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Wine amino acids of four autochthonous grape varieties from Southwest France: influencing factors and role in taste perception

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ABSTRACT

The hundreds of molecules present in wine play an important role in its complexity, including amino acids. While numerous studies exist on the role of amino acids in musts, few studies focus on wines. In the present study, the amino-acid profiles of a large sample of monovarietal white wines from three vintages of varieties from Southwest France (Colombard, Gros Manseng, Len de l'el, and Mauzac) were evaluated. Physicochemical oenological parameters were first analysed for each wine, revealing significative differences between grape varieties and within each variety. These differences included the organic nitrogen content, which was studied in greater detail. After derivatisation, the primary amino acids were identified and quantified using high-performance liquid chromatography (HPLC) with UV detection. The wines from the four grape varieties predominantly contained the same amino acids (proline, arginine, alanine and lysine) as those found in wines from other varieties. The amino acid composition in wines can be strongly influenced by various factors, such as the ripeness of the grapes, the grape variety itself, or certain parameters like vineyard treatments. In this study, it was possible to differentiate between the varieties by their proline and glutamic acid contents; both these amino acids were analysed to assess their impact on taste perception thanks to a naïve panel fitted with a nose clip, and a difference was perceived for proline. Further studies would be necessary to better understand their effect on gustatory perception.

KEYWORDS: amino acids, white wines, HPLC, wine flavour

INTRODUCTION

Wine composition is complex and involves hundreds of compounds, each contributing to the final flavour. Due to their role in this complexity, research has recently been focusing on amino acids, which account for 30 to 40 % of total nitrogen in ripe grapes. The majority of those present in musts are utilised as nutrients for yeast growth, leading to the production of volatile compounds, such as alcohols, aldehydes, esters, and other volatiles that influence the organoleptic properties of wines (Ribéreau-Gayon *et al.,* 2006a). Additionally, it is generally accepted that amino acids can serve as nutrients for bacterial growth during secondary fermentations (Lonvaud-Funel, 2001).

Amino acids present in wine originate from various sources. As well as those found in grapes, which are partially or completely metabolised by yeasts during their growth phase, some are excreted by live yeasts at the end of fermentation, others are released through proteolysis during the autolysis of dead yeasts, and others still are produced by the enzymatic degradation of grape proteins (Soufleros *et al.,* 2003). The type and concentration of these compounds in grapes depend on various factors, such as grape variety (Garde-Cerdán *et al.,* 2014), ripeness (Stines *et al.,* 2000), terroir (Gutiérrez-Gamboa *et al.,* 2017; Gutiérrez-Gamboa *et al.,* 2018; Gutiérrez-Gamboa & Moreno-Simunovic, 2019), climate and nitrogen fertilisation (Gutiérrez-Gamboa *et al.,* 2017). Numerous studies have shown that high nitrogen concentration in grapes increases the amino acid content in wines (Bell & Henschke, 2005; Gutiérrez-Gamboa *et al.,* 2017). Additionally, organic or mineral nitrogen can be added to musts to provide a sufficient quantity and quality of nitrogen to enhance the fermentation process, while also promoting the formation of positive aromas (*e.g.*, thiols in wines from Côtes de Gascogne) (Jiranek *et al.,* 1995). Furthermore, different vinification conditions (*e.g.*, fermentation temperature and kinetics, yeast strain) can influence the amino acid content of wine (Rapp & Versini, 1995; Martínez-Rodríguez *et al.,* 2002).

Despite the many factors that affect the nature and concentration of amino acids in wines, a limited number of studies (Burin *et al.,* 2016; Martínez-Pinilla *et al.,* 2013; Mirás-Avalos *et al.,* 2020; Pérez-Álvarez *et al.,* 2019; Sartor *et al.,* 2021; Stines *et al.,* 2000) have examined the amino acid profiles of musts and wines to differentiate and classify them according to grape varieties, management conditions and wine-growing regions. Pérez-Álvarez *et al*. (2019) studied four vintages to determine how soil type affects the amino acid content of Tempranillo must; they showed that the concentration of free amino acids can be used to differentiate between musts from different soils. In addition, by analysing 22 amino acids, Sartor *et al*. (2021) differentiated three non-traditional sparkling wine varieties (Niagara, Manzoni Bianco, and Goethe) from a traditional variety (Chardonnay). They demonstrated that amino acid profiles can be influenced by varietal characteristics, with all musts undergoing the same clarification process during the pre-fermentation phase. They also analysed the amino acids present in base wines from the same grape varieties. Although the concentrations of amino acids were lower than in the musts, they came to the same conclusion: the grape variety strongly affects the concentration of amino acids in wines. This conclusion was also reached by Mirás-Avalos *et al*. (2020), who differentiated between white wines from three native Galician grape varieties by analysing 22 amino acids. Their study revealed that the amino acid composition of grapes and wines can be influenced by factors such as grape variety, geographical location and vintage. Furthermore, Soufleros *et al*. (1998) successfully classified French wines from different regions (Bordeaux, Burgundy, Alsace and Champagne) based on their origin, type, and aging by analysing 21 amino acids, biogenic amines, and volatile substances, independently of vinification conditions. Another study (De La Presa-Owens *et al.,* 1995) characterised white wines from the Penedès region in Spain, showing that certain amino acids were more characteristic of specific factors, such as grape variety (tyrosine, isoleucine, glycine, and alanine), geographical origin (asparagine, proline and lysine), and vintage (alanine, histidine and methionine). Additionally, Martínez-Pinilla *et al*. (2013) demonstrated the impact of variety, vinification stage, and vintage on the amino acid and biogenic amine composition of Rioja red wines; however, it was observed that amino acids could not be used to differentiate samples that had undergone malolactic fermentation. Moreover, another study (Burin *et al.,* 2016) has shown that clarification affects the nitrogen composition of musts, which thus influences the production of volatile compounds and, consequently, the wine's specific sensory characteristics.

All these studies show that parameters like grape variety influence the amino acid composition of musts and wines. The amino acid profile may therefore be a good way of discriminating between different wine profiles. However, few studies have focused on white wines, and in particular white wines from the vineyards of Southwest France.

In France, famous vineyards have built their reputation on emblematic grape varieties: Chardonnay or Pinot noir for Burgundy, Merlot or Cabernet-Sauvignon for Bordeaux. Among the renowned wine-growing regions, the Southwest vineyards stand out, with almost 300 grape varieties, 120 of which are autochthonous. This particularity is accompanied by a production dominated by white wines (127 million litres produced in 2022) (Agreste, 2023). The two main wine-producing regions in Southwest France are the Gers (20,362 hectares, 2022) and the Tarn (6274 hectares, 2022) (Agreste, 2023), both of which produce dry white wines from grape varieties typical of the Southwest. In the Gers, Colombard (COL) and Gros Manseng (GM) grape varieties are mainly used for producing Côtes de Gascogne wines (a protected geographical indication). These two grape varieties produce acidic wines, with GM also characterised by an alcohol content (ABV) frequently higher than 13%. Nonetheless, they possess distinct aromatic qualities: COL offers substantial aromatic potential characterised by grapefruit, citrus and passion fruit aromas, while GM yields wines with quince, apricot and citrus aromas, along with floral and spicy notes (Frankel, 2013;

Robinson *et al.,* 2012). These distinct aromas are often attributed to varietal thiols, primarily derived from odourless amino precursors present in grapes, such as S-cysteine, which are mainly released through yeast enzymatic activity during alcoholic fermentation (Choné *et al.,* 2006; Roland *et al.,* 2011). Present in very low concentrations in grapes (ppb) (Peña-Gallego *et al.,* 2012), the concentrations of these thiol precursors can be altered as a result of viticultural practices; for example, it has been shown that a high nitrogen level in the vineyard can lead to a four-fold increase in concentration of cysteinylated precursors (Choné *et al.,* 2006), and thus a corresponding rise in varietal thiols in the finished wines (Geffroy *et al.,* 2016).

