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SPECIAL ISSUE

Changes in amino acid composition of still Chenin blanc wine during ageing on lees

Chantal Maury[1,*], Vanessa Lançon-Verdier[1], Julie Frachet[1], Daoud Ounaissi[1], Renata Villalobos Enriquez[1,2], Margaux Renaud[2], François Litoux-Desrues[2]

[1] GRAPPE, Ecole Supérieure des Agricultures, USC 1422 INRAE, SFR 4207 QUASAV, 55 rue Rabelais, BP 30748, 49007 Angers Cedex 01, France
[2] CHENE & CIE, 5 rue Magellan, 75008 Paris, France

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*correspondence:
c.maury@groupe-esa.com

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ABSTRACT

Amino acids are known to affect the aromatic profile of wines, mainly because amino acids contribute to yeast nutrition during winemaking. While the latter has often been addressed in research, studies are lacking regarding the effect of amino acids during wine ageing, even though wine perception depends on a wine's final composition and probably on its amino acid composition. This study thus aimed to assess changes in the amino acid composition of still Chenin blanc wine during ageing on lees, a process that occurs after alcoholic and malolactic fermentation. In 2021 and 2022, grapes were collected in three vineyards representing different terroirs in the Loire Valley. Fermentation occurred in new and old barrels. After undergoing alcoholic and malolactic fermentation, the wine was aged on lees for six months, and amino acid composition was monitored using LC-MS analysis (direct injection). The results clearly show that amino acid concentration correlated with the week of ageing and vineyard; there was also an effect of the interaction between vintage and barrel age (old *vs* new). Aspartic acid, proline, and glutamic acid were the main amino acids found in the wines. Globally, three types of changes were seen: most amino acids (including glutamic acid and serine) increased in concentration, some amino acids decreased in concentration (alanine and tryptophan), and one amino acid (Leu) displayed inconsistent patterns.

KEYWORDS: IVAS 2024, amino acid, white wine, ageing on lees, vineyard, barrel

INTRODUCTION

Wine ageing on lees is usually carried out when creating high-quality wines (Fornairon-Bonnefond *et al.*, 2001). Yeasts are not removed from the wine, and the contact between the wine and the non-viable yeasts, called lees, leads to changes in wine composition and sensory properties, notably increased roundness and fullness (Losip *et al.*, 2022) and differences in aroma characteristics (Juega *et al.*, 2015).

Wines aged on lees are richer in polysaccharides, and particularly in mannoproteins, which improves protein (Fornairon-Bonnefond *et al.*, 2001) and tartaric (Rodriguez-Nogales *et al.*, 2012) stability. Ageing on lees also induces a release in free amino acids in wine, whose profile depends on alcohol level, pH, and the identity of the yeast strains (Guilloux-Bénatier & Chassagne, 2003), as well as on grape variety (Moreno-Arribas *et al.*, 1998). Most research on this topic has focused on how amino acids influence yeast-produced aromas or bacteria catabolism (Fernández & Zúñiga, 2006; Pereira *et al.*, 2021).

Furthermore, amino acids contribute to the taste of wines and, in the context of ageing on lees, they help shape mouthfeel. For example, a few amino acids have been reported to underlie the perception of umami in beverages like tea, sake, and Champagne. Umami is the fifth taste, after acidity, bitterness, saltiness, and sweetness (Lindemann *et al.*, 2002); it means "savoury" and corresponds to the palatable taste of perceived satisfaction, which has been described using the words "amplitude", "mouth fullness", and "bloom" (Yamaguchi & Ninomiya, 2000). The reference for umami is sodium glutamate (Ferrer-Gallego *et al.*, 2014). Glutamic acid, alanine, and aspartic acid have been reported to contribute to the perception of umami (Shan *et al.*, 2024; Kaneko *et al.*, 2006; Zhao *et al.*, 2016; Rotzoll *et al.*, 2006), mainly in the case of soy sauce. It should be noted that the pH of soy sauce means that glutamic acid and aspartic acid are present in their carboxylate forms. Phenylalanine and tyrosine can enhance the perception of umami associated with monosodium glutamate but only in the presence of NaCl (Lioe *et al.*, 2005). Shan *et al.* (2024) showed that asparagine at 5.3 mg/L, glutamine at 39.00 mg/L, phenylalanine at 11.49 mg/L, or histidine at 1.57 mg/L could increase the perception of umami associated with a solution of glutamic acid at 53.2 mg/L. Furthermore, an overall high concentration of amino acids has been linked with the perception of umami (Chen *et al.*, 2020; Schmidt *et al.*, 2021).

Given this background, it would be interesting to better understand amino acid release during ageing on lees and the relationship with potential perceptions of umami in wines, a subject which lacks clarity (Fornairon-Bonnefond *et al.*, 2001; Ferrari & Feuillat, 1988) and sufficient research. A limited number of studies have explored wine ageing kinetics, and there is very little research on the comparative effects of vintages, barrel age, or vineyards. This exploratory study thus aimed to examine the impacts of still white wine ageing on amino acid composition while accounting for the influence of vintage, vineyard, and barrel age.

MATERIALS AND METHODS

1. Chemicals

Standards of 20 L-amino acids, as well as of acetonitrile and formic acid, were purchased from Sigma-Aldrich. All standards had a purity level that exceeded 99.5 %, and the solvents were LC/MS grade. Ammonium formate was obtained from Fluka. Milli-Q water was produced using a water purification system from Merck.

2. Samples

In 2021 and 2022 (the two study vintages), Chenin blanc grapes were harvested at a maturity level selected by the winemaker collaborating on the project; the grapes came from three different vineyards (hereafter called A, B and C) with surface areas of about 1 ha located in the Loire Valley of France. The soil in vineyard A was mainly sandstone schist with sandy loam on the surface; the soil in vineyard B was mainly schist with sandy loam on the surface; and the soil in vineyard C was mainly volcanic (spilite) with sandy clay on the surface.

The 2021 vintage saw early bud break due to a mild winter, followed by spring frosts and cool, wet conditions in summer, slowing down the growth and ripening of grapes. In contrast, the 2022 vintage, marked by spring frosts but a hot, dry summer, favoured early and uniform ripening, with low disease pressure despite water stress partially offset by summer rains.

The barrels used in the study were Pure T (low toasting) as well as 172M and 179M (medium toasting); they measured 400 or 500 L and were obtained from Taransaud®. Neither barrel production type nor volume was considered in this study.

