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RESEARCH ARTICLE

Grape amino acid content in 'Chenin blanc' is affected by leaf removal and harvest date

Gastón Gutiérrez-Gamboa[1] and Erna Blancquaert[2*]

[1]Instituto de Investigaciones Agropecuarias, INIA Carillanca, Temuco, Chile.
[2]Stellenbosch University, South African Grape and Wine Research Institute, Stellenbosch 7600, South Africa.
*Corresponding author (ewitbooi@sun.ac.za).
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ABSTRACT

'Chenin blanc' is a key grape (*Vitis vinifera* L. subsp. *vinifera*) variety in the South African wine industry, and its quality is influenced by some viticultural practices, including leaf removal and harvest time. This study aimed to evaluate the effects of leaf removal and harvest date on physicochemical parameters and amino acid content of 'Chenin blanc' grapes. The trial was conducted in a commercial vineyard in Stellenbosch, South Africa, during the 2018-2019 season. The results showed that leaf removal and harvest date did not affect 'Chenin blanc' berry weight. Leaf removal significantly increased the concentration of several amino acids, including isoleucine, leucine, alanine, phenylalanine, and tryptophan. Grapes harvested at the stage of berries harvest-ripe according to Eichhorn and Lorenz scale (E-L 38) + 18 d had higher soluble solids (23.63 °Brix) compared to those harvested at E-L 38 (21.15 °Brix), and leaf removal resulted in increased soluble solids (23.28 to 21.50 °Brix) compared to control. Succinic acid content was reduced in the leaf removal treatment, and total acidity was lower in grapes with leaf removal (4.85 to 7.56 g L^{-1}). The interaction between leaf removal and harvest time significantly affected the amino acid content. Grapes harvested at E-L 38 + 18 d from vines subjected to leaf removal showed high contents of several amino acids, especially phenylalanine and tryptophan (14.47 to 10.46 and 4.50 to 3.11 mg L^{-1}, respectively). These findings suggest that both leaf removal and the timing of harvest contribute to the N composition of the grapes. The optimal harvest timing at around 25 °Brix aligns with the concept of nitrogenous maturity, and further supporting information is needed to precise vineyard management to enhance wine quality.

Key words: Defoliation, phenylalanine, sugar maturity, vine microclimate, *Vitis vinifera* subsp. *vinifera*, wine.

INTRODUCTION

South Africa has the most 'Chenin blanc' wine grape (*Vitis vinifera* L. subsp. *vinifera*) plantings in the world which points to the versatile nature (SAWIS, 2023). In the recent years there has been a big focus on the certification of 'Chenin blanc' vineyards that are 35 yr and older (Old Vine Project, 2024). 'Chenin blanc' has been predominantly utilized for brandy production and bulk wine blends. However, since the 1990s, there has been a concerted effort to enhance the quality of 'Chenin blanc' wines. Studies have focused on the chemical and sensory profiling of 'Chenin blanc' wines, examining factors such as volatile aroma compounds and sensory attributes (Brand et al., 2020). Today, South African viticulture has been at the forefront of revaluating old vines, with 'Chenin blanc' leading the way, often from vineyards that are over 35 yr old and being worldwide commercialized (Mafata et al., 2020; Old Vine Project, 2024).

Leaf removal is a canopy management practice that directly impacts light exposure and berry temperature, thereby influencing the sink-to-source relationship and grape quality (Mucalo et al., 2021). This practice enhances light penetration and improves air circulation around the clusters, leaf removal can optimize berry temperature and enhance the efficacy of fungicide treatments, which reduces the prevalence of grapevine diseases (Mucalo et al., 2021). The published scientific literature about the effects of leaf removal has focused on its effectiveness on the most famous grapevine varieties (VanderWeide et al., 2021), but studies of this subject on 'Chenin blanc' are limited.