In the Tarn, Gaillac white wines (Protected Denomination of Origin wines) are primarily made from Len de l'el (LL) and Mauzac (MZ) varieties. These two varieties produce wines with lower titratable acidity concentrations than the COL or GM. LL wines have a broad aromatic range, featuring white flowers, honey, red berries and citrus fruit, while MZ variety wines are characterised by ripe apple aromas and floral notes, such as jasmine, acacia and violet (Frankel, 2013; Robinson *et al.,* 2012). The origin of these aromas remains uncertain, as these grape varieties are highly endemic to the Tarn and have hardly been studied.

Each wine has its own unique taste and sensory identity. Wine contains amino acids, which are essential in the food industry (Roudot-Algaron, 1996). Physiological studies show that above a certain concentration threshold, most amino acids and their salts affect the taste (Delompré *et al.,* 2019). Given the distinctive taste characteristics of each grape variety, it is of interest to analyse the amino acid profile of each of these wines. This approach would not only enable differentiation but also help identify which amino acids influence observed sensory variations. Furthermore, various studies (Espinase Nandorfy *et al.,* 2022; Franceschi *et al.,* 2023; Skogerson *et al.,* 2009) have attempted to investigate the impact of certain amino acids, such as proline and glutamic acid, on the organoleptic perceptions of wines. However, these studies were not solely based on taste by using, for example, nose clips.

Considering the impact of numerous factors on amino acid concentrations, this study initially focuses on examining how intra- and inter-varietal oenological parameters affect the amino acid profiles of wines from four white grape varieties cultivated in the vineyards of Southwest France. Additionally, a sensory test based solely on taste (using nose clips) was conducted to provide preliminary data on the potential impact of two amino acids (Pro and Glu) on the taste perception of the wines.

MATERIALS AND METHODS

1. Samples

This study used 69 wines from various wine estates in two main white-wine-producing areas in Southwest France: Côtes de Gascogne and Gaillac. The samples were from the 2016, 2020, and 2021 vintages and were collected at two stages of winemaking: i) directly from the tank after alcoholic fermentation (AF), and ii) after bottling (B). The four main white grape varieties of the Southwest were represented: COL (25 samples), GM (9 samples), LL (20 samples) and MZ (15 samples). The basic physicochemical parameters of each wine (alcohol by volume (ABV), pH, titratable acidity, and the concentration of malic, lactic, and tartaric acids) were determined by Fourier transform infrared (FTIR) spectrometry using a WineScan wine analyser (Foss, France SAS) and following OIV/OENO Resolution 390/2010. Free and total SO_2 were determined in wines by potentiometric titration with a Titromatic 2S instrument (Crisson Instruments, Spain). Ammonium and organic nitrogen levels were measured using Ammonia Infinity (Thermo Fisher Scientific TR60101, Waltham, USA), and Alpha Amino Nitrogen (Thermo Scientific 984342, Waltham, USA) kits integrated into a Gallery multiparameter analyser (Thermo Electron Corporation, Waltham, USA) equipped with a spectrophotometer for measurements at 340 nm.

A reference wine served to ensure repeatability in each analysis. The reference wine contained a blend of different grape varieties, most of which were Southwest varieties.

2. Chemicals

Supergradient acetonitrile and methanol were obtained from Carlo Erba (France) with an industrial purity of 99 % [high-performance liquid chromatography (HPLC) grade], and ultrapure water was obtained from a Merck MilliQ integral 15 system. Sodium azide (95 % purity) was purchased from Sigma Aldrich (St Louis, MO, USA). L-aspartic acid (Asp), L-glutamic acid (Glu), L-cysteine (Cys), L-leucine (Leu), L-phenylalanine (Phe), L-lysine (Lys), L-histidine (His), L-arginine (Arg), L-tyrosine (Tyr), L-valine (Val), L-serine (Ser), L-glutamine (Gln), L-ornithine (Orn), L-tryptophan (Try), L-asparagine (Asn), L-threonine (Thr), γ-aminobutyric acid (Gaba), L-isoleucine (Ile), L-methionine (Met), L-glycine (Gly), L-alanine (Ala), L-aminoadipic acid, and diethylethoxymethylenemalonate (DEEMM) were purchased from Merck (Darmstadt, Germany).

3. Determination of free amino acids in wine by high-performance liquid chromatography

The free-amino-acid content was determined in duplicate using HPLC and following a slightly modified method from a previous derivatisation reaction (Gómez-Alonso *et al.,* 2007; OIV, 2022).

A borate buffer was prepared beforehand: 31 g of boric acid was added to 400 mL of ultrapure water in a 500 mL flask. The buffer was adjusted to pH 9 using a 4 M solution of NaOH. The volume was adjusted to 500 mL with ultrapure water. A few minor changes have been made to the derivatisation method. The samples were vortexed every 5 min during the 30-min heating period (*i.e.*, six times). The samples were first covered with aluminium foil and then placed in a 70 °C water bath for one hour to allow complete degradation of excess DEEMM and reagent byproducts. Upon returning to room temperature, the samples were transferred to vials for analysis. The injected volume was 50 µL.

HPLC was undertaken using an Ultimate 3000 Series instrument (Thermofisher, France). The separation was performed by using a Zorbax Eclipse AAA column (C18), particle size 5 µm (250 mm × 4.6 mm, Agilent) with a pre-column (Zorbax eclipse AAA, 12.5 mm × 4.6 mm, Agilent) thermostated at 17 °C.

Two eluents were used as mobile phases: mobile phase A consisted of 25 mM acetate buffer (pH = 5.8) with 0.4 g of sodium azide (Sigma Aldrich, St Louis, MO, USA), and mobile phase B consisted of acetonitrile (Carlo Erba, France) and methanol (80:20, v/v) (Carlo Erba, France). All reagents were first filtered through a 0.1 µm PTFE Millipore filter (Merk, Darmstadt, Germany). The flow rate was 0.9 mL/min. The same gradient profile used by Gómez-Alonso *et al.* (2007) was applied. However, an additional 7 min were added to equilibrate the column to its original state.

A UV detector monitoring at 280 and 292 nm was used for detection. These chromatographic conditions enabled the separation, identification and quantification of 22 amino acids, including proline. The following compounds were identified based on their retention times and on the UV-vis spectral characteristics of the derivatives of the corresponding standards: L-Pro, L-Asp, L-Glu, L-Cys, L-Leu, L-Phe, L-Lys, L-His, L-Arg, L-Ala, L-Gly, L-Tyr, L-Val, L-Ser, L-Gln, L-Orn, L-Try, L-Asn, L-Thr, γ-aminobutyric acid (GABA), L-Iso, and L-Met (Merck, Darmstadt, Germany). Quantification was realised using the internal standard method (Table 1).

TABLE 1. Measured Wavelengths, Calibration Parameters, Repeatability and Detection Limit for Aminoenone Derivatives of Amino Acids.

		Calibration	
Compounds	Wavelenghts (nm)	Range (mg.L^{-1})	r^2
Asp	280	0-40	0,9918
Glu	280	0-40	0,9985
Pro	292	0-1500	0,9972
Asn	280	0-40	0,9625
Ser	280	0-50	0,9896
Gln	280	0-40	0,9987
His	280	0-30	0,9974
Gly	280	0-30	0,9997
Thr	280	0-30	0,9918
Arg	280	0-50	0,9983
Ala	280	0-60	0,9989
GABA	280	0-100	0,9994
Tyr	280	0-30	0,9993
Val	280	0-30	0,9986
Met	280	0-15	0,9991
Cys	280	0-30	0,9751
Ile	280	0-20	0,9995
Try	280	0-15	0,9988
Leu	280	0-40	0,9974
Phe	280	0-30	0,9993
Orn	280	0-30	0,9961
Lys	280	0-50	0,9979

4. Sensory analysis

Sensory evaluation was conducted exclusively using triangle tests, in accordance with UNI ISO 4120 standards (ISO, 2021). The testing room was maintained at a temperature of 22 °C, and kept free of external odours and noise. The tasting panel consisted of 30 naïve and experienced judges aged between 20 and 64 years. Participants were instructed to refrain from eating and smoking for one hour prior to tasting. No ethical approval was obtained, but all participants provided written informed consent before taking part in the study. To focus solely on taste, the panellists wore nose clips during the tasting session.

