz wine	2^3 full factorial	No addition, addition	Eight model wine samples and the Shiraz wine†	Model wine	Colour masking
2	QDA	L-proline, L-glutamic acid	$4^1 3^1$ full factorial	Four levels of proline, three of glutamic acid	12 Samples	Shiraz wine†	Daylight type
3	QDA	Shiraz volatile extract, L-proline	2^2 full factorial, nose clip/no nose clip evaluation condition	No addition, addition	Four reconstructed samples and the Shiraz wine†	Non-volatile SAFE retentate of Shiraz wine†	Colour masking, with- and without nose clip
4	Duo-trio, Triangle	L-proline	2^1	No addition, addition		Shiraz wine†	Daylight type

†For each study, the Shiraz wine was the same 2018 McLaren Vale Shiraz. QDA, quantitative descriptive analysis; SAFE, solvent-assisted flavour evaporation.

extracted for approximately 2 h, with the extract transferred to sealed glass containers and stored at −21.0°C. The ethanol concentration of the pooled volatile extract was determined to be 41% v/v. The non-volatile retentate was also collected and stored frozen at −21.0°C.

Polyphenolic extract

A commercial liquid grape skin tannin product GSkinEx (Tarac Technologies, Nuriootpa, SA, Australia) was de-aromatised with 25 g/L of XAD-4 food grade resin agitated by magnetic stir bar for 24 h similar to methods described by Ferreira et al. (2002). The extract was filtered using a vacuum pump (Barnant, Barrington, IL, USA) with a glass fibre filter (Millipore) with a pore size of 0.7 μm to remove any resin particles. The tannin of the de-aromatised extract was isolated by solid-phase extraction and characterised by phloroglucinolysis and gel-permeation chromatography (Kassara and Kennedy 2011).

Amino acids and glutathione

Analytical HPLC grade L-proline, L-histidine, L-glutamic acid and GSH of ≥99% purity were obtained from Sigma-Aldrich, Castle Hill, NSW, Australia.

Model wine

A concentrated solution of tartaric acid, succinic acid, lactic acid (85% natural), potassium metabisulfite, glycerol and D-(−)-fructose (Sigma-Aldrich) was prepared in Milli-Q water. This solution was added in 100 mL aliquots to each 250 mL sample to achieve the same final concentration quantified in the 2018 Shiraz wine: pH 3.50, 1.9 g/L tartaric acid, 2.8 g/L lactic acid, 1.7 g/L succinic acid, 0.6 g/L fructose, 11.5 g/L glycerol and 87 mg/L total sulfur dioxide and then supplemented with 95% food safe analytical ethanol (Rowe Scientific, Lonsdale, SA, Australia) to reach a final ethanol concentration of 14.4% v/v.

Chemical analysis

The amino acid and GSH composition of the 2018 Shiraz wine was quantified by the method of Boughton et al. (2011). The sample was derivatised with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and analysed by LC–MS/MS electrospray ionisation (positive). The wine was diluted by a factor of 100 with 0.1% formic acid in Milli-Q water to obtain the appropriate concentration range. The sample constituents were separated with an Agilent 1290SL HPLC coupled to a QQQ 6490A (Agilent Technologies, Santa Clara, CA, USA). Samples were acquired in dynamic multiple reaction monitoring in positive ionisation mode.

The concentration of total anthocyanin and tannin of the polyphenolic extract (PE) was determined following the procedure detailed in Mercurio et al. (2007).

Targeted volatile compounds were quantified by GC/MS or LC/MS stable isotope dilution analyses using previously published methods that are routinely used in-house, as detailed in Siebert et al. (2018) and with recent updates as below. All analytical methods used deuterated analogues as the internal standards, and MS in selected ion monitoring mode or MS/MS with multiple reaction monitoring except one using a GC/sulfur chemiluminescence detector (SCD) which instead used two chemically similar compounds to the analytes (Siebert et al. 2018).

Fermentation-derived aroma compounds analysed by headspace (HS)-solid phase micro-extraction (SPME)-GC/MS as described by Siebert et al. (2005) using 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. (2022) using membrane-assisted solvent extraction-GC/MS on an Agilent 7890B GC, coupled to an Agilent 5977B MS and equipped with a Gerstel MPS Robotic Pro (Lasersan) (Pisaniello et al. 2022). C6 alcohols and aldehydes were determined by HS-SPME-GC/MS as described in Capone et al. (2012) using an Agilent 6890 GC, coupled to an Agilent 5973 MS and equipped with a Gerstel MPS2 (Lasersan). Methoxypyrazines were analysed by HS-SPME-GC/MS as described by Bindon et al. (2013) using an Agilent 6890 GC, coupled to an Agilent 5973 MS, and equipped with a Gerstel MPS2. Oak-derived aroma compound were quantified according to Pollnitz et al. (2004), except 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. (2010) and Cordente et al. (2022) utilising static HS-GC/SCD on an Agilent 7890B GC, coupled to an Agilent 8355 SCD and equipped with a Gerstel MPS2 XL (Lasersan). Polyfunctional thiols were analysed by HPLC/MS/MS after derivatisation and SPE as described by Capone et al. (2015) and Cordente et al. (2022) using an Exion UHPLC coupled to a 6500 QTrap+ (Sciex, Mulgrave, Vic., Australia).

The wines were analysed for their basic composition using a Foss WineScan FT 120 as described by the manufacturer (Foss, Hillerød, Denmark).

Study 1. Evaluation of volatiles, polyphenolics and amino acids in a reconstituted model red wine system

A full factorial design consisting of three factors at two levels (2^3, present and absent) was used, so that eight treatments were prepared. The three factors involved the volatile extract from the Shiraz wine (VE), the de-aromatised commercial PE, and the amino acids L-proline, L-histidine, L-glutamic acid with GSH (AA), added to a red wine-like model wine (250 mL final volume). The Shiraz wine was also included in the sensory evaluation. During the training sessions of the QDA, the volume of the SAFE volatile extract added was adjusted to resemble the Shiraz wine aroma most closely. The volume of volatile extract used (80 mL) corresponded to double strength, that is an amount extracted from twice the volume of the original wine to account for the partial evaporation. The PE (25 mL) was added to give a final concentration of 512 mg/L anthocyanin and a tannin concentration of 1520 mg/L epicatechin equivalents. The AA solution (10 mL) was added to give a final concentration of 931 mg/L L-proline, 6 mg/L L-histidine, 18 mg/L L-glutamic acid and 13 mg/L GSH. These concentration values, which were considered low to moderate relative to those reported in red wines (Gutiérrez-Gamboa et al. 2019), targeted the summed taste activity value of each amino acid by dominant taste (sweet, bitter or umami) using reported taste active thresholds compiled in Van Gemert (2011) quantified in the 2018 Shiraz wine. Following addition of the appropriate volume of ethanol, the pH was adjusted to 3.50 with aqueous potassium hydroxide solution (20% w/v) to match that of the Shiraz wine. The Shiraz wine was also subjected to the same agitation and pH measurement.

Study 2. Discrimination test of added L-proline to the Shiraz wine

A discrimination test was conducted according to the American Society of Materials Testing Duo-Trio Test standard methods for sensory analysis (ASTM E2610-18) (American National Standards Institute 2018). Screened assessors were convened from the AWRI internal difference testing panel. The test was conducted in duplicate, with each assessor receiving two sets of the same samples, randomised, on a single tray. Samples (30 mL) were presented to assessors under normal daylight conditions. The 2018 Shiraz wine (749 mg/L proline) was compared with the same wine with 3000 mg/L added proline achieving a final concentration of 3749 mg/L. Six bottles were homogenised in two 5 L Pyrex flasks, one with anhydrous L-proline added to it, the other without, then both received agitation prior to the test.

Study 3. Addition of L-proline and L-glutamic acid to Shiraz wine

A two-component full factorial design was followed consisting of L-proline at four concentration values (749, 1825, 2900 and 3972 mg/L), and L-glutamic acid at three concentration values (17, 50 and 100 mg/L), spanning the ranges reported in the literature (Gutiérrez-Gamboa et al. 2019), added to the Shiraz wine (a $4^1 3^1$ design). Thus 12 addition permutations, including the Shiraz wine with no addition, were assessed.

Concentrated solutions of L-proline and L-glutamic acid were prepared in Milli-Q water along with a blank water solution. Multiple bottles of the Shiraz wine were mixed together each morning of the sensory evaluation days. Appropriate aliquots of proline and glutamic acid solutions were made up to 250 mL with the wine volumetrically, with Milli-Q water also added in each sample to level the small dilution of the wine arising from the various additions, including the base Shiraz wine. Fresh samples were prepared daily.