Grape N composition is crucial for yeast growth, fermentation kinetics, and flavor metabolism (Gutiérrez-Gamboa et al., 2020). Certain amino acids, especially phenylalanine and valine serve as precursors for key volatile compounds in wine, which are produced through yeast enzymatic metabolism during alcoholic fermentation (Gutiérrez-Gamboa et al., 2020). These volatile compounds, which include higher alcohols, esters, carbonyl compounds, volatile fatty acids, and S compounds, are primarily derived from the metabolism of sugars and amino acids by yeast, and they play a significant role in shaping the wine's aroma (Chua et al., 2021). Consequently, a close relationship between the amino acid composition of the must and the profile of volatile compounds in the wine has been well documented (Gutiérrez-Gamboa et al., 2020; Chua et al., 2021). In contrast, musts with insufficient N levels may lead to stuck or sluggish fermentations, a persistent issue in wine production that disrupts winery operations (Gutiérrez-Gamboa et al., 2020).

'Chenin blanc' has compact bunch morphology and is sensitive to *Botrytis cinerea* and downy mildew (Pañitrur-De La Fuente et al., 2018). Leaf removal may optimize light exposure and air circulation around the 'Chenin blanc' grape clusters, potentially increasing the concentration of amino acids in the must, which are crucial for yeast growth and fermentation. The effects of leaf removal on amino acid profiles of this variety may vary depending on the harvest date, with sequential harvests potentially showing different patterns in amino acid concentration due to variations in vine maturation and environmental conditions. To date, limited scientific literature is available regarding the effects of leaf removal on the amino acid composition of grape berries. In a previous study, Yue et al. (2019) reported that this practice applied at different phenological stages significantly influenced the amino acid composition of 'Sauvignon blanc' grapes and wines during one study season. Leaf removal performed at 72 d after flowering with moderate severity led to the highest concentrations of total amino acids, enhancing compounds such as aspartic acid, serine, arginine, alanine, aminobutyric acid, and proline in grapes. In contrast, leaf removal performed at 72 d after flowering with high severity reduced amino acid concentrations. In this study, the content of certain amino acids, including glycine, tyrosine, cysteine, methionine, and lysine remained unaffected.

Based on this, the goal of this research was to evaluate the effects of leaf removal and harvest time on amino acid content and physicochemical parameters in 'Chenin blanc' grapevines.

MATERIALS AND METHODS

Study site and plant material

The study was conducted in a 'Chenin blanc' vineyard (*Vitis vinifera* L. subsp. *vinifera*) (34°01'02.0" S, 18°81'53.5" E) located within the Stellenbosch Wine of Origin district, South Africa, during the 2018-2019 growing season. The vines used in the study were of the SN 1064 clone, planted in a North-West/South-East row orientation, grafted onto 101-14 Mgt (*Vitis riparia* × *V*. *rupestris*), trained using a six-wire vertical trellis system and spur pruned. The vines were trained on a six-wire vertical trellis system. Additionally, the block was subjected to drip irrigation as required throughout the season. The soil comprised of a deep granite gravel on laterite (coffee stone) and weathering clay (saprolite). Overall, the season was characterized by cool conditions between January and March (Figure 1) with low water stress due to the regular rain showers during this period.

Treatments and experimental design

The treatments consisted of modifying bunch microclimate as follows: i) Control, no lateral shoot or leaf removal in the bunch zone was applied and ii) leaf removal, defoliation of the leaves located on the eastern/morning side of the bunch zone. The treatment was performed when the grapevines reached the berry set stage corresponding to growth stage E-L 27 according to the Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995) and were displayed in a completely randomized design. Leaves were removed on the eastern side of the canopy (morning sun) at the fruit zone level (approximately 35 to 40 cm above the cordon) as previously by Hed and Centinari (2024). Each treatment consisted of five replicates and each replicate comprised two panels (six vines between poles) accounting a total of 120 vines (60 for the control and 60 for the treatment) in each of the vineyards. Grapes from both treatments were harvested sequentially at two different dates according to Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995): E-L 38, berries were ripe for the commercial harvest; and E-L 38 + 18 d, berries were harvested 18 d after the commercial harvest. At each harvest date, four vines from each of the replicates were harvested.