The 30 judges participated in a session consisting of two successive triangle tests of two products. For the first triangle test series, the stimulus was L-Glu (Ajinomoto foods Europe SAS©, Paris), and for the second series, the stimulus was L-Pro (Merck©, Germany). Their impact on taste was evaluated in a dry white wine. For L-Glu, the selected wine was a COL with a low L-Glu concentration (15 mg/L, pH = 3.2, ABV = 11 %), and for Pro, the wine was an MZ with a low Pro concentration (38 mg/L, pH = 3.3, ABV = 13 %). For each amino acid, the maximum concentrations found in the wines after quantification by HPLC were targeted in the wines depleted in L-Glu and L-Pro, resulting in 55 mg/L for L-Glu and 1655 mg/L for L-Pro, respectively.

For each triangle test, three samples (10 mL) were presented in wine glasses and identified by groups of three random values. Each judge was asked to taste each sample using only

their sense of taste to identify which of the three sampled products was different from the other two, and then to note the perceived difference using their own descriptor. The samples were tested according to a randomised complete block design. The evaluators were not provided with any information about the origin of the samples. The results of the sensory evaluation were analysed according to the UNI ISO 4120 standard (ISO, 2021).

5. Statistical analysis

Data were analysed using the PROC MIXED of SAS software (SAS® OnDemand for Academics, SAS Institute Inc., Cary, NC, USA) through ANOVA. The grape variety, stage of sampling and its interaction, as well as the vintage were considered as fixed effects, while the vineyard was considered as a random effect. The degrees of freedom were adjusted by the Kenward-Rodgers procedure (Loughin, 2006). Post-hoc means comparison was performed through Tukey's test, and the significance threshold was set at $\alpha \leq 0.05$, while tendencies were considered as $0.05 < p\text{-value} \leq 0.10$. The normality of the residuals was checked by QQ-plot and Kolmogorov–Smirnov test. In the case of no normal distribution, the variable was log-transformed, and the results presented after back-transformation.

Classification trees were performed using XLSTAT to create representative classes of wines from four grape varieties of the Southwest region in France, based initially on their oenological parameters and amino acid concentrations. Cross-validation was carried out, and the "Exhaustive CHAID" method with a 5 % significance level was chosen.

Sensory data (the average of each attribute evaluated by the judges) were exported to Microsoft Excel. A triangle test using a Thurstone model was used. Statistically significant differences between wines were determined by considering a p-value < 0.05.

RESULTS AND DISCUSSION

1. Characterisation of the Physicochemical Parameters of Wines: Influence of Various Factors

The four grape varieties studied exhibit distinct physicochemical characteristics influenced by various parameters, such as grape variety, vintage, and sampling stage.

1.1. Influence of Grape Variety

Grape variety influenced most of the variables ($p < 0.001$), except for certain parameters, such as tartaric acid (A_{tart}), free SO_2 and ammoniacal nitrogen (Table 2). The concentrations of ammoniacal nitrogen were not shown, as they are mostly below the threshold defined by the analytical method.

TABLE 2. Physicochemical parameters of the COL, GM, MZ, and LL wines.

Variety	ABV	pH	Titratable acidity	Tartaric acid	Malic Acid	Lactic acid	Free SO_2	Total SO_2	Organic nitrogen
	%		$g.L^{-1}$ as Tartaric acid	$g.L^{-1}$	$g.L^{-1}$	$g.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$
COL (n = 25)	11.5± 0.3 c	3.02 ± 0.05 b	5.01 ± 0.21 a	2.41 ± 0.17	3.96 ± 0.32 a	0.01 ± 0.003 b	24.2 ± 3.50	117 ± 7 a	58 ± 6 a
GM (n = 9)	13.2± 0.3 a	3.23 ± 0.06 a	4.44 ± 0.24 b	2.39 ± 0.20	3.06 ± 0.36 b	0.13 ± 0.01 b	27.7 ± 3.77	111 ± 9 a	46 ± 7 ab
LL (n = 20)	12.2 ± 0.2 b	3.29 ± 0.04 a	3.21 ± 0.18 c	2.15 ± 0.15	0.99 ± 0.28 c	0.67 ± 0.003 a	26.4 ± 3.18	90 ± 6 b	43 ± 5 b
MZ (n = 15)	12.4 ± 0.2 ab	3.25 ± 0.04 a	3.14 ± 0.19 c	2.14 ± 0.16	0.63 ± 0.28 c	0.81 ± 0.004 a	22.1 ± 3.04	82± 7 b	37 ± 6 b
AF (n = 30)	12.2 ± 0.3	3.22± 0.05	3.85 ± 0.21	2.14 ± 0.17	2.25 ± 0.31	0.22 ± 0.003	22.3 ± 3.31	96 ± 7	51 ± 6
B (n = 39)	12.4 ± 0.2	3.18± 0.04	3.89 ± 0.16	2.41 ± 0.13	2.07 ± 0.26	0.38 ± 0.002	27.9 ± 2.48	104 ± 5	41 ± 5
Vintage 16 (n = 7)	12.1 ± 0.3	3.15± 0.07	3.76 ± 0.27 b	2.22± 0.23	1.63 ± 0.39 b	0.29 ± 0.01	22.4 ± 5.12	95 ± 9	41 ± 8
Vintage 20 (n = 8)	12.9 ± 0.3	3.27± 0.06	3.63 ± 0.26 b	2.39 ± 0.22	1.80 ± 0.39 b	0.35± 0.01	27.8 ± 4.09	102 ± 10	58 ± 8
Vintage 21 (n = 54)	12.0 ± 0.2	3.18± 0.03	4.25 ± 0.14 a	2.20 ± 0.10	3.04 ± 0.26 a	0.25± 0.001	25.2 ± 1.59	102 ± 3	39 ± 5
				Factors					
Variety	<0.001	<0.001	<0.001	0.335	<0.001	<0.001	0.476	0.001	0.011
Stage of sampling (AF or B)	0.601	0.487	0.845	0.163	0.563	0.019	0.129	0.280	0.13
Vintage	0.080	0.363	0.020	0.726	0.001	0.576	0.680	0.759	0.129

Data are the average values for the standard errors. The p-values for the following factors are shown: variety, vintage, sampling stage (AF (after alcoholic fermentation) vs B (after bottling)), and their interactions. Significant differences between data for different parameters are indicated by different letters according to Tukey's HSD test.

For the alcohol content (ABV), wines made from the GM grape variety had a ABV (13.2 % ± 0.3, n = 9) relatively close to that of wines made from MZ (12.4 % ± 0.2, n = 15), which shared common characteristics with the LL grape variety (12.2 % ± 0.2, n = 20), but differed significantly from wines made from COL (TAV = 11.5 % ± 0.3, n = 25). Furthermore, the COL variety exhibited the highest titratable acidity (TA) and the lowest pH. As mentioned in the introduction, these characteristics are often sought after and associated with early grape harvesting to achieve higher yields (Robinson *et al.,* 2012). Consequently, COL grapes are generally less ripe than those of other varieties.

Regarding the data collected for malic (A_{mal}) and lactic acids (A_{lac}), the COL and GM varieties tended to show high concentrations of malic acid ($A_{mal\ (COL)}$ = 3.96 ± 0.32 g/L; $A_{mal\ (GM)}$ = 3.06 ± 0.36 g/L) but low concentrations of lactic acid ($A_{lac\ (COL)}$ = 0.01 ± 0.003 g/L; $A_{lac\ (GM)}$ = 0.13 ± 0.006 g/L), unlike the LL and MZ varieties ($A_{mal\ (LL)}$ = 0.99 ± 0.28 g/L; $A_{mal\ (MZ)}$ = 0.63 ± 0.28 g/L; $A_{lac\ (LL)}$ = 0.67 ± 0.003 g/L; $A_{lac\ (MZ)}$ = 0.81 ± 0.004 g/L). The completion of malolactic fermentation (MLF) in these latter two varieties explains this result. This biological reaction converts malic acid, (a dicarboxylic acid) into lactic acid (a monocarboxylic acid), thus reducing the wine's acidity.