For each vintage, grapes were harvested at maturity (at around 22 °Brix and a pH of 3.2). In 2021, the °Brix, pH, and titratable acidity (eq. H_2SO_4 g/L) values were 23.0, 3.3, and 5.10 respectively for vineyard A; 23.0, 3.2, and 5.62 for vineyard B; and 21.6, 3.1, and 6.84 for vineyard C. In 2022, they were 21.2, 3.4, and 4.17 for vineyard A; 23.9, 3.3, and 4.53 for vineyard B; and 22.1, 3.3, and 4.26 for vineyard C.

Before the study, the winemaker had selected yeasts from his vineyards and then prepared inocula using these selected yeasts and autochthonous yeasts. These inocula were added to different musts: the 02/10, 27/09, and 29/09 musts in 2021 for vineyards A, B and C, respectively, and the 17/09 musts in 2022 for vineyard A and the 11/09 musts in 2022 for vineyards B and C. After settling and at the start of alcoholic fermentation (AF), the musts were transferred into new barrels (NB) and old barrels (OB), in which fermentation and ageing proceeded. The wine cellar was kept at a temperature of 20 °C during fermentation (AF and malolactic fermentation (MLF)). Following AF, density values were measured. MLF was spontaneous and was monitored using periodic analyses of malic and lactic acid levels. AF and MLF reached completion about a month after inoculation for AF, marking the start of ageing.

Ten and eleven wines (representing the combinations of vineyards × barrels) for 2021 and 2022, respectively, were analysed over the 29 weeks of ageing. The study started after AF and MLF had been completed (*i.e.*, week 6 for the 2021 vintage and week 7 for the 2022 vintage) and finished 29 weeks later (*i.e.*, week 35 for the 2021 vintage and week 36 for the 2022 vintage). This study applied a partial factorial design, because it was conducted under real-life conditions in an actual winery with low yields (Figure 1): old barrels were used in all combinations (since old barrels are the most common ones used in the region), and new barrels were used in a subset of combinations.

In 2021, 15 mg/L of OptiFlore® (Lamothe–Abiet) was added to the must of vineyard B, whose level of assimilable nitrogen was only 142 mg/L. In 2022, the musts of all three vineyards had assimilable nitrogen levels of around 100 mg/L. They were thus supplemented with NUTRISTART® (Laffort): with 40 g/hL in the case of vineyards B and C and with 50 g/hL in the case of vineyard A. Just after MLF had finished, 1 g/hL of SO_2 was added to all the wines. During ageing, light stirring was performed approximately four times. All the wines were collected in 75 cL bottles, after very light stirring to avoid moving the lees in the deepest part of the barrels. Upon arrival in the laboratory, samples of the wines were distributed among 50 mL Eppendorf tubes and stored at –70 °C.

3. Amino acid analyses

Amino acid analyses were carried out using an Agilent LC 1260 Infinity II System with a mass detector and a simple quadrupole Agilent LC-MSD iQ System. Chromatographic separation was performed using an Agilent InfinityLab Poroshell 120 HILIC-Z (150 × 2.1 mm, 2.7 µm particle size) with a guard column. The two mobile phases were each composed of 10 % ammonium formate buffer (pH 3.4) and either 90 % water (mobile phase A) or 90 % acetonitrile (mobile phase B). A gradient mixture of the two mobile phases was used in the analysis (flow rate of 0.4 mL/min at 30 °C) as follows: 0–10.5 min: 0 to 11 % of A; 10.5–15 min: increase to 12 % of A; 15–24 min: increase to 25 % of A. Three microlitres were injected into the chromatographic system.

The amino acids present (Table 1) were detected by mass spectroscopy. Electrospray ionisation (spray voltage: 3,500 V) was applied, and SIM spectra were recorded in positive ion mode. During the analysis, the source temperature was 350 °C, the fragmentation voltage was 80 V, and the gas flow was fixed at 8 L/min. A stock solution (the 20 amino acid standards in the A mobile phase) was used for calibration. Before the injection, each solution was filtered through a PTFE 0.22 µm filter. Calibration was carried out on the mass spectra using the specific ion extracted chromatograms and employing nine points of calibration.

The method has been validated by the fact that all amino acids were clearly identified thanks to their RT and their *m*/*z*. The calibration curves were all linear ($R^2 \geq 0.99$ except for Arg, 0.97) except for Pro, a quadratic equation was preferred ($R^2 = 1.00$). The LOD (Limit of Detection) and LOQ (Limit of Quantification) are available in Table S1. All standards were injected at days 1, 2, and 3 and no significant difference was observed. The variation coefficients of the repeatability of the standard preparation and injection comprised between 0 and 4 %, and 6 % was reached for Asp with respect to the repeatability of the preparations.

The wines were thawed the day prior to running the analyses and were then kept at 4 °C. The analyses were carried out on three 50 mL samples of each wine that had been centrifuged and filtered (PTFE 0.22 µm filter) beforehand.

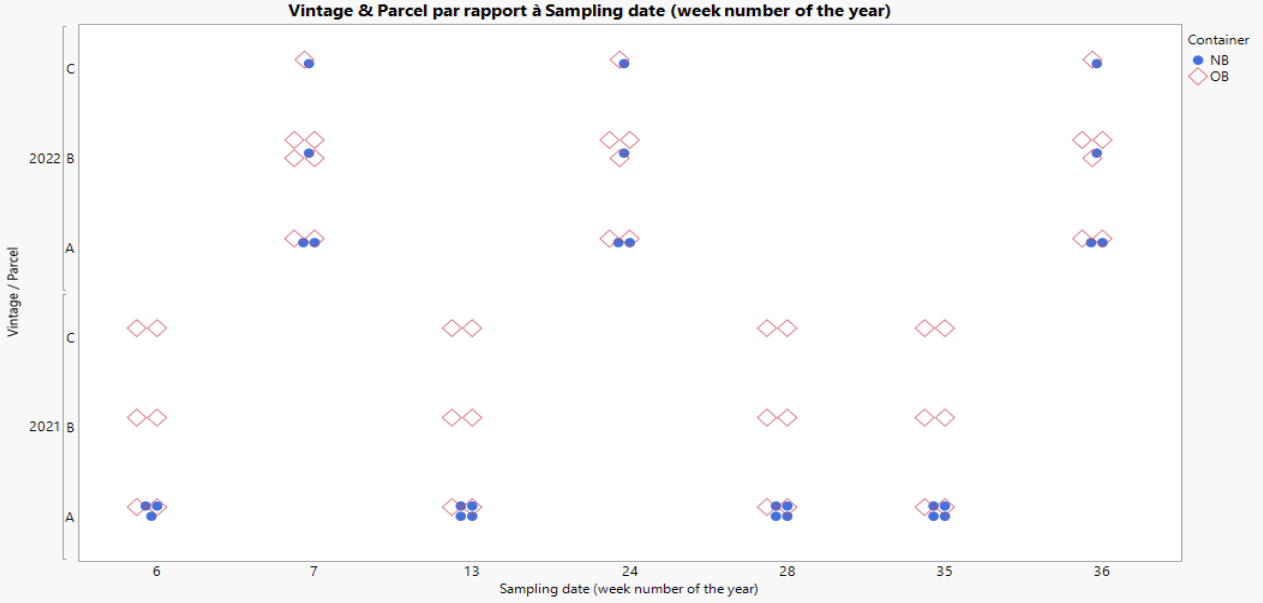


FIGURE 1. Experimental design: analyses were conducted on wines of two vintages (2021 and 2022) created with grapes from three vineyards (A, B, and C) sampled over 29 weeks of ageing. NB = new barrel, OB = old barrel.