Study 4. Reconstruction of Shiraz wine with added L-proline and volatiles, assessed under two conditions

A 2^2 full factorial design was constructed consisting of two concentration values of L-proline (749 and 3749 mg/L) and VE (corresponded to half and single strength), each added to the 2018 Shiraz retentate left over from the modified SAFE process. The frozen retentate was thawed to room temperature overnight and thoroughly mixed before use. An alcohol concentration of 13% v/v was equalised across the treatments. The four sample permutations, with the Shiraz wine, were assessed by the panel as normal and with a nose clip condition (nose clip from Speedo, Sydney, NSW, Australia). The assessors were allocated randomly to one of two groups which were closely balanced for gender (four females and one or two males in each group). These two groups either evaluated the samples normally, followed by nose clip or vice versa in a cross-over-like design.

General methods for sensory analysis

Samples were evaluated in covered, three-digit-coded ISO glasses and presented to assessors in 20 mL aliquots at 22–24°C, in isolated booths. For each study, a randomised monadic presentation order was followed.

A generic descriptive analysis approach was followed for Studies 1, 3 and 4 (Heymann et al. 2014), with initial discussion sessions to agree on attributes, followed by practice

rating and formal evaluation sessions. Panels were convened from the external permanent AWRI trained descriptive analysis panel group, with assessor experience ranging from 18 months to over 10 years of previous involvement in wine sensory descriptive analysis. Given that wine colour has been shown to influence wine odour (Morrot et al. 2001) black glasses and black spittoons were used in Studies 1 and 4. Studies 2 and 3 were conducted with clear glasses in daylight-type light conditions as no colour changes were evident. Samples were evaluated three times on separate days by assessors in a Williams Latin Square random block design provided by Compusense Cloud sensory evaluation software (Compusense, Guelph, ON, Canada). A 60 s rest was enforced between samples, with water given as a palate cleanser, and there was a minimum 10 min rest between sets of three samples, where assessors were requested to leave the booths.

For each of the three QDA studies, a list of attributes was established independently, by each panel, through a consensus discussion process. The terms used for each study are given in Table 2.

The intensity of each attribute was rated using an unstructured 15 cm line scale (0–10), with indented anchor points of 'low' and 'high' placed at 10 and 90%, respectively. Data were acquired using Compusense Cloud sensory evaluation software. Specific details for each of the studies are detailed below.

Following formal data collection, assessor performance was measured using Compusense 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 SD of each assessor by attribute.

Sensory panels. 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. Of the three QDA studies, ten assessors were common to two studies, and three assessors were common across all three QDA studies.

Study 1. Quantitative descriptive analysis. The panel consisted of 12 assessors (11 females, 1 male) with an average age of 50 (SD = 9.4). Assessors attended six 2 h training sessions, with formal data collection over three 2 h sessions.

Study 2. Discrimination test. The panel consisted of 36 assessors (22 females, 14 males) with an average age of 36 (SD = 11.8).

Study 3. Quantitative descriptive analysis. The panel consisted of 12 assessors (10 females, 2 males) with an average age of 48 (SD = 12.6). Assessors attended three training sessions and three formal sessions which were 2 h in duration.

Study 4. Quantitative descriptive analysis. The panel consisted of 11 assessors (8 females, 3 males) with an average age of 55 (SD = 13.0). Assessors attended three training sessions and three formal sessions which were 2 h in duration.

Statistical analysis and interpretation. All QDA data were examined by ANOVA for the effects of sample, assessor, presentation replicate and their two-way interactions, before the ANOVA was re-run for the effects and interactions of interest in each study individually. Principal component analysis (PCA) was used to visualise the sample mean data using the correlation matrix. The factorial designed experiment data sets were explored using design of experiment (DOE) and response surface regression (RSR) and modelling (RSR-M) functions in Minitab 18 (Minitab, Sydney, NSW, Australia). The functions DOE and RSR-M were used to detect and visualise the main and interactive effects of sensory significance.

The duo-trio discrimination test was analysed using XLSTAT (Addinsoft, Paris, France); data analysis to determine the level of statistical evidence was carried out using a Thurstonian binomial model and used the Clopper–Pearson test statistic. Due to the assessment being completed in duplicate with 36 assessors, the approach of Smith (1981) as outlined in Lawless and Heymann (2010) was used to test whether there was significantly more correct choices on one replication, or whether they are not significantly different from each other and could be therefore combined to increase statistical power. This assessment concluded that judgements from both replicates could be combined.

Regarding statistical interpretation of sensory data, consideration was given to the level of statistical evidence (*P*-value), magnitude of effect size (*F*-value) and absolute effect value (mean values) to interpret and draw conclusions about effects of sensory significance (Sullivan and Feinn 2012, Wasserstein et al. 2019), as recommended in the American Statistical Association Statement on *P*-values (Wasserstein and Lazar 2016). Statements ascribing the level of statistical evidence 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' (***).

Results

Attributes for quantitative descriptive analysis

For each of the three QDA studies (summarised in Table 1), a list of attributes was established, by each panel, through a consensus discussion process. The final list of terms, providing an overall description of the samples for each study is given in Table 2. Six attributes were used only in Study 1 including Overall red wine aroma and flavour, Eucalyptus aroma, Nail polish remover/Vinegar aroma, and Smoky aroma and flavour, while the appearance term Opacity was only used in Study 3.

Study 1. Evaluation of volatiles, polyphenolics and amino acids in a reconstituted model red wine system

This investigation aimed to understand the influence of amino acids relative to volatiles and polyphenolics on the sensory properties of model wines. The study used the approach of adding volatiles extracted from a Shiraz wine; adding a purified grape skin de-aromatised polyphenolic extract (PE); and adding several amino acids together, all at a concentration relevant to the commercially produced Shiraz wine. The pH of the model wine and the concentration of acids, alcohol, glycerol and fructose matched that of the Shiraz wine.

To assess whether the PE had a perceptible aroma, an initial aroma-only triangle test, conducted in duplicate,

Table 2. Sensory attributes, definitions and composition of reference standards for the three quantitative descriptive analysis studies.

Study	Attributes	Definitions/synonyms	Standards
Appearance			
3	Opacity	The degree to which light cannot pass through the sample (colour intensity)	Conceptual standard
Aroma			
1	Overall red wine	The intensity of total characteristic wine aroma in the sample	Conceptual standard
All	Red fruit	The intensity of the aromas of strawberries and raspberries	5× Sliced frozen strawberries (46.4 g), 5× frozen raspberries (9.0 g)
All	Dark fruit	The intensity of the aromas of blackberries, blackcurrants and plums	10× Frozen blackberries (18.5 g), 15× frozen blueberries (13.8 g), 4× frozen cherries (27.0 g), 5 mL Ribena Syrup
All	Woody/Spice	The intensity of the aroma including baking spices, oak wood, coconut, vanilla and cedar	1.5 g American oak chips and 1 teaspoon vanilla paste (Queens), ½ tsp. mixed spice, 1 tsp. coconut shavings
1	Eucalyptus	The intensity of the aroma including eucalyptus, 'green' and fresh herbs	3 mL of 1–8 Cineole (0.98 g/L)
1	NPR/vinegar	The intensity of the aroma of nail polish remover and vinegar	1 mL Acetic acid (1000 g/L), 100 µL ethyl acetate (1000 g/L)
3 and 4	Savoury	The intensity of savoury aroma	1× Beef style stock cube (Massel)
All	Drain (reduction)	The intensity of the aroma including dirty drain, LPG (liquid propane gas) and boiled egg	2 g Wood ash mixed fresh each day with wine to release sulfidic aroma
1	Smoky	The intensity of the aroma of smoke, medicinal, barnyard and Band-Aids	150 µL Guaiacol (605.3 mg/L), 100 µL 4-ethyl phenol (1.01 g/L)
All	Pungency	The intensity of the aroma of a warming alcohol sensation	15% v/v Ethanol solution in water (95% ethanol (Rowe Scientific) stored in glass)
3 and 4	Medicinal	The intensity of the aroma of medicinal, barnyard and Band-Aids	100 µL 4-ethyl phenol (1.01 g/L) and 50 µL of 4-ethyl guaiacol (1.06 g/L)
3 and 4	Earthy	The intensity of the aroma of earth and dust	1× Teaspoon of soil with a drop of water
In-mouth			
All	Sourness	The intensity of perceived sour taste	1 g/L L-(+)-Tartaric acid (Chem-Supply) in water
All	Astringency	The intensity of the drying sensation in the mouth	0.5 g/L Aluminium sulfate (Ajax fine Chem Supply in water
All	Bitterness	The intensity of perceived bitter taste	15 mg/L Quinine sulfate (Sigma-Aldrich) in water
All	Viscosity	The perceived thickness and 'weight' of the sample in the mouth	1.5 g/L Carboxymethylcellulose sodium salt (Sigma-Aldrich) in water
All	Hotness	The intensity of the alcohol burning sensation, including aftertaste	15% v/v Ethanol solution in water [95% ethanol (Rowe Scientific) stored in glass]
All	Sweetness	The intensity of perceived sweet taste	5 g/L Table sugar in water
All	Umami/Savoury	The intensity of the perceived umami/savoury taste	0.35 g/L Monosodium glutamate (Ajino Moto)
All	Body	The intensity of perceived overall 'mouthfulness', impression of fullness and mouthfilling	Agreed upon by the judges to be represented by a 'light bodied' McLaren Vale Sangiovese, 'medium bodied' South Australian Shiraz and a 'full bodied' Barossa Valley Shiraz
1	Overall red wine flavour	The intensity of the total perception of characteristic red wine flavours including taste and mouthfeel sensations	Conceptual standard
All	Red fruit	The intensity of the flavour of strawberries and raspberries	Conceptual standard
All	Dark fruit	The intensity of the flavour of blackberries, blackcurrants and plums	Conceptual standard
All	Woody/Spice	The intensity of the flavour including baking spices, oak wood, coconut, vanilla and cedar	Conceptual standard
1	Smoky	The intensity of the flavour of medicinal, barnyard, smoke and Band-Aids	Conceptual standard
3 and 4	Earthy/Dusty	The intensity of the flavour of earth and dust	Conceptual standard