Since freshness and acidity are key elements in GM and COL wines, malolactic fermentation is actively prevented (Ribéreau-Gayon *et al.,* 2006b). This is reflected in the higher total SO_2 levels for the COL and GM varieties ($SO_{2(COL)}$ = 117 ± 7 mg/L; $SO_{2(GM)}$) = 111 ± 9 mg/L) compared to the MZ and LL varieties ($SO_{2(LL)}$ = 90 ± 6 mg/L; $SO_{2(MZ)}$ = 82 ± 7 mg/L).

The organic nitrogen content also varied depending on grape variety (p = 0.011). The organic nitrogen level in COL (58 ± 6 mg/L) was relatively similar to that in GM (46 ± 7 mg/L). The latter also shown a similar organic nitrogen content to wines from MZ (43 ± 5 mg/L) and LL (37 ± 6 mg/L). Organic nitrogen, primarily composed of primary amino acids, is the most common form of nitrogen in wines (Ribéreau-Gayon *et al.,* 2006b) and is influenced by various factors, such as viticultural practices (e.g., foliar nitrogen treatments). Grapes from COL, whose wines are known for their thiol aromas, are often subjected to nitrogen application in the vineyard (Dufourcq *et al.,* 2011). These treatments could contribute to the organic nitrogen content of COL wines analysed in this study, which were higher than in MZ and LL wines. Similar organic nitrogen content was found in GM, wines that are also known for being rich in thiol precursors, and which are grown in the same production region as COL wines (Dournes *et al.,* 2022).

Oenological parameters differed in the four varieties studied, with similarities in the wines originating from the same production area being more marked in COL and GM wines. The reason for these findings may be related to the regional viticultural and winemaking practices.

In addition, the discriminant analysis conducted on all the data allowed the differences between grape varieties to be visualised in a classification tree. This tree, presented in Figure 1, shows how different discriminating variables, such as TA, A_{mal} concentration, and pH, effectively separated the observed classes. For example, TA appeared to be the variable that best discriminated between the Côtes de Gascogne grape varieties and the Tarn varieties, the COL grape variety showing higher values for this factor. As mentioned in the introduction, the COL grape is harvested very early to preserve all its acidity (Robinson *et al.,* 2012). Conversely, the GM grape is harvested later while still maintaining good acidity, a distinctive feature of this variety. Moreover, wines from LL and MZ varieties were differentiated based on their A_{mal} concentration, as these wines undergo malolactic fermentation.

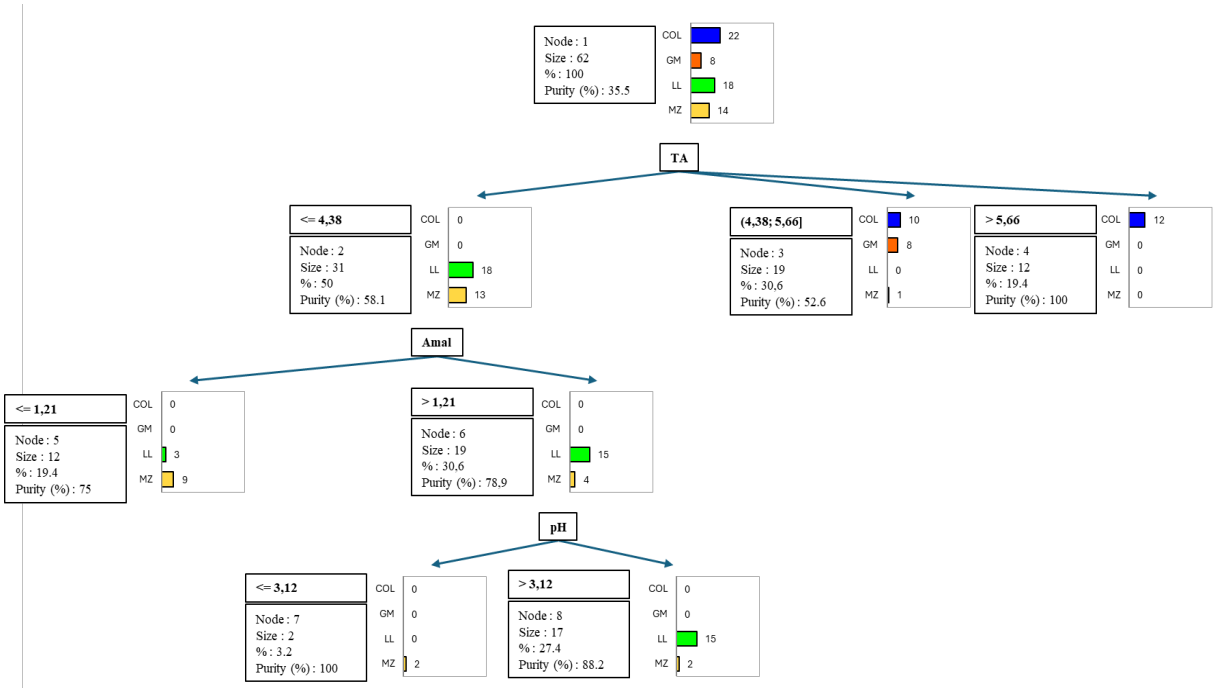


FIGURE 1. Classification and regression tree analysis performed using all the oenological parameters measured in the wines.

COL = Colombard, GM = Gros Manseng, MZ = Mauzac, LL = Len de l'El, TA = titratable acidity, Amal = malic acid.

Thus, wines from the Côtes de Gascogne and the Tarn were both differentiated by the grape varieties used. As the climate significantly impacts grape ripening, three vintages were analysed to assess their influence on classical oenological parameters.

1.2. Influence of the vintage

The vintage directly influenced the TA (p-value = 0.20) and the malic acid concentration in the wines (p-value = 0.001) (Table 2).

The 2021 vintage showed a higher titratable acidity (TA) compared to the 2016 and 2020 vintages. Indeed, 2021 was characterised by environmental disruptions, such as spring frosts and a cool, rainy summer, which led to diseases and less advanced berry ripeness (Météo France, 2021). In comparison, 2020 and 2016 were warmer, with generally favourable climatic conditions for berry maturation (Météo France, 2016; Météo France, 2020); thus, the grapes from 2021 were likely less ripe than those from 2020 and 2016.

The higher concentration of malic acid in 2021 can also be attributed to this lower level of ripeness, as malic acid is present in large quantities in grapes after veraison and is gradually metabolised during maturation, reducing its concentration as the berries ripen (Ribéreau-Gayon, *et al.,* 2006a). However, most samples from the 2016 and 2020 vintages were primarily MZ and LL wines, which are generally harvested later (resulting in lower TA) and whose technical protocols involve completing malolactic fermentation (MLF).

Climate influenced wine classification, particularly in terms of total acidity. However, in this study, the impact appeared to be related to sampling variations rather than to the climate itself. The sampling stage may have also exerted an influence. To study this parameter, wines sampled at the end of alcoholic fermentation (FA) (n = 30) and wines sampled from bottles (n = 39) were analysed.

1.3. Effect of Sampling Stage

The sampling stage greatly influenced the A_{lac} concentrations in wines (Table 2). Indeed, A_{lac} concentrations were higher in bottled wines (B) compared to those sampled at the end of alcoholic fermentation (FA), a phenomenon once again linked to the MLF stage.

Four distinct grape varieties were analysed in this study. The results showed a variability in oenological parameters, one of which was organic nitrogen, whose content was correlated with grape variety. In wine, most of the organic nitrogen consist of amino acids. Numerous studies have focused on the amino acid profiles of grapes and musts from various grape varieties (Burin *et al.,* 2016; Mirás-Avalos *et al.,* 2020; Pérez-Álvarez *et al.,* 2019; Sartor *et al.,* 2021). However, no research has yet been conducted on the grape varieties examined in this work, let alone on the wines made from these varieties.