TABLE 1. Amino acids characterised (quantifier *m/z* in positive mode, retention time (RT), and qualifier ions).

Molecule	Short name	*m/z*	RT	Other *m/z* qualifiers
Alanine	Ala	90.1	13.6	none
Arginine	Arg	175.2	22.0	70.1; 60.1
Asparagine	Asn	133.1	15.3	none
Aspartic acid	Asp	134.1	20.0	88.1; 116.1
Cysteine	Cys	122.1	13.2	76.1
Glutamine	Gln	147.1	15.2	none
Glutamic acid	Glu	148.1	18.2	130.1; 84.1
Glycine	Gly	76.1	14.6	none
Histidine	His	156.1	19.9	110.1
Isoleucine	Ile	132.2	9.5	86.1
Leucine	Leu	132.2	9.7	86.1
Lysine	Lys	147.1	23.4	84.1
Methionine	Met	150.2	10.1	104.1; 61.0
Phenylalanine	Phe	120.1	7.6	166.1; 103.1
Proline	Pro	116.1	12.3	70.1
Serine	Ser	106.1	15.2	60.1; 88.1
Threonine	Thr	120.1	13.6	74.1; 56.1; 103.1
Tryptophan	Trp	205.1	7.8	146.1
Tyrosine	Tyr	182.2	11.0	136.1
Valine	Val	118.1	12.3	72.1; 55.1

4. Data analysis

The replicates comprised the wines in the new barrels and the wines in the old barrels that had undergone the various combinations of vintage and vineyard in the partial factorial design.

Two-way ANOVAs were performed for each vintage-vineyard combination where the concentration of a given amino acid was the response variable, and the explanatory variables were sampling period (*i.e.*, week of ageing) and barrel age. When sampling period was significant, post-hoc pairwise comparisons (Bonferroni correction) were conducted to identify which periods differed significantly. All the tests employed a 95 % confidence level.

To evaluate all the variables simultaneously, a series of linear mixed-effects models was performed, where the concentration of a given amino acid was the response variable; vintage, vineyard, and sampling period (and their interactions) were the fixed effects; and barrel age was a random effect. Barrel age was treated as a random effect, because repeated measurements were obtained for the same barrels over time and barrel number was limited. This approach aimed to account for the unbalanced design resulting from the fact that not all factor combinations were present, which prevents the inclusion of all higher-order interactions in a standard ANOVA. All data analysis was performed using R software.

RESULTS AND DISCUSSION

The study's objective was to characterise the amino acid content of still Chenin blanc wine during ageing on lees in oak barrels. As Cys was not detected in the samples, it was excluded from the analyses.

1. Amino acid contents

There were clear differences in amino acid concentrations between the two vintages (Table 2), all other factors combined (sampling period, barrel age, and vineyard).

First, total amino acid concentrations were 962.29 ± 237.27 mg/L in 2021 and 569.77 ± 93.87 mg/L in 2022. These values fell within the expected ranges of 235–1,812 mg/L and 417–2,121 mg/L, respectively, according to Mirás-Avalos *et al.* (2020) and Moreno-Arribas *et al.* (1998).

Asp occurred at very high concentrations in the wines of both vintages (435.30 mg/L in 2021). It was also the amino acid with the highest relative levels, since it accounted for 45 % and 59 % of total amino acid content in 2021 and 2022, respectively. Pro was the second most abundant amino acid in terms of concentration (299.23 mg/L in 2021) and accounted for 31 % and 20 % of total amino acid content in 2021 and 2022, respectively. The third most abundant amino acid was Glu (34.04 mg/L in 2021), which accounted for 4 % and 5 % of total amino acid content in 2021 and

TABLE 2. Mean concentrations (mg/L) of all the amino acids present in the wines of each vintage (2021 and 2022; all other factors combined). The presence of different letters (A, B) within a row indicates a significant difference due to vintage.

Amino acid	Vintage	
	2021	2022
Ala	24.43 ± 5.59 ^{A}	12.47 ± 1.91 ^{B}
Arg	20.37 ± 8.17 ^{A}	17.49 ± 8.33 ^{B}
Asn	11.87 ± 3.00 ^{A}	6.75 ± 1.17 ^{B}
Asp	435.30 ± 103.39 ^{A}	336.63 ± 70.22 ^{B}
Gln	7.59 ± 2.77 ^{A}	2.52 ± 0.86 ^{B}
Glu	34.04 ± 5.79 ^{A}	25.73 ± 4.02 ^{B}
Gly	9.14 ± 2.59 ^{A}	3.06 ± 0.68 ^{B}
His	17.52 ± 6.70 ^{A}	7.23 ± 3.17 ^{B}
Ile	5.81 ± 2.64 ^{A}	2.04 ± 0.57 ^{B}
Leu	23.33 ± 5.62 ^{A}	12.63 ± 4.18 ^{B}
Lys	9.58 ± 3.54 ^{A}	3.28 ± 1.03 ^{B}
Met	5.86 ± 1.57 ^{A}	2.04 ± 0.47 ^{B}
Phe	20.37 ± 4.78 ^{A}	9.54 ± 1.59 ^{B}
Pro	299.23 ± 120.02 ^{A}	112.52 ± 21.89 ^{B}
Ser	7.78 ± 2.53 ^{A}	3.30 ± 0.91 ^{B}
Thr	7.22 ± 2.08 ^{A}	3.46 ± 0.70 ^{B}
Trp	6.25 ± 1.36 ^{A}	0.93 ± 0.26 ^{B}
Tyr	9.54 ± 2.77 ^{A}	5.20 ± 1.18 ^{B}
Val	7.93 ± 2.54 ^{A}	2.96 ± 0.80 ^{B}
Total	962.29 ± 237.27 ^{A}	569.77 ± 93.87 ^{B}

2022, respectively. These three amino acids (*i.e.*, Asp, Pro, and Glu) accounted for 80–83 % of the total amino acid content in wines of both vintages.