Attributes used in all studies unless otherwise noted. All red wine standards were added to 500 mL of 2019 Yalumba premium selection bag-in-box Shiraz (Angaston, SA, Australia) unless otherwise noted.

showed virtually no evidence that the extract, diluted at the same level as used in the reconstitution QDA study, could be distinguished from an ethanol/water control solution ($n = 11$ assessors × 2 replicate tests, $P = 0.12$, $P = 0.29$). The extract was found to contain 5.13 g/L of anthocyanin and 15.2 g/L of tannin. The commercial 2018 Shiraz wine selected for the reconstitution study had an anthocyanin concentration of 0.49 and 1.54 g/L of tannin. The ratio of anthocyanin to tannin was similar for the phenolic extract (0.33) and the commercial Shiraz wine (0.32), which was also close to the average found for the analysis of >200 Shiraz wine samples (0.34 ± 0.13) (Table S3). Tannin molecular mass, mean degree of polymerisation and the proportion of prodelphinidin were comparable between the phenolic extract and the wines included in the survey, but the degree of galloylation was lower. Since most of the galloylated tannins in red wine are derived from grape seeds (Peyrot des Gachons and Kennedy 2003), this supports that the extract was derived from grape skins.

The sensory attribute differences among the eight factorially designed model wines (Table 1) compared to the Shiraz wine were evaluated with an ANOVA that was

calculated evaluating the attribute ratings across the nine samples, accounting for the assessor and replicate effects and their interactions (Table S4). Sample means are shown in Table S5. Inspection of the mean data showed that, as expected, many aroma and flavour attributes primarily contributed by volatiles were strongly positively correlated with each other (Table S6), with the broad attributes Overall red wine aroma and flavour closely correlated with Red fruit aroma and flavour, Dark fruit aroma and flavour, Woody and Eucalyptus ($r > 0.91$). Smoky aroma and flavour were both strongly negatively correlated with Overall red wine aroma and flavour ($r < -0.85$).

The PCA of the mean data for the nine samples is shown in Figure 1, with 94% of the variance accounted for. The attributes highly positively correlated with Overall red wine flavour and aroma are not shown, to simplify the interpretation. Figure 1 shows that samples with added volatiles, and the Shiraz wine, are separated along PC1, and were rated highly for the attributes Overall red wine aroma and flavour, Sweetness, Body and Viscosity, which were strongly negatively loaded on the first PC. Conversely, samples plotted to the right of the figure, without added volatiles, were rated low in these attributes and highly for Umami/Savouriness, Pungency, Sourness and Smoky flavour. The vertical separation of the samples along PC2 was determined by addition of polyphenols, with Astringency and to a lesser extent Bitterness and Sourness, attributes rated highly for the Shiraz wine and the samples with added polyphenols, while samples with polyphenols absent rated higher in Hotness. The influence of the several amino acids and GSH was more subtle, with an effect of AA increasing Body, Sweetness and Viscosity ratings for the added volatiles with no polyphenolics sample, while increasing Bitterness for the no added volatiles with polyphenolics sample (Table S5). Thus the sensory properties of the Shiraz wine, rated highest in attributes negatively loaded on PC1, were most similar to the sample with added volatiles and added AA, with the addition of polyphenolics to the model increasing the astringency rating but lowering the Body, Viscosity and Sweetness ratings (Table S5).

A subsequent ANOVA assessing the effect of the compositional factors and their interactions provided clear evidence regarding the effect of the components. As seen in Tables 3 and 4 the addition of the volatile extract was highly significant, enhancing all the aroma and flavour attributes except for Nail polish remover/vinegar and Drain (sulfidic, reductive) aromas, which were not significantly influenced by any factor and had a low intensity. Neither of the other factors or their interactions influenced the aroma and flavour attributes. Surprisingly, very strong evidence showed that volatiles contributed to increased intensity of the taste and mouthfeel attributes Sweetness, Body and Viscosity. Very strong evidence was also found that the polyphenolics increased Astringency, Sourness and Bitterness but the *F*-ratios for the latter two attributes were relatively small. Very strong evidence was uncovered indicating that Sweetness was suppressed with added polyphenolics, while some evidence also indicated suppression of Hotness. Virtually, no evidence was found that polyphenolics influenced Body or Viscosity. No evidence was found that the addition of amino acids resulted in any direct effects, but evidence of a moderate effect size was found for a volatile by amino acid interaction effect for Bitterness. Inspection of the interaction plot (Figure 2) revealed that in the absence of volatiles, amino acids increased the Bitterness rating, while amino acids and volatiles combined significantly lowered Bitterness.

Study 2. Discrimination test of added L-proline to the Shiraz wine

Duo-trio discrimination testing was used to further assess the effect of L-proline, at a concentration of 3 g/L, added to

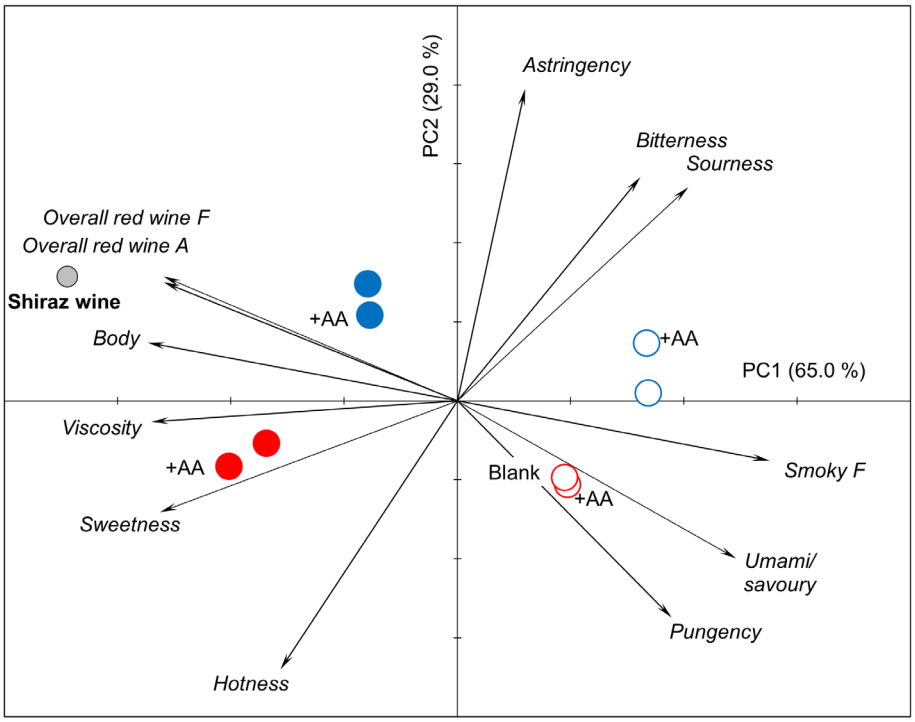

Figure 1. Principal component (PC) biplot of taste and mouthfeel sensory attributes as well as overall red wine aroma (A), flavour (F) and smoky flavour for the eight Shiraz reconstitutions and the target Shiraz wine as a supplementary sample (◉) showing the influence of added volatiles, polyphenolics and amino acids. Samples with volatiles 'present' shaded (●, ●). Polyphenolics 'present' coloured blue (●, ○) and 'absent' coloured red (●, ○). Added amino acids and glutathione indicated as +AA.