TABLE 3. Minimum and maximum concentrations (mg/L of free amino acids in the wines from four white grapevine varieties (n = 69)).

Compounds	COL (25 x 2 samples)		GM (9 x 2 samples)		LL (20 x 2 samples)		MZ (15 x 2 samples)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Asp	8.43 ± 0.64	60.4 ± 2.48	15.6 ± 1.29	54.8 ± 3.12	7.76 ± 0.23	34.0 ± 2.67	3.91 ± 0.08	49.3 ± 1.10
Glu	5.72 ± 0.03	70.0 ± 0.72	18.6 ± 1.13	48.5 ± 0.33	4.05 ± 0.21	22.4 ± 2.05	3.48 ± 0.12	17.6 ± 0.59
Pro	179 ± 12.4	817 ± 64.1	736 ± 8.84	1693 ± 80.9	56.7 ± 5.20	254 ± 16.3	7.04 ± 0.26	323 ± 23.4
Asn	5.09 ± 0.46	28.7 ± 1.95	11.8 ± 14.0	22.7 ± 2.89	3.65 ± 0.33	13.8 ± 1.49	2.42 ± 0.11	12.8 ± 1.07
Ser	8.27 ± 0.07	32.7 ± 2.24	16.9 ± 0.33	42.1 ± 2.80	4.84 ± 0.37	19.0 ± 0.19	5.38 ± 0.08	22.8 ± 0.36
Gln	0.52 ± 0.27	28.5± 1.18	5.64 ± 0.29	29.1 ± 0.58	2.74 ± 0.04	10.5 ± 0.84	2.10 ± 0.09	16.1 ± 0.50
His	20.0 ± 0.82	81.9 ± 61.74	19.5 ± 1.09	85.6 ± 6.40	10.9 ± 0.29	32.4 ± 0.83	11.6 ± 0.23	40.4 ± 1.65
Gly	5.65 ± 0.05	37.9 ± 2.24	15.2 ± 1.13	39.4 ± 1.07	5.22 ± 0.23	19.3 ± 1.04	4.48 ± 0.29	21.4 ± 0.77
Thr	5.55 ± 0.09	26.3 ± 1.57	10.2 ± 0.50	28.3 ± 1.91	5.15 ± 0.34	14.8 ± 2.25	4.34 ± 0.12	18.2 ± 1.51
Arg	15.5 ± 1.05	523 ± 12.9	28.8 ± 1.59	146 ± 7.68	7.10 ± 0.65	78.6 ± 2.62	8.56 ± 0.12	157 ± 4.04
Ala	10.4 ± 0.77	171 ± 17.7	23.8 ± 0.43	85.6 ± 4.76	5.55 ± 0.48	54.8 ± 0.36	6.99 ± 0.12	63.9 ± 0.16
GABA	1.55 ± 0.10	7.67 ± 0.52	2.47 ± 0.15	7.39 ± 0.39	1.61 ± 0.10	4.59 ± 0.03	1.00 ± 0.05	5.98 ± 0.07
Tyr	5.60 ± 0.03	35.6 ± 2.75	13.5 ± 0.34	40.2 ± 1.23	5.60 ± 0.34	19.6 ± 0.80	3.49 ± 0.01	26.3 ± 0.96
Val	4.65 ± 0.31	24.4 ± 0.56	9.26 ± 0.28	23.2 ± 0.88	2.81 ± 0.10	15.5 ± 1.92	2.38 ± 0.04	18.1 ± 0.36
Met	1.32 ± 0.03	10.0 ± 0.37	3.46 ± 0.22	7.03 ± 0.17	1.00 ± 0.05	3.03 ± 0.17	0.73 ± 0.05	4.09 ± 0.27
Cys	15.0 ± 1.02	129 ± 1.65	23.5 ± 1.56	129 ± 3.21	8.29 ± 0.54	73.4 ± 5.59	10.8 ± 0.89	70.4 ± 2.59
Ile	4.51 ± 0.15	25.7 ± 1.69	10.4 ± 0.06	33.3 ± 2.04	3.52 ± 0.08	19.1 ± 1.81	3.16 ± 0.51	18.7 ± 1.26
Try	1.34 ± 0.04	11.2 ± 0.86	1.85 ± 0.13	12.9 ± 0.01	0.88 ± 0.66	4.99 ± 0.11	0.72 ± 0.01	6.09 ± 0.05
Leu	9.46 ± 0.70	64.7 ± 4.34	19.7 ± 1.60	83.0 ± 6.96	6.95 ± 0.41	53.5 ± 4.94	5.94 ± 0.26	46.9 ± 0.88
Phe	7.49 ± 0.14	41.8 ± 2.0	11.9 ± 0.01	46.0 ± 2.30	5.08 ± 0.40	21.8 ± 4.01	3.25 ± 0.21	32.3 ± 0.28
Orn	2.73 ± 0.49	69.8 ± 8.32	7.23 ± 0.23	64.9 ± 2.40	1.95 ± 0.01	68.0 ± 2.69	1.95 ± 0.09	59.4 ± 1.85
Lys	21.0 ± 0.97	172 ± 12.6	10.5 ± 0.35	206 ± 12.0	11.9 ± 0.50	77.5 ± 4.85	9.86 ± 0.45	84.7 ± 6.61
TOTAL AA	339 ± 28.9	2479 ± 204	1015 ± 35.5	2928 ± 114	163 ± 11.6	915 ± 57.6	104 ± 4.19	1087 ± 53.4

2. Quantification of amino acids in the wines

To differentiate the amino acid profiles of the 69 white wines from Southwest France, an analytical method developed by Gómez-Alonso *et al*. (2007) was used to identify and quantify twenty-two amino acids in wine samples from the four grape varieties COL, GM, LL, and MZ. The average total concentrations of free amino acids in wines from the four studied grape varieties ranged from 104 ± 4 to 2928 ± 114 mg/L (Table 3). The same amino acids were present in each of the four groups of wines, but their concentrations differed.

The amino acids Pro, Lys, Arg, Cys, and Ala were present in most of these grape varieties. Empirically, certain amino acids are predominant in wine, particularly α-Ala, Ser, Arg, Pro, and Glu (Ribéreau-Gayon, *et al.,* 2006a). Pro accounted for the largest proportion of total amino acids in all the grape varieties studied.

Additionally, these results were consistent with the amino acid content of other white grape varieties. For example, Kelly *et al*. (2017) studied the white grape variety Petit Manseng, a close relative of GM (Ribéreau-Gayon *et al.,* 2006a). They showed that Arg, Ala, and His are the predominant amino acids in Petit Manseng wines. Moreover, Chardonnay wines also contain most of these amino acids (Chen *et al.,* 2019). Gly, Glu, Met, Iso, and Lys found in the wines from this study have also been reported in wines made from European white grape varieties. For instance, Mirás-Avalos *et al*. (2020) demonstrated that white wines from Galicia were rich in Lys, Arg, Glu, Ala, and His. Furthermore, Soufleros *et al*. (2003) analysed the amino acid

TABLE 4. Concentration (mg/L) of free amino acids in the 69 wines studied.