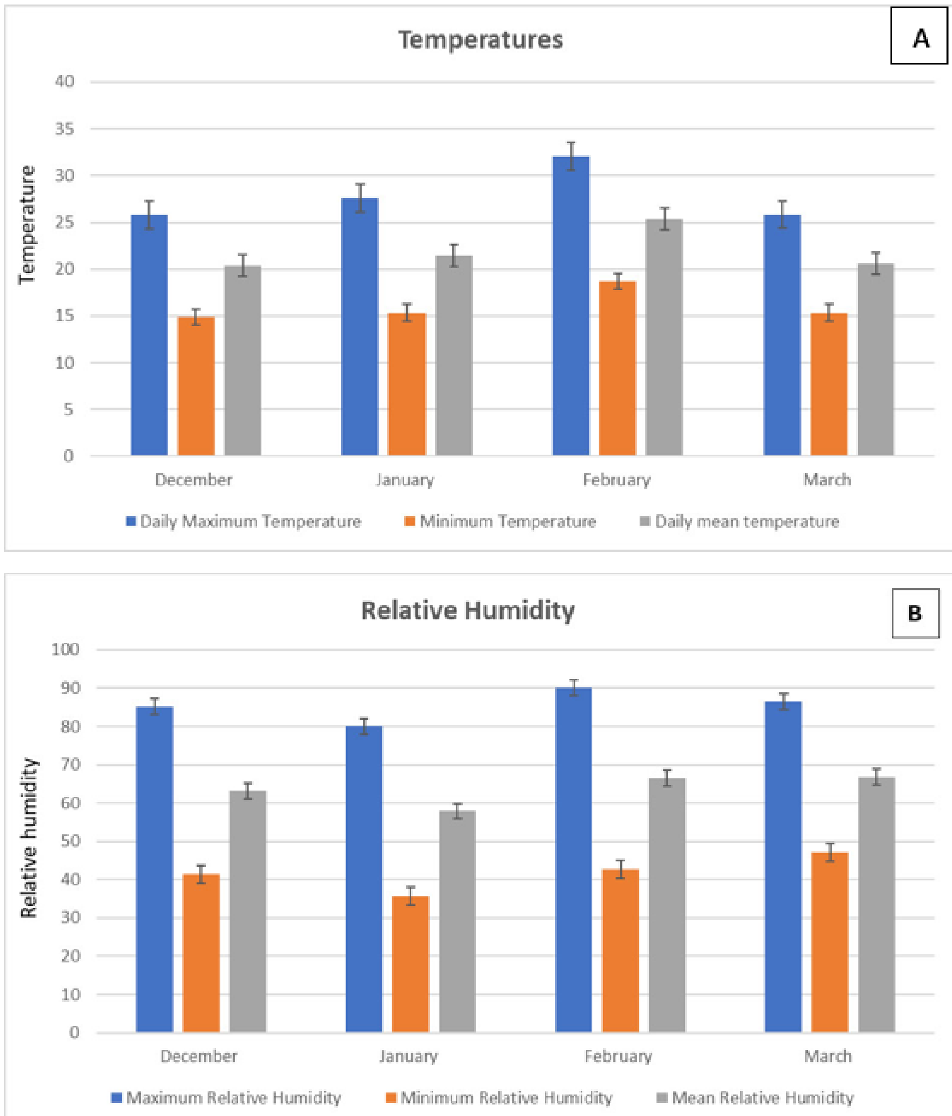

Figure 1. (A) Daily maximum, minimum and mean temperatures (ºC), and relative humidity (%) during the 2018-2019 growing season December - March.

Harvest and technological parameters measurements in grapes and wines

The harvesting was conducted between 06:00 and 10:00 h. Thirty berries from each of the treatment replicates (n = 5) were sampled at each of the harvest dates. Each berry was weighed individually (n = 150) using an analytical balance (AG204 Delta Range, Mettler Toledo, Columbus, Ohio, USA). Subsequently, the grape samples were processed for analytical assessments as physicochemical parameters, organic acids, and amino acids.

The determinations of soluble solids (°Brix), pH and titratable acidity (g L^{-1} tartaric acid) in grape and alcohol degree (% v/v) of wines were performed according to the OIV (International Organisation of Vine and Wine, Dijon, France) methods (OIV, 2003).

Amino acid determination in grapes and organic acids and sugars in grapes and wines

Free amino acids in grape juice were derivatized, separated, and quantified using HPLC with a fluorescence detector, employing a Poroshell HPH-C18 column (4.6 × 150 mm, 2.7 µm) and a UHPLC Guard 3PK Poroshell HPH-C18 guard column (4.6 × 5 mm, 2.7 µm). The analytical method was adapted according to the steps described in the report published by Šuklje et al. (2016).