Table 3. Results of the factorial ANOVA for Study 1: *F*-ratios for main effects, two-way and three-way interactions, probability values, degrees of freedom and mean square error.

	Main effects			Interactions				
Attributes	**VE**	**PE**	**AAs**	**VE × PE**	**VE × AAs**	**PE × AAs**	**VE × PE × AAs**	**Error**
Overall red wine A	150.63***	0.50	0.16	0.88	0.03	0.08	0.50	1.055
Red fruit A	116.86***	0.51	0.44	0.53	0.28	0.25	0.29	1.421
Dark fruit A	119.02***	0.01	0.21	0.75	0.08	0.01	0.54	1.210
Woody A	68.52***	0.22	2.31	0.19	0.38	1.07	0.60	0.598
Eucalyptus A	60.86***	1.79	0.36	0.01	0.06	0.03	0.46	0.332
NPR/Vinegar A	1.76	0.04	0.69	0.01	1.29	0.50	0.16	1.059
Drain A	0.55	0.03	1.18	0.01	0.29	0.01	0.80	0.491
Smoky	67.17***	2.01	0.31	1.05	0.71	0.01	0.92	1.294
Pungency	2.92ǂ	0.39	0.03	0.19	0.38	0.01	0.34	0.915
Sourness	3.56ǂ	11.67***	0.40	0.26	0.07	0.01	0.09	0.418
Astringency	2.4	170.47***	0.01	0.07	0.02	0.35	1.25	0.632
Bitterness	1.89	7.34**	0.00	0.71	4.19*	0.51	0.96	0.305
Viscosity	20.01***	3.94ǂ	0.14	0.01	0.48	0.10	1.44	0.239
Hotness	0.39	4.83*	0.09	0.12	0.00	0.09	0.04	0.492
Sweetness	26.53***	16.52***	0.68	0.90	0.15	0.55	0.29	0.410
Umami	37.56***	0.66	0.02	0.12	0.19	0.41	0.74	1.584
Body	81.38***	1.11	0.92	1.22	0.20	0.25	0.16	0.376
Overall red wine F	109.07***	0.52	0.03	0.47	0.09	0.20	0.03	0.905
Red fruit F	71.68***	0.14	0.21	0.76	0.00	0.09	0.18	1.033
Dark fruit F	113.78***	0.02	0.21	0.44	0.04	0.06	0.00	0.786
Woody F	54.86***	0.18	1.41	0.07	0.68	0.94	0.00	0.659
Smoky F	73.47***	1.67	0.40	0.00	0.03	0.06	0.07	0.753
df	1	1	1	1	1	1	1	77

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ǂ, $P < 0.10$; assessor effects were significant for all attributes ($P < 0.005$). A, aroma; AAs, solution of L-histidine, L-proline, L-glutamic acid and glutathione; df, degrees of freedom; F, flavour; NPR, nail polish remover; PE, de-aromatised polyphenolic extract; VE, solvent-assisted flavour evaporation extract.

Table 4. Mean ratings and SE of the sensory attributes rated for the Shiraz wine and the reconstitutions by factor for Study 1.

	Shiraz wine		Volatiles				Polyphenolics				Amino acids			
Attribute	**Mean**	**SE**	**Absent**	**SE**	**Present**	**SE**	**Absent**	**SE**	**Present**	**SE**	**Absent**	**SE**	**Present**	**SE**
Overall red wine A	5.2	0.3	**1.3**	0.1	**3.9**	0.2	2.5	0.2	2.7	0.2	2.6	0.2	2.5	0.2
Red fruit A	4.0	0.3	**0.5**	0.1	**3.1**	0.2	1.7	0.2	1.9	0.2	1.9	0.2	1.7	0.2
Dark fruit A	4.6	0.3	**0.4**	0.1	**2.9**	0.2	1.6	0.2	1.6	0.2	1.7	0.2	1.6	0.2
Woody A	4.6	0.2	**1.0**	0.1	**2.3**	0.1	1.6	0.1	1.7	0.1	1.8	0.1	1.5	0.1
Eucalyptus A	1.2	0.3	**0.3**	0.1	**1.2**	0.1	0.7	0.1	0.8	0.1	0.8	0.1	0.7	0.1
NPR/Vinegar A	0.4	0.2	1.2	0.1	1.5	0.1	1.4	0.1	1.4	0.1	1.3	0.1	1.5	0.1
Drain A	0.3	0.1	0.9	0.1	0.8	0.1	0.9	0.1	0.9	0.1	0.8	0.1	0.9	0.1
Smoky	0.9	0.2	**2.6**	0.2	**0.7**	0.1	1.5	0.2	1.8	0.2	1.7	0.2	1.6	0.2
Pungency	4.6	0.2	**5.6**	0.2	**5.2**	0.1	5.5	0.1	5.4	0.2	5.4	0.1	5.4	0.1
Sourness	4.5	0.3	**5.4**	0.2	**5.2**	0.2	**5.1**	0.2	**5.5**	0.2	5.4	0.2	5.3	0.2
Astringency	5.0	0.2	4.6	0.2	4.8	0.2	**3.7**	0.1	**5.8**	0.1	4.7	0.2	4.7	0.2
Bitterness	2.8	0.3	3.1	0.1	2.9	0.1	**2.8**	0.1	**3.1**	0.1	3.0	0.1	3.0	0.1
Viscosity	4.4	0.2	**3.3**	0.1	**3.7**	0.1	**3.6**	0.1	**3.4**	0.1	3.5	0.1	3.5	0.1
Hotness	4.9	0.2	5.6	0.1	5.7	0.1	**5.8**	0.1	**5.5**	0.1	5.6	0.1	5.6	0.1
Sweetness	2.2	0.2	**0.7**	0.1	**1.3**	0.1	**1.3**	0.1	**0.7**	0.1	0.9	0.1	1.0	0.1
Umami/ Savoury	0.8	0.2	**2.6**	0.2	**1.0**	0.1	1.9	0.2	1.7	0.2	1.8	0.2	1.8	0.2
Body	5.0	0.2	**2.3**	0.1	**3.5**	0.1	3.0	0.1	2.8	0.1	2.8	0.1	3.0	0.1
Overall red wine F	5.5	0.3	**1.7**	0.1	**3.7**	0.2	2.6	0.2	2.8	0.2	2.7	0.2	2.7	0.2
Red fruit F	4.0	0.3	**0.9**	0.1	**2.7**	0.2	1.9	0.2	1.8	0.1	1.8	0.2	1.9	0.2
Dark fruit F	4.9	0.3	**0.9**	0.1	**2.8**	0.2	1.8	0.2	1.9	0.2	1.8	0.2	1.9	0.2
Woody F	4.5	0.2	**1.1**	0.1	**2.3**	0.1	1.6	0.1	1.7	0.1	1.8	0.1	1.6	0.1
Smoky F	0.6	0.2	**2.3**	0.1	**0.8**	0.1	1.5	0.1	1.7	0.1	1.6	0.1	1.5	0.1

Mean values that were significantly different from the factorial ANOVA for each factor are in bold. A, aroma; F, flavour; NPR, nail polish remover.

the Shiraz wine which had a base concentration of proline of 0.75 g/L (Table S1). This concentration was chosen based on data from a small survey of commercially produced Shiraz wines and from data previously reported (Ough and Stashak 1974), with 3 g/L considered a commonly observed value for red wines. The test showed a P value of 0.022 (72

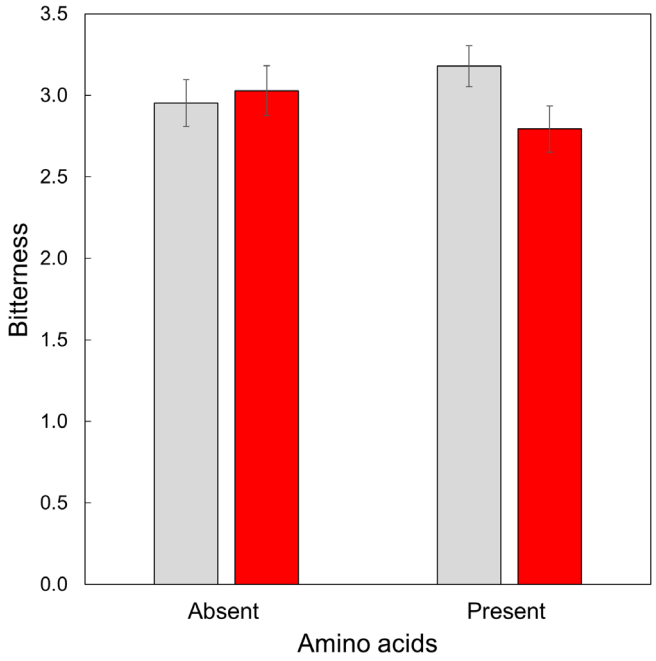

Figure 2. Mean bitterness rating for the significant volatile by amino acid interaction effect from Study 1 ANOVA. Volatile extract 'present' (■) and 'absent' (□). Error bars are ±1 SE.

responses, 45 correct responses, power 0.646, d-prime 1.27) providing evidence of a clear sensory effect of proline in red wine at this concentration.