Compounds	COL n = 25 x 2	GM n = 9 x 2	LL n = 20 x 2	MZ n = 15 x 2	VARIETY	Stage of sampling	Vintage	Variety * stage of sampling
	mg.L^{-1}							
Asp	39.7 ±1.21 a	42.6 ±1.24 a	21.8 ±1.18 b	20.6 ±1.18 b	0.0022	0.225	0.145	0.035
Glu	28.2 ±1.20 a	29.6 ±1.22 a	12.7 ±1.17 b	9.16 ± 1.17 b	<0.0001	0.089	0.083	0.343
Pro	521 ± 1.29 b	1371 ± 1.31 a	125 ± 1.26 c	118 ± 1.27 c	<0.0001	0.070	0.012	0.140
Asn	19.2 ± 1.11 a	21.5 ± 1.16 a	9.37 ± 1.10 b	7.99 ± 1.12 b	<0.0001	0.059	0.030	0.561
Ser	21.4 ±1.14 a	28.3 ±1.16 a	12.0 ±1.12 b	12.5 ±1.12 b	<0.0001	0.036	0.026	0.502
Gln	9.00 ±1.24 a	11.3 ±1.29 a	4.99 ±1.21 b	4.29 ±1.22 b	0.0015	0.041	0.255	0.053
His	43.4 ±3.48 a	54.7 ±4.84 a	24.9 ±3.20 b	28.6 ±3.78 b	<0.0001	0.070	0.065	0.878
Gly	23.8± 1.22 a	32.2 ± 1.18 a	12.0 ± 1.19 b	11.9 ± 1.15 b	<0.0001	0.248	0.040	0.299
Thr	16.6 ± 1.14 b	22.4 ± 1.16 a	11.3 ± 1.12 c	11.5 ± 1.12 c	0.0003	0.634	0.005	0.178
Arg	87.1 ±1.33 a	84.7 ± 1.33 a	25.1 ± 1.29 b	27.5 ± 1.26 b	<0.0001	0.144	0.176	0.029
Ala	59.4 ± 1.24 a	57.6 ± 1.28 a	23.3 ± 1.21 b	26.4 ± 1.21 b	0.0002	0.077	0.048	0.091
GABA	4.84 ± 1.13 a	4.62± 1.15 a	3.06± 1.12 b	2.62± 1.12 b	<0.0001	0.127	0.061	0.031
Tyr	22.4 ± 1.19 a	29.5 ± 1.21 a	13.7 ± 1.16 b	12.1 ± 1.16 b	0.0012	0.156	0.023	0.041
Val	16.3 ±1.19 a	18.0 ±1.22 a	9.18 ±1.16 b	7.89 ±1.16 b	0.0007	0.092	0.027	0.521
Met	4.76 ± 1.15 a	6.82 ± 1.17 a	2.28± 1.13 b	2.20 ± 1.14 b	<0.0001	0.518	0.050	0.266
Cys	70.5 ± 7.62 a	65.7± 8.79 a	40.1± 6.89 b	43.9± 7.00 b	0.0004	0.008	0.023	0.033
Ile	16.7 ± 1.75 a	20.0 ± 1.97 a	11.2 ± 1.58 b	11.8 ± 1.58 b	0.0003	0.019	0.006	0.216
Try	5.83 ± 1.22 a	7.22± 1.25 a	3.21± 1.18 b	2.80± 1.19 b	0.0008	0.016	0.026	0.163
Leu	42.6 ± 4.85 a	46.3± 5.69 a	30.0± 4.33 b	28.6± 4.48 b	0.0096	0.032	0.022	0.200
Phe	26.9 ± 2.79 a	26.6± 3.22 a	16.1± 2.51 b	16.3± 2.55 b	0.0004	0.041	0.062	0.050
Orn	27.3 ± 1.35 a	34.3 ± 1.40 a	6.64 ± 1.29 b	9.49 ± 1.30 b	0.0001	0.052	0.007	0.198
Lys	97.4 ± 1.24 a	85.6 ± 1.28 a	47.0 ± 1.21 b	41.0 ± 1.22 b	0.0016	0.186	0.020	0.152
TOTAL average concentration	1203 ± 41.08	2100 ± 45.52	464.6 ± 38.61	457.5 ± 39.50	0.001	0.134	0.055	0.230
TOTAL average concentration (without Pro)	683 ± 40	729 ± 44	340 ± 37	339 ± 38	0.001	0.137	0.044	0.230

Data are the average for the different samples from one grapevine variety ± standard errors. The p-values for the following factors are shown: variety, stage of sampling (end of AF or after bottling), and their interactions. Significant differences between varieties according to the Tukey's HSD test for a given parameter are indicated by different letters.

profiles of various white grape varieties and found that the most abundant amino acids in Roditis wines were Arg and Lys. Specifically, the most abundant amino acids were Arg, Lys, and Glu in Muscat of Alexandria; Ala and Thr in White Muscat; and Arg, Ala, Glu and GABA in Chardonnay.

On average, across the three vintages studied, the amino acid concentrations were 1203 ± 41 mg/L for COL wines, 2100 ± 46 mg/L for GM wines, 465 ± 39 mg/L for LL wines and 458 ± 40 mg/L for MZ wines (Table 4). These wines exhibited higher amino acid concentrations than those reported in the literature for wines made from white grape varieties (Chen *et al.,* 2019; Kelly *et al.,* 2017; Mirás-Avalos *et al.,* 2020; Soufleros *et al.,* 2003). Moreover, significant differences were observed in the concentration of most amino acids, which may be due to vintage, grape variety, and the various viticultural and oenological practices employed during winemaking. These factors were examined in greater detail in relation to the amino acid composition data of the wines analysed in the present study.

2.1. Effect of Vintage

Thirteen amino acids were correlated (p-value < 0.05) with the vintage (Table 4): Pro, Ser, Asn, Gly, Thr, Tyr, Val, Cys, Ile, Try, Leu, Orn, and Lys. The 2020 vintage stood out due to generally higher concentrations of most amino acids than in 2016 and 2021 (Table 5). The year 2020 was the warmest, with high temperatures and significant water stress leading to early maturity (Météo France, 2020). This could explain the richness in amino acids associated with that vintage, particularly the high concentrations of Pro and Orn (an intermediate in the synthesis of arginine from Glu). It has also been well established that water stress impacts berry volume, thus affecting yield. Grapes harvested under such conditions have higher concentrations of sugar, potentially leading to higher alcohol levels in the wines. Indeed, the wines from the 2020 harvest (12.9 ± 0.3 %, n = 8) showed higher alcohol degrees than those from the 2016 (12.1 ± 0.3 %, n = 7) and 2021 (12 ± 0.2 %, n = 54) vintages (Section 1.2). However, this difference was not statistically significant (p = 0.008 > 0.05). Although the 2020 season was drier, the specific conditions at the plot level remain unknown.

TABLE 5. Concentration (mg/L) of free amino acids in the studied 69 wines in each vintage year.

Vintage	2016 n = 7 x 2	2020 n = 8 x 2	2021 n = 54 x 2	Vintage p-values
	mg.L^{-1}			
Pro	209 ± 1.34 b	663 ± 1.36 a	237 ± 1.27 b	0.01
Asn	12.8 ± 1.16 a	18.1 ± 1.17 a	10.1 ± 1.06 b	0.03
Ser	16.0 ±1.18 a	23.7 ±1.17 a	13.8 ±1.10 b	0.03
Gly	18.1± 1.33 ab	16.8 ± 1.17 a	11.0 ± 1.10 b	0.04
Thr	17.6 ± 1.18 a	22.4 ± 1.16 a	11.3 ± 1.12 b	0.01
Tyr	19.2 ± 1.25 ab	24.0 ± 1.23 a	13.0 ± 1.12 b	0.02
Val	11.4 ±1.24 a	17.1 ±1.24 a	9.62 ±1.13 b	0.04
Cys	53.7 ± 10.9 a	70.7± 9.55 a	40.8± 3.87 b	0.02
Ile	14.7 ± 2.52 ab	19.0 ± 2.15 a	11.1 ± 0.86 b	0.01
Try	3.75 ± 1.29 ab	7.11± 1.27 a	3.22± 1.14 b	0.03
Leu	33.1 ± 6.62 a	48.8± 6.17 a	28.7± 2.87 b	0.02
Orn	8.25 ± 1.45 b	39.9 ± 1.44 a	11.5 ± 1.22 b	0.01
Lys	56.4 ± 1.32 ab	105 ± 1.31 a	42.8 ± 1.15 b	0.02
Average total concentration	67 ± 2.5	149 ± 2.6	26 ± 1.5	/

Data are the average for the different samples from different vintages ± standard errors. The p-values for the vintage factor is shown. Significant differences between the vintages according to the Tukey's HSD test for a given parameter are indicated by different letters.

The 2021 vintage (26 ± 1.5 mg/L, n = 54) showed lower amino acid levels than the 2020 vintage (67 ± 2.5 mg/L, n = 7). The year 2021 was particularly difficult for viticulturists, with a series of environmental disturbances, such as spring frosts and diseases, significantly affecting the grape components (Météo France, 2021).