All the amino acids had significantly higher concentrations in the 2021 wines than in the 2022 wines, indicating the effect of vintage (Table 3; $p < 0.05$); the only exceptions were Asp and Arg (Table 2).

Previous studies have observed an overall effect of vintage on amino acid concentrations (Ferrari & Feuillat, 1988), which appears to be particularly related to weather conditions (Ortega-Heras *et al.*, 2014). Local climate conditions in combination with soil composition (notably available nitrogen levels) can induce different grape ripening levels, as has already been shown for red grapes (Hernandez-Orte *et al.*, 1999; Garde-Cerdán *et al.*, 2018). To compensate for that effect, grapes were harvested later in 2021 than in 2022. The ripeness level was generally higher in 2022 than in 2021, suggesting that grape maturity could not explain the differences in amino acid concentrations observed in this study.

It is important to note the relatively large standard deviation values (Table 2), in particular those for Arg (40–48 %), Gln (34–36 %), His (38–44 %), and Lys (31–37 %) for both vintages and those for Pro (40 %) and Ile (45 %) for the 2021 vintage. This fact suggests heterogeneity in the data that is linked to the different combinations of factors, including vineyard, sampling period, and barrel age.

2. Changes in amino acid concentrations during wine ageing in old barrels

Patterns were seen in the amino acid concentrations in the wines from 2021 (Tables 4, S2 and S3) and the wines from 2022 (Tables 5, S4 and S5) The significance (*p*-value) of the factors (sampling period, barrel age, and their interactions) are indicated in the tables as well.

First, wines of the two vintages and the three vineyards aged in old barrels were considered.

Some amino acids displayed the same patterns across vintages and vineyards. Ser concentrations increased significantly across the 29 weeks of ageing (+46 %). For instance, this rise was 61 % in 2021 wines from vineyard C. While Glu, Lys, Phe, and Thr concentrations displayed different patterns over the course of ageing, they were all significantly higher at the end than at the beginning across the two vintages and three vineyards. For example, Glu concentrations rose by 40 % in 2021 wines from vineyard A (Table 4). Thr is a precursor of sotolon, a lactone associated with nutty, curry, and oxidised aromas (Thuy *et al.*, 1995).

TABLE 3. Significance of the vintage, vineyard, and period effects and their interactions (*p*-values). ns: not significant.

Amino acid	Vintage	Vineyard	Period	Vintage: vineyard	Vintage: period	Vineyard: period	Vintage: vineyard: period
Ala	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001
Arg	ns	< 0.05	< 0.0001	ns	< 0.0001	ns	< 0.05
Asn	< 0.01	< 0.01	< 0.001	< 0.05	< 0.05	< 0.01	ns
Asp	ns	< 0.0001	< 0.0001	ns	< 0.0001	< 0.001	< 0.0001
Gln	< 0.0001	< 0.0001	< 0.0001	< 0.05	< 0.0001	< 0.05	ns
Glu	< 0.05	ns	< 0.01	ns	< 0.0001	ns	ns
Gly	< 0.0001	< 0.0001	< 0.0001	< 0.01	< 0.0001	< 0.0001	< 0.0001
His	< 0.0001	0.0075	< 0.0001	< 0.05	< 0.0001	< 0.05	ns
Ile	< 0.01	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001
Leu	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Lys	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Met	< 0.0001	< 0.0001	< 0.0001	< 0.01	< 0.01	< 0.01	< 0.001
Phe	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001
Pro	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001
Ser	< 0.001	< 0.001	< 0.0001	< 0.01	< 0.0001	< 0.01	< 0.001
Thr	< 0.0001	< 0.01	< 0.0001	< 0.01	< 0.0001	< 0.0001	< 0.001
Trp	< 0.0001	< 0.0001	< 0.0001	< 0.01	< 0.0001	< 0.0001	< 0.0001
Tyr	< 0.001	< 0.001	< 0.0001	< 0.05	< 0.0001	ns	< 0.001
Val	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001
Total	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001

Although Thr concentrations generally increased over the 29 weeks of ageing, there were periods of decline, probably due to acid degradation; the latter could potentially have led to the formation of volatile compounds like sotolon in the presence of acetaldehyde, after a step of deamination (Thuy *et al.*, 1995). Acetaldehyde could have been produced by the oxidation of ethanol in the presence of H_2O_2, itself generated by O_2 oxidation in the presence of copper or iron, and in the absence of ascorbic acid (Barril *et al.*, 2016). The absence of ascorbic acid was indeed verified. In red wines, acetaldehyde react with phenolic compounds (Han *et al.*, 2019). In a white wine matrix with a low content of phenolic compounds, acetaldehyde might accumulate longer.

Certain amino acids displayed inconsistent patterns over the course of ageing. Gly concentrations generally increased, except in 2022 wines from vineyards A and C, for which there was no change. Similarly, Tyr concentrations generally increased, except in 2022 wines from vineyards A and B, in which there was no change; His and Asp concentrations generally increased, except in 2022 wines from vineyard B and in 2021 wines from vineyard A, in which they were unchanged. Fiechter and Mayer (2011) also observed an increase in Asp concentrations among control wines and wines that had aged three and six months on lees and that had been made using three different fermentation yeasts and Grüner Veltliner grapes; similar results were seen by Ferrari and Feuillat (1988) in Chardonnay. Val concentrations generally increased, except in 2022 wines from vineyard A and in 2021 wines from vineyard B, in which there was no change. Val is one of the main amino acids released by peptide hydrolysis (Alexandre *et al.*, 2001). Asn concentrations generally increased, except in 2022 wines from vineyard A, in which a decrease was seen, and in 2022 wines from vineyard C, in which no change was seen.

Consequently, total amino acid content (*i.e.*, the sum of all the amino acid concentrations) also significantly increased during ageing: from 12.7 % (2021 vineyard B) to 35.4 % (2022 vineyard B), the greatest increases being in 2022 wines. The release of nitrogen indicates that there was autolytic activity linked to protease activity during ageing (Kemp *et al.*, 2015). Indeed, in model solutions, Asp, His, Thr, and Ser have been seen to appear during the autolysis of lees (Alexandre *et al.*, 2001). The occurrence of Thr and Ser could reflect the degradation of mannoproteins that had been released by glucanase activity during ageing (Alexandre *et al.*, 2001). In addition, Asn, Asp, Gln, Glu, Ser, and His were found to originate from the activity of peptide hydrolyses (Alexandre *et al.*, 2001) and from passive diffusion through the cellular membrane. This could explain the variability of the changes in Asn, Gln, and His concentrations over the course of ageing.