Study 3. Sensory effect of the addition of L-proline and L-glutamic acid to Shiraz wine

Following from Study 1 and the discernible effect of L-proline detected by the discrimination test (Study 2), the amino acids L-histamine, L-proline and L-glutamic acid and GSH were added to the Shiraz wine at several concentration values and assessed in a sensory-guided approach using a small sensory panel. From these assessments, it was indicated that L-histamine and GSH had only a slight sensory effect, even at the highest concentration previously reported in red wine. A further study tested L-proline and L-glutamic acid at higher concentration values than that measured in the 2018 Shiraz wine, at a concentration range previously found in red wines (Huang and Ough 1991, Gutiérrez-Gamboa et al. 2019).

L-proline at four concentration values and L-glutamic acid at three concentration values and their combinations were added to the Shiraz wine (Table 1) targeting sub-, peri- and supra-threshold values reported in water (Van Gemert 2011) and within the reported concentration range of red wines (Huang and Ough 1991, Gutiérrez-Gamboa et al. 2019). Again, to assess the sensory attribute differences among the 12 factorially designed wines, including the Shiraz, an ANOVA was conducted, accounting for the assessor and replicate effects and their interactions (Table S7). Following QDA and from the response surface modelling (Table 5) and inspection of mean values (Table S8), no evidence was found that amino acids influenced fruity or woody aroma attributes in the wine. Some evidence indicated that glutamic acid slightly suppressed a low-intensity Savoury aroma and weaker evidence was found that proline suppressed the intensity of Drain (reductive off-odour term) aroma, but the mean intensity scores were low.

Of the changes of in-mouth attributes evidenced by the RSR-M in Table 5 and visualised by Figure 3, very strong evidence highlighted that proline increased Sweetness and Red fruit flavour, with the size of these effects, as indicated by the *F*-ratios, the largest of the study. These two attributes

Table 5. Results of the response surface regression ANOVA for amino acid addition Study 3: *F*-ratios for main effects, quadratic and two-way interaction effects, probability values, degrees of freedom and mean square error.

	Probability values					
	Main effects		Quadratic effects		Two-way interaction	
Attributes	Pro	Glu	Pro × Pro	Glu × Glu	Pro × Glu	MSE
Opacity	1.35	0.05	0.86	0.21	0.09	0.103
Dark fruit A	0.03	0.89	1.16	0.03	1.30	0.320
Red fruit A	0.05	1.29	1.16	1.97	0.29	0.832
Woody A	1.11	0.61	0.42	0.61	0.00	0.620
Savoury A	0.32	5.38*	1.20	0.10	1.47	0.497
Drain A	3.19‡	0.19	0.11	0.76	0.25	0.807
Medicinal A	0.00	0.08	1.98	0.01	1.98	0.631
Earthy A	0.01	1.78	1.13	0.93	0.36	0.425
Pungency	1.31	0.07	0.60	0.00	0.61	0.234
Sourness	2.54	0.69	0.00	0.12	2.15	0.198
Sweetness	14.35***	1.14	0.17	3.44‡	0.70	0.451
Astringency	6.14*	3.46‡	2.18	1.64	0.17	0.302
Bitterness	6.97**	0.17	0.33	0.02	0.00	0.352
Umami	0.37	5.24*	3.12‡	1.11	0.81	0.290
Viscosity	5.02*	2.43	1.62	2.93‡	0.00	0.136
Hotness	0.48	0.04	0.17	0.48	3.84‡	0.170
Body	2.47	0.00	0.00	0.14	0.57	0.157
Dark fruit F	0.51	0.03	0.01	0.03	0.16	0.246
Red fruit F	7.76***	0.20	1.25	3.73‡	0.14	0.453
Woody F	2.80‡	1.06	0.06	1.46	3.50‡	0.303
Earthy F	2.24	0.02	0.18	0.82	1.35	0.367
df	1	1	1	1	1	127

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ‡, $P < 0.10$; model and block (assessor) effects were significant for all attributes ($P < 0.005$). A, aroma; df, degrees of freedom; F, flavour; Glu, L-glutamic acid; MSE, mean square error; Pro, L-proline.

were correlated ($r = 0.78$, CI = 0.38, 0.94, $P < 0.01$). Strong evidence was also found that L-proline suppressed Bitterness. Evidence also supported decreased Astringency and increased Viscous mouthfeel with added L-proline. The addition of L-glutamic acid increased Umami/Savoury taste, with a moderate effect size. Weak evidence supported a small effect of proline to increase Woody flavour, and glutamic acid to modestly decrease Astringency. Weak quadratic effects of L-glutamic acid were found to slightly influence Sweetness, Red fruit flavour and Viscosity, while a quadratic effect for L-proline indicated an augmented Umami/Savoury taste at intermediate concentration. These relatively subtle effects are apparent in the convex or concave planes in Figure 3. Weak evidence pointed to interactive effects to alter Hotness and Woody flavour for both amino acids. Mean values for the study samples can be found in Table S8.

Study 4. Reconstruction of Shiraz wine with added L-proline and volatiles, assessed under two conditions

For the fourth sensory investigation (Table 1), the origin of the observed proline and volatile interactions were further tested, using the volatile extract added to the SAFE retentate at two levels, providing a means of assessing the volatile and non-volatile fractions, with proline added at 749 mg/L and 3749 mg/L, and the Shiraz wine with no additions also evaluated. The 'high' level of the volatile extract was half that used in Study 1. Sensory data were collected under two conditions (Table 6), where assessors wore a nose clip to obstruct their sense of smell and did not rate orthonasal attributes, and also evaluated the samples normally, including aroma attributes, using a crossover study design. Tables S9 and S10 show the ANOVAs for assessing the effect of the samples, assessors and replicates, under normal and the nose clip condition, respectively. Mean sensory ratings of each sample for each condition are also displayed (Table S11), along with basic chemical composition of a batch of the five samples (Table S12).

Mean values by factor are shown in Table 7, together with the mean data for the Shiraz wine with no addition. For both conditions, there was only a negligible effect of the volatile extract on the sensory attributes, with evidence that the volatiles at the higher level decreased Woody/Spice aroma in the nose-clip off (normal) condition. The addition of the volatile extract increased aroma and flavour attribute ratings in the normal condition compared to the base wine (Table 7), but differences between the volatile extract levels were minimal. Some evidence was found supporting a volatile extract by L-proline interaction effect, with the high L-proline addition decreasing Dark fruit aroma at low volatile concentration but increasing the aroma in the high volatile samples.