A favourable climate seems to significantly influence amino acid concentrations, especially those of Pro. However, wines made from COL, theoretically harvested earlier and with lesser maturity (Robinson *et al.,* 2012), showed high Pro levels. This could either be a characteristic of the grape variety or a consequence of the winemaker's management choices, such as nitrogenous fertilisation (Bell & Henschke, 2005; Gutiérrez-Gamboa *et al.,* 2017). It would also be interesting to further explore whether each grape variety can be characterised by the presence of one or more specific amino acids. Furthermore, to obtain more precise data, grape and vinification processes should be monitored, particularly those related to vineyard management and winemaking operations.

2.2. Effect of grape variety

Amongst all the varieties, the wines from the Côtes de Gascogne (COL and GM) stood out the most due to their having predominant concentrations of most amino acids. The average concentrations of all amino acids for each variety are detailed in Table 4. In the studied wines, Pro was the predominant amino acid, which can be explained by the fact that yeasts do not utilise it during fermentation (Long *et al.,* 2012; Nishimura *et al.,* 2022). The concentrations of Pro varied depending on the variety: it represented nearly 70 % of the total amino acid content in GM wines (Figure 2), 40 % in COL wines, and 25 % in LL and MZ wines.

Pro is an amino acid that is highly characteristic of grape maturity: its concentrations can increase by a factor of 25 to 30 during maturation due to the transformation of Arg. Furthermore, Pro is highly characteristic of certain grape varieties due to genetic factors, which are more determining than environmental factors (Stines *et al.,* 2000). Indeed, here, the concentrations of Arg and Pro vary by a factor of 10 to 15, depending on the variety (from 300 to 4600 mg/L for Pro). The Pro/Arg ratio remains relatively constant from one vintage to another within the same variety, which may be due to a more marked variety effect and/or technical practices

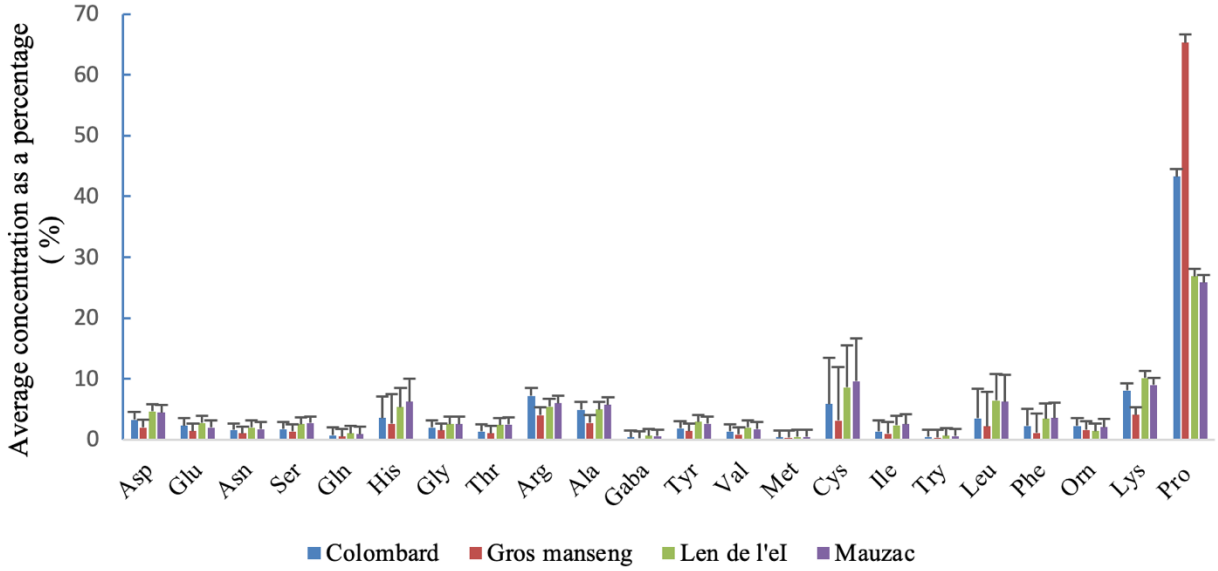


FIGURE 2. Average percentage of each amino acid relative to the total free amino acids in COL, GM, MZ and LL wines.

The bars represent the standard errors. Abbreviations: Asp (aspartic acid), Glu (glutamic acid), Lys (lysine), Arg (arginine), Asn (asparagine), Ala (alanine), His (histidine), GABA (gamma-aminobutyric acid), Leu (leucine), Gly (glycine), Val (valine), Phe (phenylalanine), Thr (threonine), Ser (serine), Ile (isoleucine), Tyr (tyrosine), Met (methionine), Try (tryptophan), Orn (ornithine), Gln (glutamine), Cys (cysteine), Pro (proline).

rather than to the vintage itself. Beyond a higher proportion of Pro in relation to the total amino acid content, wines from GM and COL stood out for their high concentrations of Pro. As mentioned earlier in the introduction, the GM and COL wines of the Côtes de Gascogne are highly sought after for their thiol aromas. Therefore, to optimise thiol concentrations, winemakers may add organic nitrogen to the musts (Dufourcq, 2018), in addition to the foliar nitrogen by spraying as described earlier, naturally impacting amino acid concentrations, including those of Pro. By contrast, wines from the Tarn did not stand out in terms of significant concentrations of Pro.

In addition to Pro, other amino acids were predominantly present in the four wine varieties (Figure 2): Lys, Arg, Cys, Ala, and His. The amino acid present in the highest concentrations after Pro was Lys, an amino acid generally found in wines. Most Lys content may be a result of yeast metabolism (McKinnon, 2013), but it is not preferred as a nitrogen source by *Saccharomyces cerevisiae* yeast (Mandl *et al.,* 2017). Arg was another major amino acid, with higher concentrations in COL and GM wines compared to LL and MZ wines. Differences in Arg content between the wines are likely due to differing Arg levels in the grape berries and resulting musts, which are influenced by nitrogen fertilisation (Soufleros *et al.,* 2003) and grape maturity (Ribéreau-Gayon *et al.,* 2006b). During grape maturation, this Arg is more often stored in the grape skin (Stines *et al.,* 2000). Therefore, it is likely that the COL wines exhibited higher concentrations of Arg, because they often undergo a cold pre-fermentation maceration phase after crushing, aimed at increasing thiol precursors in the must.

The wines from the Côtes de Gascogne showed lower levels of Cys, an amino acid involved in thiol precursor compounds (Roland *et al.,* 2011). Ala was also very abundant in the studied wines from Southwest France. One of the main nitrogen sources for yeasts, it is usually derived from pyruvic acid, either after the decarboxylation of aspartic acid or through transamination reactions (Ribéreau-Gayon *et al.,* 2006b).

It was possible to differentiate the four studied wine varieties from indigenous grapes of the Southwest of France by their amino acid content (Figure 3), with Pro being the variable that defined three distinct groups. GM wines had higher Pro levels compared to COL wines and the typical Tarn varieties, LL and MZ. This can be attributed either to the inherent characteristics of the grape variety itself or to the effects of any foliar treatments and, more generally, the winemaking process. Tarn wines, with lower Pro concentrations, were differentiated by their Glu content. LL wines seemed to be richer in Glu compared to MZ wines. Depending on the variety, Glu concentrations can increase during grape maturation (Kliewer, 1969): thus, it is possible that the advanced maturity of LL grapes at harvest - potentially linked to the winemaker's production objectives - was responsible for the higher Glu content in LL wines compared to that in MZ wines.