TABLE 4. Amino acid concentrations (mg/L) in wines in barrels of different ages (new barrels [NB] *vs* old barrels [OB]) over the course of ageing (sampling period—week) for the 2021 vintage from vineyard A (mean ± SD). In green: concentrations that were significantly higher than during the prior sampling period; in blue: concentrations that were significantly lower than during the prior sampling period. Within rows, the different letters (a, b, etc.) indicate a significant difference between sampling periods, and the different Greek letters (α, β , etc.) indicate a significant difference due to barrel age. Significance was based on *p*-values (variables: sampling period, barrel age, and sampling period-by-barrel age interaction [∩]). *: $0.001 \leq p < 0.05$, **: $p \geq 0.05$, ns: not significant.

Amino acid	Barrel	Vineyard A (2021)				Significant effect		
		Week 6	Week 13	Week 28	Week 35	∩	Period	Barrel
Ala	NB	28.98 ± 4.56	28.38 ± 2.42	21.77 ± 4.48	25.87 ± 4.90	**	**	*
	OB	35.41 ± 0.19	26.08 ± 1.82	21.96 ± 2.93	26.24 ± 0.73			
Arg	NB	21.68 ± 5.38	16.08 ± 5.67	7.40 ± 3.22	9.41 ± 2.68	**	**	**
	OB	32.41 ± 1.05	31.48 ± 1.13	23.36 ± 0.49	29.17 ± 0.70			
Asn	NB	12.03 ± 0.61	14.6 ± 0.40	13.82 ± 0.82	14.47 ± 0.85	*	**	**
	OB	12.14 ± 0.26	15.51 ± 0.23	14.73 ± 0.26	16.31 ± 0.10			
Asp	NB	493.53 ± 27.21 $^{ab,\alpha}$	538.14 ± 29.63 $^{a,\alpha}$	373.7 ± 95.72 $^{c,\alpha}$	474.25 ± 52.87 $^{b,\alpha}$	ns	**	*
	OB	500.25 ± 39.24 $^{ab,\beta}$	548.02 ± 17.24 $^{a,\beta}$	431.02 ± 12.18 $^{c,\beta}$	521.26 ± 7.17 $^{b,\beta}$			
Gln	NB	6.52 ± 1.01 $^{a,\alpha}$	12.73 ± 0.60 $^{b,\alpha}$	6.64 ± 0.62 $^{a,\alpha}$	6.31 ± 0.82 $^{a,\alpha}$	ns	**	**
	OB	6.52 ± 0.26 $^{a,\alpha}$	13.3 ± 0.07 $^{b,\beta}$	6.97 ± 0.17 $^{a,\beta}$	7.25 ± 0.23 $^{a,\beta}$			
Glu	NB	32.90 ± 2.47	38.47 ± 1.55	35.26 ± 1.22	42.44 ± 1.53	**	**	ns
	OB	31.36 ± 0.56	39.51 ± 0.34	35.78 ± 0.60	43.92 ± 0.34			
Gly	NB	9.41 ± 0.52 $^{a,\alpha}$	10.84 ± 0.56 $^{b,\alpha}$	11.10 ± 0.77 $^{b,\alpha}$	12.02 ± 0.76 $^{c,\alpha}$	ns	**	*
	OB	9.39 ± 0.18 $^{a,\beta}$	11.31 ± 0.17 $^{b,\beta}$	11.7 ± 0.23 $^{b,\beta}$	12.94 ± 0.13 $^{c,\beta}$			
His	NB	19.79 ± 2.46	23.14 ± 1.21	16.19 ± 0.46	27.54 ± 1.80	**	**	**
	OB	14.66 ± 4.03	21.82 ± 1.38	15.66 ± 0.36	28.13 ± 0.91			
Ile	NB	6.32 ± 0.78 $^{a,\alpha}$	6.59 ± 0.62 $^{a,\alpha}$	6.89 ± 0.31 $^{a,\alpha}$	7.02 ± 0.45 $^{a,\alpha}$	ns	ns	ns
	OB	6.37 ± 0.63 $^{a,\alpha}$	6.43 ± 0.43 $^{a,\alpha}$	6.99 ± 0.22 $^{a,\alpha}$	7.23 ± 0.13 $^{a,\alpha}$			
Leu	NB	26.49 ± 1.89 $^{a,\alpha}$	27.12 ± 2.22 $^{a,\alpha}$	27.61 ± 1.61 $^{a,\alpha}$	28.79 ± 1.76 $^{a,\alpha}$	ns	ns	ns
	OB	26.94 ± 0.49 $^{a,\alpha}$	27.58 ± 1.49 $^{a,\alpha}$	25.91 ± 6.39 $^{a,\alpha}$	27.93 ± 6.18 $^{a,\alpha}$			
Lys	NB	8.89 ± 0.32 $^{a,\alpha}$	12.13 ± 0.54 $^{b,\alpha}$	14.34 ± 1.08 $^{c,\alpha}$	12.59 ± 0.87 $^{b,\alpha}$	ns	**	ns
	OB	9.86 ± 0.31 $^{a,\beta}$	12.25 ± 0.62 $^{b,\alpha}$	14.77 ± 0.34 $^{c,\alpha}$	12.46 ± 0.15 $^{b,\alpha}$			
Met	NB	6.57 ± 0.90	6.56 ± 0.58	7.07 ± 0.58	7.45 ± 0.52	**	*	*
	OB	6.13 ± 0.38	6.80 ± 0.25	7.48 ± 0.09	8.35 ± 0.35			
Phe	NB	20.87 ± 1.06 $^{a,\alpha}$	22.67 ± 1.66 $^{b,\alpha}$	24.62 ± 1.31 $^{c,\alpha}$	26.63 ± 1.21 $^{d,\alpha}$	ns	**	ns
	OB	21.79 ± 0.22 $^{a,\alpha}$	23.14 ± 1.3 $^{b,\alpha}$	24.3 ± 0.16 $^{c,\alpha}$	27.4 ± 0.43 $^{d,\alpha}$			
Pro	NB	348.88 ± 30.73 $^{a,\alpha}$	453.50 ± 18.02 $^{b,\alpha}$	384.29 ± 7.56 $^{c,\alpha}$	390.05 ± 6.87 $^{d,\alpha}$	ns	*	ns
	OB	315.63 ± 5.13 $^{a,\alpha}$	432.66 ± 33.29 $^{b,\alpha}$	359.52 ± 11.35 $^{c,\alpha}$	379.36 ± 6.97 $^{d,\alpha}$			
Ser	NB	7.26 ± 0.56 $^{a,\alpha}$	8.98 ± 0.48 $^{b,\alpha}$	10.06 ± 0.67 $^{c,\alpha}$	11.32 ± 0.80 $^{d,\alpha}$	ns	**	ns
	OB	7.09 ± 0.11 $^{a,\alpha}$	9.16 ± 0.2 $^{b,\alpha}$	10.17 ± 0.19 $^{c,\alpha}$	11.95 ± 0.16 $^{d,\alpha}$			
Thr	NB	8.42 ± 0.47 $^{a,\alpha}$	8.22 ± 0.69 $^{a,\alpha}$	7.73 ± 0.67 $^{b,\alpha}$	9.72 ± 0.54 $^{c,\alpha}$	ns	**	ns
	OB	8.56 ± 0.33 $^{a,\alpha}$	8.16 ± 0.29 $^{a,\alpha}$	7.2 ± 0.27 $^{b,\alpha}$	10.00 ± 0.26 $^{c,\alpha}$			
Trp	NB	5.09 ± 1.92	7.32 ± 0.35	7.60 ± 0.31	7.74 ± 0.14	**	**	**
	OB	6.76 ± 0.26	7.16 ± 0.22	7.53 ± 0.11	7.60 ± 0.12			
Tyr	NB	7.45 ± 0.74 $^{a,\alpha}$	9.68 ± 0.9 $^{b,\alpha}$	12.03 ± 0.83 $^{c,\alpha}$	13.30 ± 1.18 $^{d,\alpha}$	ns	**	*
	OB	8.17 ± 1.31 $^{a,\beta}$	10.34 ± 0.32 $^{b,\beta}$	12.69 ± 0.47 $^{c,\beta}$	14.55 ± 0.29 $^{d,\beta}$			
Val	NB	8.20 ± 0.43 $^{a,\alpha}$	9.30 ± 0.74 $^{b,\alpha}$	10.20 ± 0.49 $^{c,\alpha}$	11.07 ± 0.56 $^{d,\alpha}$	ns	**	ns
	OB	8.43 ± 0.10 $^{a,\alpha}$	9.49 ± 0.51 $^{b,\alpha}$	10.30 ± 0.18 $^{c,\alpha}$	11.45 ± 0.11 $^{d,\alpha}$			
Total	NB	1,079.26 ± 67.67 $^{a,\alpha}$	1,254.45 ± 25.29 $^{b,\alpha}$	998.32 ± 98.36 $^{c,\alpha}$	1,105.52 ± 119.54 $^{d,\alpha}$	ns	**	ns
	OB	1,067.87 ± 40.26 $^{a,\alpha}$	1,260.21 ± 56.28 $^{b,\alpha}$	999.25 ± 129.95 $^{c,\alpha}$	1,203.50 ± 9.30 $^{d,\beta}$			