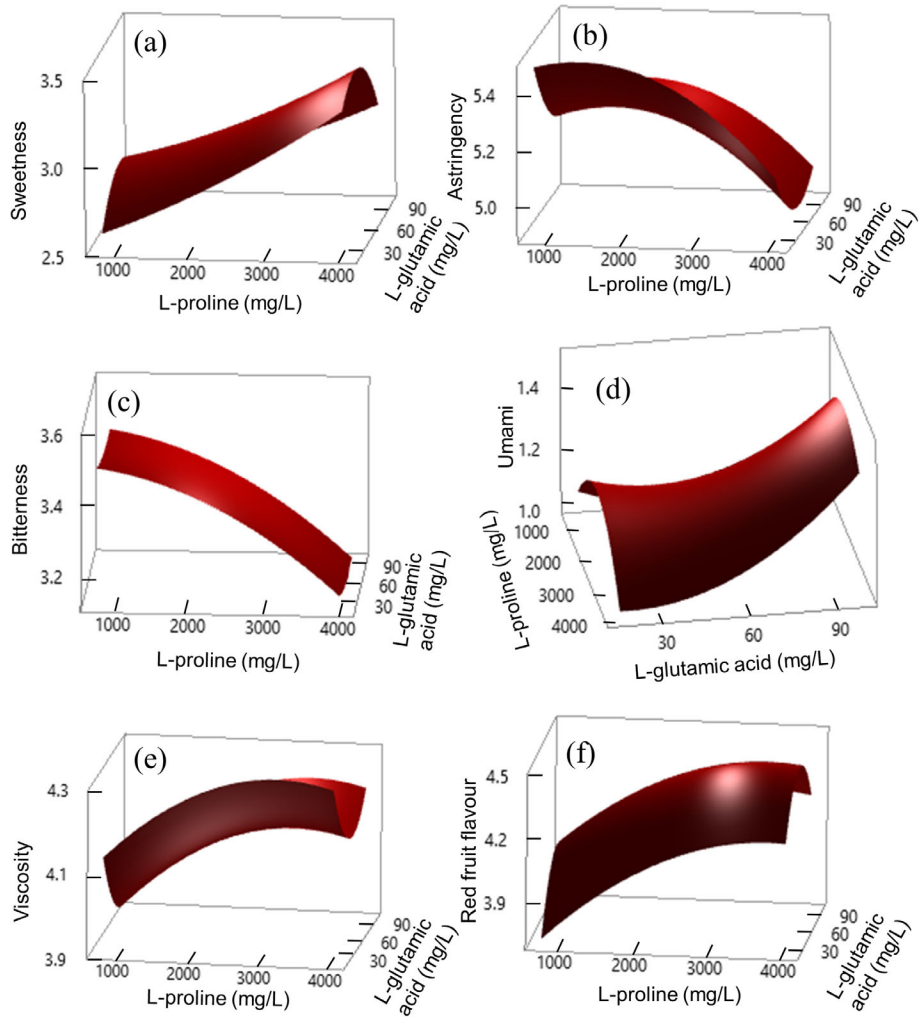

Figure 3. Response surfaces relating the intensity of in-mouth sensory properties: (a) Sweetness, (b) Astringency, (c) Bitterness, (d) Umami (Savoury), (e) Viscosity and (f) Red fruit flavour to the concentration of added L-proline and L-glutamic acid in Shiraz wine from Study 3.

Table 6. Results of the ANOVA from the reconstruction Study 4: *F*-ratios for main effects and two-way interaction effects, probability values, degrees of freedom and mean square error, assessing the effect of two levels of volatile extract and L-proline added to a Shiraz wine solvent-assisted flavour evaporation retentate, assessed with or without a nose clip.

	Assessment condition							
	Nose-clip off (normal)				Nose-clip on			
Attribute	VE	Pro	VE × Pro	MSE	VE	Pro	VE × Pro	MSE
Dark fruit A	0.06	0.43	6.24*	0.816	–	–	–	–
Red fruit A	0.01	0.16	0.92	1.619	–	–	–	–
Woody/Spice A	4.78*	2.36	0.00	0.495	–	–	–	–
Savoury A	3.91‡	0.38	0.00	0.373	–	–	–	–
Drain A	2.90‡	0.01	0.68	1.058	–	–	–	–
Medicinal A	2.18	0.00	0.01	0.657	–	–	–	–
Earthy/Dusty A	0.08	0.39	0.13	0.559	–	–	–	–
Pungency A	0.32	1.28	0.29	0.283	–	–	–	–
Sourness T	1.16	0.18	14.53***	0.175	0.60	2.58	2.09	0.178
Sweetness T	0.20	13.70***	0.01	0.522	0.00	12.25***	0.99	0.511
Astringency MF	1.04	0.11	0.12	0.324	0.35	7.58**	2.05	0.218
Bitterness T	0.78	4.16*	0.34	0.219	0.09	3.91‡	0.75	0.424
Umami/Savoury T	0.07	0.02	3.94‡	0.564	1.50	0.00	0.72	0.336
Viscosity MF	2.30	1.65	0.04	0.191	0.22	0.02	0.05	0.095
Hotness MF	0.81	0.25	0.14	0.140	2.34	12.82***	1.07	0.162
Body	0.29	0.61	0.06	0.154	0.34	0.01	0.12	0.150
Dark fruit F	0.31	0.01	2.49	0.261	3.34‡	3.43‡	1.98	0.108
Red fruit F	0.32	3.37‡	0.11	0.907	0.22	1.90	0.84	0.167
Woody/Spice F	0.00	0.18	0.10	0.362	1.69	0.11	0.17	0.162
Earthy/Dusty F	0.97	2.59	1.07	0.181	0.62	8.48**	0.02	0.151
df	1	1	1	30	1	1	1	30

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ‡, $P < 0.10$; assessor effects were significant for all attributes ($P < 0.005$). A, aroma; df, degrees of freedom; F, flavour; MF, mouthfeel; MSE, mean square error; Pro, L-proline; T, taste; VE, solvent-assisted flavour evaporation extract.

There was very strong evidence under both evaluation conditions that added proline increased Sweetness while weaker evidence supported decreased Bitterness. There was very weak evidence that both volatile extract and L-proline may have had an effect of increasing Viscosity, with this effect, if real, not evident in the nose clip condition. In the nose-clip condition, strong evidence was found for added L-proline to decrease Astringent mouthfeel and Hotness, with these effects not evident when assessed normally.

A volatile extract × L-proline interaction effect for Sourness was supported by very strong evidence for the normal condition, resulting in an increased rating when both the volatile extract and L-proline were low, while increasing the amount of volatile extract suppressed Sourness in the low L-proline condition but not when more L-proline was present. Weak evidence was found supporting an interaction influencing Umami/Savoury taste, with high L-proline and high volatile extract decreasing Umami/Savoury, and low volatile extract and high L-proline increasing Umami/Savoury taste. Surprisingly, weak evidence was found for both an increased level of the volatile extract and L-proline to increase Dark fruit flavour in the nose clip condition despite the assessors' sense of smell being blocked. Weak evidence supported added L-proline increasing Red fruit flavour in the normal assessment but not in the nose clip condition. Finally, strong evidence was found for L-proline to suppress Earthy/Dusty flavour in the nose-clip condition.

Relationship of proline with in-mouth sensory properties in additional Shiraz wines. Two recent published studies (Teng et al. 2020, Bekker et al. 2021) reported the results of QDA and gave amino acid concentration values. In addition, a set of commercially produced Barossa Valley South Australia Shiraz from an unpublished study was examined. Partial least squares regression modelling—commonly used to identify associations of chemical composition with wine sensory properties—found significant and high regression coefficients relating L-proline concentration with Viscosity and fruit flavour attributes in one set (Teng et al. 2020). Figure 4a shows mean Viscosity data plotted against L-proline concentration for these 36 wines with very strong evidence ($P < 0.0001$) found of a linear regression relationship. Other evidence for a positive association between L-proline concentration and Viscosity was found in commercially produced small lot Shiraz wines (Figure 4b), but only when highly 'reductive' wines also high in 'brown colour' were excluded. No evidence was found for a relationship between L-proline and wine Viscosity when wine L-proline concentration was below 1 g/L (Figure 4c).

Discussion

Influence of volatiles

As expected, the addition of volatiles in Studies 1 and 4 increased the intensity of almost all aroma attributes, particularly fruity wine attributes. The only exceptions were the decreased rating of Smoky aroma and Pungency in Study 1 and Woody aroma in Study 4. Such a decrease is likely due to the suppression exerted by aroma compounds in the volatile extract acting on the Pungency of ethanol and low Smoky aroma in Study 1 and a slight woody note of the retentate from the SAFE process. The masking effects from volatile–volatile interactions are common and well documented, such as those demonstrated for woody/fruity wine odorants (Atanasova et al. 2005).

Evidence was found in Study 1 that addition of volatiles strongly influenced in-mouth attributes of a model wine, attributes commonly considered to be directed mainly by non-volatile compounds, notably perceived Sweetness, Viscosity and Body, while suppressing Sourness in Study 1. A

Table 7. Study 4 mean intensity values and SE of the sensory attributes rated for the Shiraz wine and volatile extract and L-proline by factor.