Amino acids present in wines from different wine regions were distinguished mainly based on two amino acids: Pro and Glu. These amino acids are known for their roles in the perception of taste in wines (Delompré *et al.,* 2019). Pro has been identified as contributing a sweet flavour and influencing the sensory properties of red wines by reducing astringency and bitterness, while increasing sweetness and viscosity (Espinase Nandorfy *et al.,* 2022). Furthermore, the presence

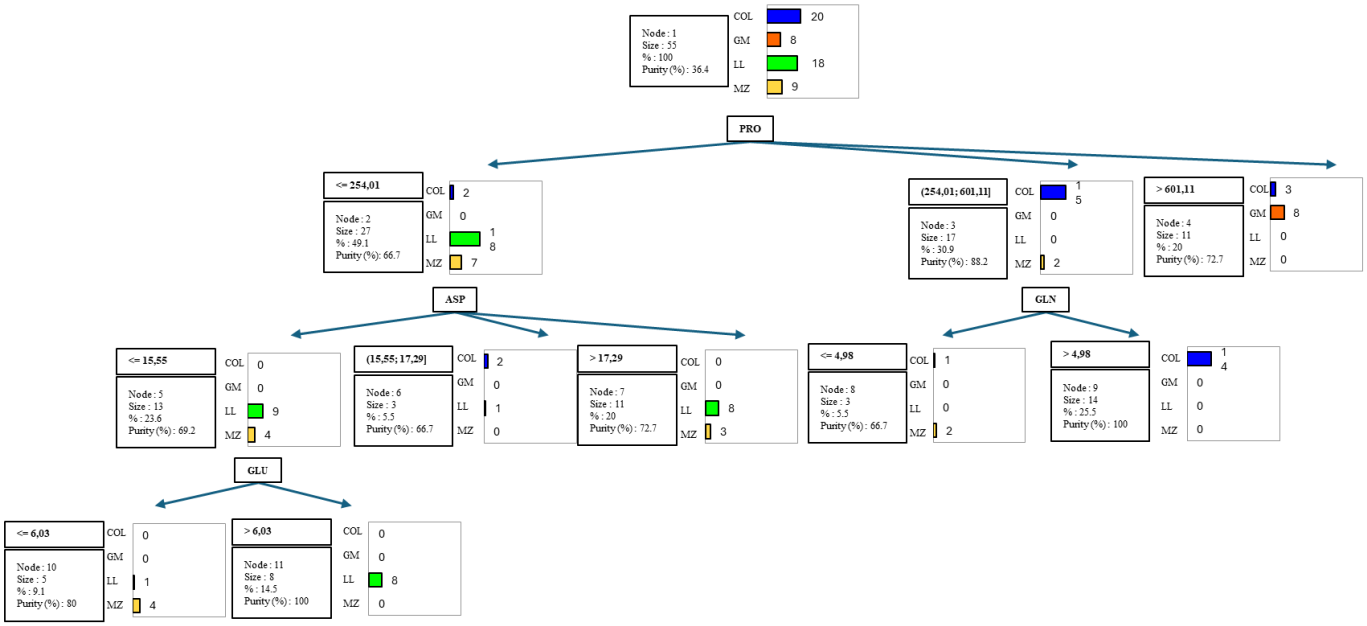


FIGURE 3. Classification and regression tree of all the amino acids measured in the wines.

COL = Colombard, GM = Gros Manseng, MZ = Mauzac, LL = Len de l'El, Pro = proline, Gln = glutamine, Asp = aspartic acid, Glu = glutamic acid.

of L-Glu affects the perception of umami in red wines (Espinase Nandorfy *et al.,* 2022). However, another study has shown that Glu influences the sensory profile of Italian wines but does not affect the umami taste (Franceschi *et al.,* 2023). Nonetheless, Glu is essential for enhancing other taste perceptions, such as saltiness and flavour intensity, as well as olfactory perceptions by increasing the intensity and persistence of aromas (Franceschi *et al.,* 2023).

Pro was present in high concentrations in the study wines (Figure 2). In the literature, there is no consensus on the role of Glu in white wines. In addition, no sensory study has been reported exclusively on the gustatory role of either Pro and Glu. Therefore, in the present study, these two amino acids were subject to a preliminary sensory study to assess their impact on the taste perceptions of a COL wine enriched with Glu and a MZ wine enriched with Pro.

3. Sensory characterisation

To assess the effect of Pro and Glu on the taste of the wine, the tasting panel was asked to perform a triangle test. The results are summarised in Table 6.

TABLE 6. Results of the triangle tests based on Glu and Pro (n = 30).

	Effect of Glu	Effect of Pro
Series	1	2
Number of correct answers/30 judges	14	15
α	5%	5%
p-value	0.09	0.043

Regarding Series 1 (Glu), the sensory data from the 30 judges (p-value = 0.09) were not significant, meaning the judges did not perceive a difference in taste between the COL wine and the COL + Glu wine. However, the panel used in this study was mostly untrained, thus it would be interesting to repeat this test with a trained panel.

Furthermore, to highlight the nature of the perceived difference, the panel was asked to rate a specific descriptor for each sample. When analysing the descriptors provided by the panellists who differentiated the samples (n = 14), it was found that, in most cases, the same descriptor was reported (Table 7). Indeed, the judges primarily distinguished the samples based on their acidity. The base wine was perceived as acidic, and this descriptor was not found in the wine supplemented with Glu, which was characterised by a lower perception of acidity. Additionally, the presence of Glu tended to enhance the bitterness for two panellists. Although the perceived difference between these two aspects was not significant, the observed descriptors suggest that Glu plays a role in balancing the wines by softening the acidity. In the literature, umami flavour in wine has not been clearly established (Klosse, 2013; Vilela, 2017), but the role played by Glu in enhancing or modulating other gustatory perceptions has been previously highlighted (Franceschi *et al.,* 2023).

Regarding Series 2 (Pro), the sensory data (p = 0.043) indicated that the judges perceived a taste difference between MZ and MZ + Pro. The descriptors listed by each judge to discriminate the samples are shown in Table 7.

Among the 30 judges who participated in the sensory test, the majority differentiated the samples when the distinctive one was the base wine enriched with Pro. The judges who perceived a gustatory difference (n = 15) primarily described the enriched Pro sample as sweeter, while the base wine was

TABLE 7. Summary table of the sensory tests (triangle test) studying the impact of Glu and Pro on the taste perceptions of Col and MZ wine.

COL wine		COL wine + Glu	
Enumerated descriptors	Occurrence	Enumerated descriptors	Occurrence
Sparkling	3	Bitterness	1
Acidic	9	Low acidity	2
Low bitterness	1	Less sparkling	1
MZ wine		MZ wine + Pro	
Enumerated descriptors	Occurrence	Enumerated descriptors	Occurrence
More astringent	2	Sweeter	7
Less sweet	1	Less astringent	3
Strong bitterness	2		

Only the descriptors listed by the judges who discriminated the samples are summarised in this table.

perceived as being more astringent and less sweet, and as having a marked bitterness. These results are consistent with existing studies on the impact of proline on wine sensory perceptions. For example, Espinase Nandorfy *et al*. (2022) showed that the presence of proline in red wines increased their sweetness and viscosity while decreasing the sensations of astringency and bitterness. The present results are particularly interesting since proline has been identified as having a sweet flavour (Schiffman & Sennewald, 1981), and sweetness is generally positively appreciated in white wine tasting, being the first impact that proline has on Mauzac wine sensory perceptions (particularly on taste), when using a nose clip.

CONCLUSION

Using a large sample of wines, this study aimed to examine the specificities of monovarietal white wines in terms of their amino acid composition. Grape variety seemed to be the main factor differentiating MZ, LL, COL and GM wines.

The HPLC analysis was carried out to differentiate these four grape varieties based on two key amino acids: Pro and Glu, known for their effects on the sensory perception of wines. A triangle test showed that Pro increased the sweetness of the wines, which aligns with the findings in the literature. On the other hand, the effect of Glu on the gustatory perception was not significant. This can be partly due to the lack of training of the sensory panel and to difficulties associated with tasting using a nose clip.

The role of Pro in the gustatory perception of a white wine was clearly established in this study. This amino acid, which is not consumed by yeasts during AF, is abundant in wines, and its concentrations are influenced by grape variety. Therefore, this sweet flavour could be sought after by winemakers and adjusted by modifying certain parameters, including choices made in the vineyard and during the winemaking process.

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