TABLE 5. Amino acid concentrations (mg/L) in wines in barrels of different ages (new barrels [NB] *vs* old barrels [OB]) over the course of ageing (sampling period—week) for the 2022 vintage from vineyard A (mean ± SD). In green: concentrations that were significantly higher than during the prior sampling period; in blue: concentrations that were significantly lower than during the prior sampling period. Within rows, different letters (a, b, etc.) indicate a significant difference between sampling periods, and differences in Greek letters (α, β, etc.) indicate a significant difference due to barrel age. Significance was based on *p*-values (variables: sampling period, barrel age, and sampling period-by-barrel age interaction [∩]). *: $0.001 \leq p < 0.05$, **: $p \geq 0.05$, ns: not significant.

Amino acid	Barrel	Vineyard A (2022)			Significant effect		
		Week 7	Week 24	Week 36	∩	Period	Barrel
Ala	NB	13.69 ± 0.25 $^{a,\alpha}$	13.22 ± 0.20 $^{a,\alpha}$	11.71 ± 0.18 $^{b,\alpha}$	ns	*	ns
	OB	12.87 ± 1.32 $^{a,\alpha}$	13.91 ± 1.30 $^{a,\alpha}$	12.10 ± 0.84 $^{b,\alpha}$			
Arg	NB	9.40 ± 1.35	26.81 ± 0.68	25.95 ± 0.64	*	**	ns
	OB	6.18 ± 8.47	13.74 ± 3.05	18.17 ± 7.67			
Asn	NB	6.73 ± 0.42 $^{a,\alpha}$	6.77 ± 0.13 $^{a,\alpha}$	7.32 ± 0.13 $^{a,\alpha}$	ns	ns	ns
	OB	6.36 ± 0.58 $^{a,\alpha}$	6.61 ± 0.29 $^{a,\alpha}$	6.88 ± 0.90 $^{a,\alpha}$			
Asp	NB	322.09 ± 27.55 $^{c,\alpha}$	397.98 ± 2.36 $^{b,\alpha}$	440.17 ± 9.16 $^{c,\alpha}$	ns	**	**
	OB	309.77 ± 33.34 $^{a,\beta}$	365.71 ± 16.12 $^{b,\beta}$	383.30 ± 15.69 $^{c,\beta}$			
Gln	NB	3.51 ± 0.22 $^{a,\alpha}$	1.84 ± 0.06 $^{b,\alpha}$	1.98 ± 0.13 $^{b,\alpha}$	ns	**	ns
	OB	3.16 ± 0.27 $^{a,\alpha}$	1.92 ± 0.14 $^{b,\alpha}$	1.77 ± 0.17 $^{b,\alpha}$			
Glu	NB	23.48 ± 0.41	26.12 ± 0.35	28.54 ± 0.34	**	**	ns
	OB	24.29 ± 3.06	24.26 ± 2.05	29.73 ± 3.25			
Gly	NB	3.48 ± 0.17 $^{b,\alpha}$	4.13 ± 0.14 $^{a,\alpha}$	3.25 ± 0.09 $^{b,\alpha}$	ns	**	*
	OB	3.58 ± 0.27 $^{b,\beta}$	4.12 ± 0.29 $^{a,\beta}$	3.71 ± 0.22 $^{b,\beta}$			
His	NB	4.57 ± 0.21	7.91 ± 0.66	10.72 ± 0.77	**	**	**
	OB	9.37 ± 1.14	7.58 ± 1.34	13.13 ± 1.48			
Ile	NB	2.47 ± 0.15	2.62 ± 0.03	2.95 ± 0.13	*	**	**
	OB	2.42 ± 0.18	2.27 ± 0.22	2.62 ± 0.21			
Leu	NB	20.62 ± 0.72 $^{a,\alpha}$	12.64 ± 0.11 $^{b,\alpha}$	11.38 ± 0.28 $^{b,\alpha}$	ns	**	ns
	OB	18.06 ± 5.24 $^{a,\alpha}$	12.03 ± 0.74 $^{b,\alpha}$	12.62 ± 0.32 $^{b,\alpha}$			
Lys	NB	4.53 ± 0.07	4.58 ± 0.17	4.26 ± 0.10	**	ns	ns
	OB	4.00 ± 0.51	4.14 ± 0.52	4.90 ± 0.16			
Met	NB	2.29 ± 0.04 $^{a,\alpha}$	2.40 ± 0.04 $^{a,\alpha}$	2.17 ± 0.16 $^{a,\alpha}$	ns	**	ns
	OB	2.01 ± 0.13 $^{a,\alpha}$	2.15 ± 0.09 $^{a,\alpha}$	2.39 ± 0.30 $^{a,\alpha}$			
Phe	NB	9.27 ± 0.30	9.34 ± 0.08	11.56 ± 0.08	*	**	ns
	OB	10.28 ± 0.95	9.11 ± 0.30	11.64 ± 0.53			
Pro	NB	127.48 ± 5.15 $^{a,\alpha}$	122.01 ± 1.60 $^{b,\alpha}$	109.01 ± 0.96 $^{c,\alpha}$	ns	**	ns
	OB	130.54 ± 5.58 $^{a,\alpha}$	118.18 ± 9.58 $^{b,\alpha}$	114.62 ± 7.60 $^{c,\alpha}$			
Ser	NB	2.93 ± 0.07	4.21 ± 0.14	3.64 ± 0.15	**	**	ns
	OB	2.81 ± 0.02	3.95 ± 0.37	4.29 ± 0.19			
Thr	NB	3.28 ± 0.08	4.26 ± 0.07	3.28 ± 0.09	**	**	**
	OB	3.43 ± 0.13	4.34 ± 0.12	4.62 ± 0.11			
Trp	NB	1.33 ± 0.01 $^{a,\alpha}$	0.94 ± 0.13 $^{b,\alpha}$	0.87 ± 0.09 $^{c,\alpha}$	ns	**	ns
	OB	1.36 ± 0.03 $^{a,\alpha}$	1.10 ± 0.03 $^{b,\alpha}$	0.89 ± 0.09 $^{c,\alpha}$			
Tyr	NB	4.65 ± 0.20	4.36 ± 0.13	3.94 ± 0.18	**	**	*
	OB	5.30 ± 0.34	4.18 ± 0.24	5.77 ± 0.37			
Val	NB	3.41 ± 0.20	3.98 ± 0.04	3.64 ± 0.05	*	**	ns
	OB	3.44 ± 0.27	3.67 ± 0.38	3.87 ± 0.13			
Total	NB	569.22 ± 34.51	656.13 ± 2.23	686.35 ± 9.80	*	**	**
	OB	559.23 ± 41.6	602.96 ± 26.72	637.00 ± 14.69			