	Nose clip off (normal)										Nose clip on									
	Wine		Volatile extract				L-proline				Wine		Volatile extract				L-proline			
	Shiraz	SE	Low	SE	High	SE	Low	SE	High	SE	Shiraz	SE	Low	SE	High	SE	Low	SE	High	SE
Dark fruit A	4.1	0.3	4.4	0.4	4.3	0.3	**4.4**	0.3	**4.3**	0.3	–	–	–	–	–	–	–	–	–	–
Red fruit A	2.5	0.3	2.9	0.3	2.8	0.3	2.9	0.3	2.8	0.3	–	–	–	–	–	–	–	–	–	–
Woody/Spice A	2.6	0.2	**3.3**	0.2	**2.9**	0.2	3.3	0.2	3.0	0.2	–	–	–	–	–	–	–	–	–	–
Savoury A	0.8	0.3	**0.9**	0.2	**0.6**	0.1	0.7	0.2	0.8	0.2	–	–	–	–	–	–	–	–	–	–
Drain A	2.0	0.4	**0.9**	0.2	**1.4**	0.3	1.1	0.3	1.1	0.3	–	–	–	–	–	–	–	–	–	–
Medicinal A	1.5	0.3	0.5	0.1	0.9	0.2	0.7	0.2	0.7	0.2	–	–	–	–	–	–	–	–	–	–
Earthy/Dusty A	1.5	0.3	1.7	0.2	1.7	0.2	1.8	0.2	1.6	0.2	–	–	–	–	–	–	–	–	–	–
Pungency A	4.2	0.2	3.9	0.3	4.0	0.3	4.0	0.3	3.8	0.3	–	–	–	–	–	–	–	–	–	–
Sourness T	4.6	0.2	4.5	0.2	4.4	0.3	4.5	0.2	4.4	0.2	4.5	0.2	4.5	0.2	4.4	0.2	4.6	0.2	4.4	0.2
Sweetness T	2.3	0.2	2.5	0.2	2.6	0.3	**2.2**	0.2	**3.0**	0.2	2.5	0.3	2.3	0.2	2.3	0.2	**1.9**	0.2	**2.7**	0.2
Astringency MF	5.5	0.3	5.6	0.2	5.4	0.3	5.5	0.3	5.4	0.3	5.1	0.3	5.4	0.2	5.3	0.2	**5.5**	0.2	**5.2**	0.2
Bitterness	2.6	0.3	2.3	0.3	2.4	0.3	**2.5**	0.3	**2.2**	0.3	1.9	0.3	2.2	0.2	2.2	0.2	**2.4**	0.2	**2.0**	0.2
Umami/Savoury T	1.0	0.3	1.3	0.2	1.2	0.2	1.3	0.2	1.2	0.2	0.9	0.2	0.9	0.2	1.2	0.2	1.1	0.2	1.1	0.2
Viscosity MF	4.1	0.2	4.0	0.3	4.2	0.2	4.0	0.3	4.2	0.2	4.1	0.2	4.0	0.2	4.0	0.2	4.0	0.2	4.0	0.2
Hotness MF	4.5	0.4	3.9	0.4	3.8	0.4	3.8	0.4	3.9	0.4	4.0	0.3	3.6	0.2	3.8	0.2	**3.9**	0.2	**3.5**	0.2
Body	4.2	0.2	4.2	0.2	4.2	0.2	4.2	0.2	4.3	0.2	4.1	0.2	4.1	0.1	4.0	0.2	4.0	0.1	4.0	0.2
Dark fruit F	4.3	0.2	4.5	0.3	4.4	0.2	4.5	0.3	4.5	0.3	1.4	0.3	**1.3**	0.2	**1.5**	0.2	**1.3**	0.2	**1.5**	0.2
Red fruit F	2.6	0.3	3.0	0.3	2.9	0.3	**2.7**	0.3	**3.2**	0.3	0.9	0.3	0.8	0.2	0.8	0.1	0.7	0.1	0.9	0.2
Woody/Spice F	3.2	0.2	3.3	0.2	3.3	0.2	3.3	0.2	3.3	0.2	1.2	0.2	0.9	0.1	1.1	0.2	1.0	0.2	1.0	0.2
Earthy/Dusty F	1.2	0.3	1.1	0.3	1.2	0.2	1.2	0.2	1.0	0.3	0.7	0.2	0.7	0.1	0.6	0.1	**0.8**	0.1	**0.5**	0.1

Main effects supported by statistical evidence from factorial ANOVA are indicated in bold. A, aroma; F, flavour; MF, mouthfeel; T, taste.

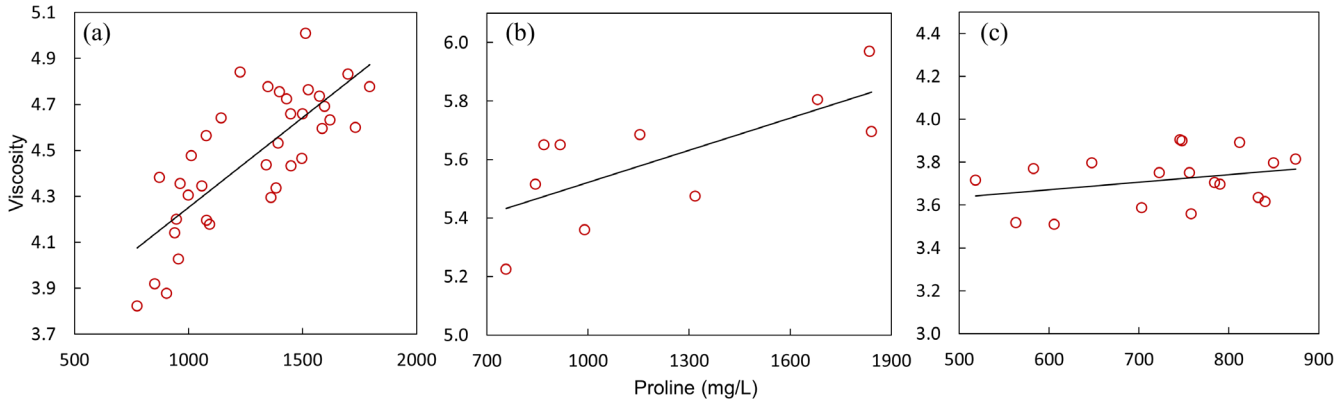

Figure 4. Relationship between proline concentration and rated viscosity of experimental and commercial Shiraz wines by quantitative descriptive analysis (QDA) panel. (a) Experimental wines investigating ripeness and amelioration approaches from Teng et al. (2020) ($R^2 = 0.5913$, $F = 49.19$, $P < 0.0001$). (b) Commercially produced Shiraz small lot wines from the Barossa valley 2017 vintage ($R^2 = 0.5151$, $F = 8.50$, $P = 0.019$) (Dr Karen Bindon, unpubl. data, 2018). (c) Experimental wines investigating strategies to mitigate 'reductive' fermentations in Shiraz ($R^2 = 0.0885$, $F = 1.55$, $P = 0.231$) (Bekker et al. 2021).

similar effect of volatile extracts increasing 'sweetness' rated in white wine reconstitution has been demonstrated (Sáenz-Navajas et al. 2010), but the effect was not evident in red wine systems in that study. A recent comparison, however, of 74 Italian wines assessed normally and after dearomatising also found evidence that olfactory cues modulated the perception of astringent subqualities, 'sweetness' and 'bitterness' (Pittari et al. 2020). Other evidence in Chardonnay wines also supports the influence of volatiles on mouthfeel attributes (Sereni et al. 2016). Recently the finding of functioning olfactory receptors on human taste cells (Malik et al. 2019) has renewed interest in the possibility of volatiles influencing tastes directly through possible changes in signalling. More likely, however, is their indirect influence through cognitive cross-modal effects on congruent tastes and mouthfeel sensations—a phenomenon investigated in Study 4.

In Study 4, which tested the volatile by L-proline interaction further in a red wine base, and with more panel training, evidence of the influence of volatiles on in-mouth sensory properties declined or disappeared. This diminishment of in-mouth enhancement might have been due to the lower amount of volatile extract added in Study 3 compared to Study 1. It is therefore unlikely that wine volatiles influence taste or mouthfeel directly in wine. Instead, the studies presented here add to the growing evidence that volatiles play an important role in wine and other products by modulating perceptually congruent tastes and mouthfeel properties likely through strong, learnt cognitive associations. These types of cross-modal interactions might be diminished with an increasing level of descriptive panel training (de-la-Fuente-Blanco et al. 2017), or pre-exposure to samples (Stevenson and Case 2003). Despite that the source of these interactions is most likely cognitive in origin, these findings nevertheless emphasise the importance of volatile compounds to wine in-mouth sensory properties. Better understanding of these cross-modal interactions and congruent odour–taste–mouthfeel combinations would improve the ability of winemakers to achieve desired flavours and styles.