Two amino acids generally decreased in concentration over the course of ageing: Ala and Trp. For Ala, the decrease occurred in all wines, with the exception of 2022 wines from vineyard B. Previous research on sparkling wine has found Ala concentrations to slightly decrease between three and six months of ageing, followed by steady values (Sun *et al.*, 2024). Trp concentrations also significantly decreased in all wines, with the exception of 2021 wines from vineyards A and B.

For other amino acids, concentration patterns were consistent within vintage across vineyards but varied between vintages. Arg concentrations significantly increased over ageing in 2022 wines from all vineyards. In 2021 wines, the pattern was more varied: Arg concentrations decreased between week 6 and week 28 and then increased between week 28 and week 36, with final concentrations that were equal to concentrations in week 6 in the case of vineyard B or that were lower in the cases of vineyards A and C. In 2021 wines, Pro concentrations significantly increased over the course of ageing; in 2022 wines, they dropped between week 7 and week 24 and then remained relatively steady.

Met concentrations increased over the course of ageing in 2021 wines; meanwhile, in 2022 wines, they were stable in the cases of vineyards A and B, but decreased significantly in the case of vineyard C. Met is the precursor of methionol (*i.e.*, 3-methylthio-1-propanol), which imparts a boiled or cooked potato or cauliflower aroma in wine, and of methanethiol, which has sewage and rubber aromas (Cordente *et al.*, 2021). The former is generated via the Ehrlich pathway while the latter derives from a demethiolation step. Competition occurs between the enzymatic and the non-enzymatic reactions. The authors showed the impacts of the strains used for AF, methionol and methanethiol during Chardonnay ageing: the concentration of methionol was stable between 3 and 15 months of aging, whereas the concentration of methanethiol increased (Cordente *et al.*, 2021). The consumption of Met generated by the Strecker degradation has been shown in wine conditions in the presence of gallic acid, caffeic acid, or catechin (Oliveira *et al.*, 2017). That reaction needs a first step consisting in the oxidation of phenolic compounds into quinones. In addition, Met could also have been adsorbed by cell walls of lees via its -SH group by creating bisulfite bridge with the Cys of mannoproteins as thiols do (Fornairon-Bonnefond *et al.*, 2001). The decreased concentrations in 2022 wines from vineyard C could have resulted from Met's conversion into volatile compounds.

A clear vineyard effect, independent of vintage, was seen in Ile concentrations ($p = 1.85.10^{-8}$): they were stable in wines from vineyard A, decreased in wines from vineyard B, and increased in wines from vineyard C. From a study concerning model wine solutions close to our wines, the decrease of Ile could be due to non-enzymatic reaction leading to 2-methylbutanal having fruity odours (Pripis-Nicolau *et al.*, 2000). Aldehydes could be formed by Strecker degradation, which is a slow reaction.

Gln concentrations were relatively stable in wines from vineyards A and B. They significantly increased in 2021 wines from vineyard C up until week 13; they then decreased, but still remained higher than they had been at week 5. In 2022 wines, Gln concentrations declined during ageing. The patterns of Gln concentrations in 2021 wines from vineyard C were relatively consistent with results seen in Niagara and Goethe sparkling wines from the 2015 vintage (Sartor *et al.*, 2021): Gln concentrations increased in Chardonnay between three and six months of ageing and displayed a strong decrease afterwards; *i.e.*, after the second fermentation had finished. It may be possible to explain this heterogeneity by the fact that Gln is mainly the product of peptide hydrolysis (Alexandre *et al.*, 2001), and wine protein and peptide contents are linked to vintage (*i.e.*, weather and vine health) rather than grape ripeness (*i.e.*, ripening level indicator values).

Finally, it was not possible to explain the concentration patterns of one amino acid by vintage effect or vineyard effect alone: Leu concentrations generally increased over the course of ageing in 2021 wines from vineyards B and C, but remained steady in 2021 wines from vineyard A and in 2022 wines from all vineyards. Indeed, the statistical analysis showed that there was a significant interaction between all the factors (Table 3). The decrease in Leu concentrations during ageing could be due to the amino acid's conversion into volatile compounds (Sun *et al.*, 2024), and in particular into 3-methylbutanal – which is associated with an amylic odour – by non-enzymatic reactions, as suggested by the work of Pripis-Nicolau *et al.* (2000).