Influence of phenolics

Red wine polyphenolic compounds are commonly considered as major contributors to the in-mouth sensory properties and colour of red wines (Cheynier and Sarni-Manchado 2010). We hypothesised that the combination of the volatile extract and the non-volatile phenolic extracts in Study 1 would interact to mimic the sensory experience of the target Shiraz wine. In particular, the PE was anticipated to contribute positively to attributes such as Overall red wine flavour, Viscosity and Body. No evidence, however, was found in Study 1 for the phenolic extract to increase these attributes. Rather, the extract was simply observed to increase the perceived Astringency, Bitterness and Sourness of the model wine, and to instead suppress Viscosity and Sweetness. This result is in agreement with other reports investigating the role of phenolics in red wine (Arnold et al. 1980, Fischer and Noble 1994, Noble 1994). Also notable was the relatively high Astringency and Bitterness scores of the model wine without the contribution of the PE, suggesting that the influence of organic acids, pH and ethanol might currently be underestimated as contributors to Astringency and Bitterness in red wines, although organic acids have been determined to have astringent qualities (Sowalsky and Noble 1998). These compositional factors have been highlighted as important contributors to white wine mouthfeel (Gawel et al. 2018).

Influence of amino acids

For the first time, the amino acids L-proline and L-glutamic acid were demonstrated to strongly influence wine in-mouth sensory properties. The QDA studies confirmed the results of the discrimination testing, which showed that there was a discernible effect of 3 g/L added proline. As L-proline concentration was increased, the attributes Sweetness, Viscosity and Red fruit flavour were enhanced while Astringency and Bitterness decreased. Increased L-proline concentration has previously been linked to higher ratings of 'body' or 'viscous mouthfeel' in a metabolomic correlation study investigating white wine cultivars (Skogerson et al. 2009), but this was not followed up by more direct experimentation or in red wines, which generally contain a higher concentration of L-proline than white wines. An enhancement of sweetness in wine due to L-proline gives sensory definition to the anecdotal use of terms such as 'fruit sweetness' in sugar-dry red wines.

The taste recognition thresholds reported for L-proline in water range from 1.5 to 15 g/L (Van Gemert 2011), with

most values closer to 2 g/L. A review of amino acids and amines in wines reported a range of 300–1300 mg/L of proline in red wine and up to 780 mg/L in seven commercial French wines (Lehtonen 1996). A higher concentration has been measured in California wines, particularly for Cabernet Sauvignon from the Davis, Santa Ynez and Oakville regions, in some cases exceeding 4000 mg/L (Ough and Stashak 1974, Huang and Ough 1991). From these studies, cultivar variation in L-proline concentration was found, with lower L-proline concentration in cultivars, such as Riesling, Sauvignon Blanc and Pinot Noir, and a much higher concentration in Chardonnay, Petite Sirah, Zinfandel and Cabernet Sauvignon, but with notable within-cultivar variation. L-proline concentration in the berry is associated with climate conditions, water management, soil and foliar fertilisation, and to a lesser extent, cover crop regimens in the vineyard as reviewed in Gutiérrez-Gamboa et al. (2019). It is also an amino acid that is not utilised in yeast metabolism during fermentation due to the lack of oxygen needed to by proline oxidase (Duteurtre et al. 1971) and therefore a high concentration of grape L-proline can be found in some wines.

Recently, L-proline has been proposed as a sugar replacement in foods, not only for its sweet taste and similar reported taste threshold but due to its zwitterionic plasticiser properties and high solubility which increase the physical viscosity of food products (van der Sman et al. 2020). Although the link between dry red wine physical viscosity and sensed in-mouth viscosity is sparse, one study has shown a relationship, which was closely linked to ethanol concentration (Danner et al. 2019). Additionally, taste-oral touch cognitive associations are likely to be at play given the overlapping brain areas that represent taste and oral food textures (Rolls 2019).

Sweet–bitter taste suppression may explain the decreased bitterness ratings in the present study, with this effect commonly reported in the psychophysical literature [Keast and Breslin (2003) and references within]. The suppression of astringency, as observed here, has been reported for other viscous sweeteners, but not by sweet taste alone (Lyman and Green 1990, Smith et al. 1996). Although the complete mechanism of the astringency phenomenon is not completely understood (Gawel 1998), one aspect that is widely accepted is the binding of tannin to proline-rich salivary proteins (PRPs). It would be of interest to determine whether free L-proline in wine might chemically interact with tannins or saliva. This effect could potentially decrease co-precipitation of PRPs with tannins, thereby modulating astringent sensation, and warrants further research. This theory may provide insight into circumstantial accounts of 'smooth and silky tannin' descriptions commonly sought by winemakers. Additionally, the increased Red fruit flavour intensity due to the L-proline addition in Study 2 and to some extent in Study 3, suggests that a congruent taste–aroma cognitive enhancement occurred. This is not surprising in light of the strong evidence for perceived 'flavour' to be a multi-modal percept integrating taste, oral-somatosensory and olfactory signals in the orbitofrontal cortex based on prior associations (Small and Prescott 2005, Small 2008, 2012).

There is little published evidence which links L-glutamic acid/glutamate with wine sensory properties, including 'savoury' or 'umami' characters, however, a comprehensive case for its investigation was proposed by Klosse (2013). In the present study, L-glutamic acid addition imparted Umami/savoury taste to red wine. More work is needed to understand the proportion of protonated and deprotonated forms of glutamic acid at wine-like and in-mouth pH values as well as interactions with the abundance of K^+ ions found in wine. The concentration reported for L-glutamic acid/glutamate in white wines is 5–140 mg/L and in red wines, 6–112 mg/L (Gutiérrez-Gamboa et al. 2019). The reported taste detection and recognition thresholds are 9–110 and 162–590 mg/L, respectively, in water (Van Gemert 2011). 'Umami' is an uncommon wine descriptor in Western cultures and is not generally included in wine appreciation tasting lexicons. 'Savoury', however, is fairly commonly used to describe red wines, particularly those from the northern Rhone (Robinson et al. 2012, Robinson and Harding 2015). Some limited work on savoury odorants in aged red wines has been conducted (Beatty 2013), but at present, it is unclear if these descriptions are directed by odorants, tastants or both. The source, occurrence and modulation of L-glutamic acid/glutamate in wine are virtually unstudied in wine science.

The present series of studies was limited in the investigation of the composition of a single Shiraz wine, with only one source of polyphenols. Further work should assess the proline and glutamic acid concentration in a wider set of wines and evaluate relationships with sensory attributes.

Conclusions

Overall, this series of sensory studies has highlighted interactions of volatiles and non-volatiles in red wine in-mouth characteristics and showed the relative effect of volatiles and polyphenols in conferring red wine flavour. Importantly, the amino acids L-proline and L-glutamic acid were found to play a key role in Sweet and Savoury/Umami tastes in red wine. These are grape-derived compounds, and the recognition of these amino acids as contributors to desirable in-mouth sensory properties in red wines opens new options for wine producers to enhance their contribution through viticultural and postharvest practices.

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Supporting information

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Table S1. Chemical composition, volatile and non-volatile compounds measured in the Shiraz wine and SAFE volatile extract.

Table S2. A subset of sensory and chemical data obtained from 14 commercial Barossa Shiraz wines produced in the 2017 season.

Table S3. Polyphenolic composition of the de-aromatised phenolic extract and a sample of 215 Shiraz wines.

Table S4. *F*-ratios, probability values, degrees of freedom and mean square error from the preliminary analysis of variance for reconstitution Study 1.

Table S5. Sample mean intensity values and standard error of the sensory attributes rated for the samples assessed in Study 1 reconstitution investigating the contribution of volatiles, polyphenolics and amino acids with glutathione added to model wine.

Table S6. Pearson correlation matrix for all sensory attributes rated in reconstitution Study 1.

Table S7. *F*-ratios, probability values, degrees of freedom and mean square error from the preliminary analysis of variance for amino acid addition Study 3.

Table S8. Sample mean intensity values and standard error of the sensory attributes rated for the samples assessed in Study 3 investigating the contribution of different concentration values of L-proline and L-glutamic acid addition to Shiraz wine.

Table S9. *F*-ratios, probability values, degrees of freedom and mean square error from the preliminary analysis of variance for reconstruction Study 4 under the normal condition.

Table S10. *F*-ratios, probability values, degrees of freedom and mean square error from the preliminary analysis of variance for reconstruction Study 4 under the nose clip condition.

Table S11. Sample mean intensity values and standard error of the sensory attributes rated for the samples assessed in Study 4 investigating the proline addition and SAFE proportion evaluated with- and without a swimmer's nose clip.

Table S12. Basic chemical composition of the reconstructed samples and Shiraz wine.