The effect of barrel age is complex, since amino acid concentrations were influenced by vintage, vineyard, and sampling period, and their interactions (Table 3), making it difficult to explain the resulting patterns. However, the dynamic shifts in amino acid concentrations seen for the two vintages could be explained by a previously described phenomenon (Fornairon-Bonnefond *et al.*, 2001; Guilloux-Bénatier & Chassagne, 2003; Gnoinski *et al.*, 2021) that comprises different stages. First, yeast starvation (due to the lack of reducing sugars) induces autophagy, which generates macromolecule degradation within cell vacuoles and an increase in membrane porosity. Autophagy seems to activate autolysis. Second, compounds in the cytoplasm, including peptides, fatty acids, nucleotides, and amino acids, are released through the cell membrane by passive diffusion. Third, after a lag period, cell wall constituents, such as proteins and polysaccharides, are released by the irreversible action of proteases, with glucanases releasing mannoproteins. Fourth, peptides are hydrolysed, generating new free amino acids in the medium (Alexandre *et al.*, 2001). Alexandre *et al.* (2001) proposed that the release of amino acids starts during the stationary growth phase of AF and is associated with cell death (Kemp *et al.*, 2015). Enzymatic activities are slow, which implies that ageing durations ranging from months to years could have a significant effect on wine quality (Gnoinski *et al.*, 2021). Tudela *et al.* (2012) observed visible changes in the ultrastructure of

sparkling wine ageing on lees after 18 months, but the cell walls remained unbroken even after 40 months. Fornairon-Bonnefond *et al.* (2001) reported different studies that indicate that enzymatic activities still occur during ageing; for example, proteases have been found to be active until five to seven months, but they showed maximum activity after two months (Alexandre *et al.*, 2001); meanwhile, the activity of esterases has been found to be significant only after 20 days on lies, and β-glucanases has shown activities for up to five months (Gnoinski *et al.*, 2021). This suggests that enzymatic reactions may occur during ageing, but probably to release amino acids, peptides and polysaccharides rather than to directly produce volatiles. Nonetheless, the consumption of amino acids seems to be mainly due to non-enzymatic reactions that result in the production of volatiles, from Ile, Leu, and Met (Pripis-Nicolau *et al.*, 2000). Further explanation cannot be given due to the lack of data concerning the evolution of amino acids during still-wine ageing on lees. The results of this study suggest that the decrease in concentrations of some amino acids over the course of ageing could be linked to their conversion to other molecules, like volatile compounds, or to their adsorption to the lees before being released again, due to further membrane enlargement.

3. Changes in amino acid concentration during wine ageing in new *versus* old barrels

Similar trends were observed in amino acid concentrations over the course of ageing, whether wines were in new or old barrels (vineyard A: Table 4 for 2021 and Table 5 for 2022; 2022 vineyard B: Table S4; 2022 vineyard C: Table S5).

Examining the results in greater detail, it is clear that barrel age did not significantly affect Phe and Trp concentrations during ageing or at the end of ageing, regardless of vineyard or vintage. However, Gly concentrations were significantly higher in wines aged in old barrels *versus* new barrels, regardless of vineyard or vintage; this difference was mainly observed at the end of ageing.

Barrel age had a significant effect on the concentrations of the main amino acids, regardless of vintage or vineyard, but its impact was rarely independent of sampling period (sampling period-by-barrel interaction; Tables 3, 4, 5, S4, and S5). Barrel age appeared to have the greatest influence on His, Trp, Ile, and Thr. Their later concentrations were not correlated with their initial concentrations at the start of ageing.

The results are unclear and they suggest that wine amino acid composition does not depend on whether the barrel is new or old, since it is likely that interactions occur between initial wine composition, period of ageing, barrel type, and barrel age. The main difference between new and old barrels is the oxygen transfer rate, which is higher for new barrels. Lees consume oxygen (Salmon *et al.*, 2000; Pons-Mercadé *et al.*, 2021), but oxygen is not considered to have an impact on autolysis at its start, given that protein degradation occurs, which protects wine from oxidation (Pons-Mercadé *et al.*, 2021). Oxygen spurs the production of free radicals via chemical reactions, which generate metabolites that are cytotoxic (Gnoinski *et al.*, 2021), leading to lower levels of cell viability and the earlier occurrence of autolysis (Salmon *et al.*, 2000).

Once ageing had ended (at week 35 or 36), one of two main results were obtained: 1) barrel age (NB/OB) had no difference on the evolution of amino acid concentrations; 2) wines in old barrels had globally higher amino acid concentrations than did wines in new barrels (except for four values). Therefore, the hypothesis that there was more oxygen in the new barrels may be an explanation for the latter results. The presence of oxygen in contact with lies generates oxygen peroxide (Salmon *et al.,* 2000), which can react with phenolic compounds leading to quinones (Barril *et al.*, 2016). These quinones can react with amino acids to produce volatile compounds (Oliveira *et al.*, 2017). Since the levels of phenolic compounds in a white wine and the concentration of oxygen are relatively limited, the aforementioned phenomenon may have occurred, but with a moderate magnitude, hence the changes in low amino acid concentrations. Another explanation could be that new and old barrels affected wine composition during the two stages of fermentation, leading to differences in wine composition at the start of ageing that were maintained over the course of aging. While this may explain most of the results when the sampling period and barrel age interaction was not significant ($p > 0.05$; *e.g.*, Gln, Glu, and His concentrations in 2022 wines from vineyard C), it does not explain the other results, notably Asp concentrations in 2022 wines from vineyard B. In the latter case, wines in the old barrels exhibited higher Asp concentrations in week 7 than did wines in new barrels, but this relationship was reversed in week 36, showing the impacts of ageing.

CONCLUSION

This study examined the amino acid composition of still Chenin blanc wine over the course of ageing on lees – the first time that such research has been conducted on wine made from this grape variety. Across the two vintages and three vineyards, aspartic acid was clearly the most abundant free amino acid, followed by proline and glutamic acid. This could be a characteristic of Chenin blanc, a hypothesis to be tested. Although amino acid levels were dependant on many factors, their proportions were generally similar in both vintages. This work is the first to show the influence and interactions of vintage × vineyard × sampling period × barrel age, all operating together. In addition, the ripeness level of grapes at harvest could not explain the differences in how amino acid concentrations changed over the course of ageing, underscoring that other factors need to be considered. There was a high level of variability in the amino acid content of wines from different vineyards, which was not offset by alcoholic and malolactic fermentation. Lastly, there was an effect of barrel age, which remains to be better understood.

In conclusion, sensory analyses should now be performed on wines and model solutions of amino acids to explore the broader impacts of the changes in amino acid concentrations during wine ageing on lees.

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