The major sugars (glucose and fructose) in grapes and wines, as well as the organic acids (tartaric, succinic, and citric acids) in grape juice, were analyzed using an HPLC method previously described by Eyéghé-Bickong et al. (2012) and expressed in g L^{-1}.

Winemaking

Standard winemaking procedures were followed at the experimental cellar of the Department of Viticulture and Oenology, Stellenbosch University. Grapes from each harvest date and replicate were vinified separately. The grapes were crushed and destemmed into 20 L plastic drums, and 30 mg L^{-1} SO_2 were added. Must samples for pH, titratable acidity, and soluble solids were taken prior to the addition of SO_2. The grapes were pressed immediately at 1.5 bar and juice was clarified for 24 h at 4 °C using clarification enzymes (Rapidase, Oenobrands, Montpellier, France). The juice was racked into sterile 4 L glass bottles and the fermentation inoculated with 30 g h L^{-1} *Saccharomyces cerevisiae* (VIN 13, Oenobrands) was performed at 15 °C. The fermentation rate was monitored daily using a hydrometer. Afterward, 0.25 g L^{-1} Fermaid K (Lallemand, Montreal, Canada) was added. Fermentation lasted approximately 5 d. Following alcoholic fermentation, the wines were racked off the lees, and 50 mg L^{-1} SO_2 was added. The wines underwent cold stabilization for 3 wk at -4 °C, followed by an adjustment of free SO_2 to 40 mg L^{-1}. The wines were then bottled in 750 mL dark green glass bottles, sealed with screw caps, and stored at 15 °C 1 d after bottling.

Statistical analysis

The variables were analyzed using a completely randomized design with a factorial arrangement, consisting of two treatments (control and leaf removal) and two harvest dates (E-L 38 and E-L 38 + 18 d). An ANOVA was conducted, and the significance of differences was determined using Duncan's test (p-value ≤ 0.05) using Statgraphics Centurion XVI (Statgraphics Technologies, The Plains, Virginia, USA). Additionally, a matrix correlation was performed to determine relationships among variables according to treatments. The analysis was made using the InfoStat software (Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina; www.infostat.com.ar).

RESULTS AND DISCUSSION

Berry weight was not affected by the treatments, harvest date and their interaction, whereas the rest of the physicochemical parameters and organic acids were affected by at least one of the factors. The levels of pH, tartaric acid and total acids were higher in the grapes harvested at E-L 38 + 18 d than in the E-L 38 stage. Soluble solids and succinic acid grape contents were individually affected by the treatment and harvest date in which leaf removal increased the soluble solid content and decreased the succinic acid content compared to control. In addition, grapes harvested at E-L 38 + 18 d showed higher content of soluble solids and succinic acid than the grapes harvested at E-L 38. Leaf removal also statistically decreased total acidity in 'Chenin blanc' grapes (Table 1).

Similar results were obtained by Alatzas et al. (2023) when applied leaf removal at E-L 27 + 72 d after flowering to 'Xinomavro' grapes. The former authors reported that defoliation resulted in the up regulation of genes at specific phenological stages such as *VviUFGT* and *VviCCD1* at veraison and *VviLOXA* at the green berry. Specifically, the induction of *VviUFGT* at veraison suggests an enhancement in flavonoid biosynthesis, particularly anthocyanins, which are critical for berry color and antioxidant properties (Griesser et al., 2018). Similarly, the increased expression of *VviCCD1*, a gene involved in carotenoid cleavage, may contribute to the accumulation of aroma precursors, potentially influencing the aromatic complexity of the primary wine aroma (Alatzas et al., 2021). The activation of *VviLOXA*, associated with lipid metabolism and the production of volatile compounds (He et al., 2020). This suggests that leaf removal may modulate the sensory attributes of grape berries through microclimatic alterations within the canopy, which in turn influence metabolic pathways associated with aroma, flavor, and phenolic compound biosynthesis. Lukić et al. (2017) found that leaf removal after berry set leads to greater exposure of grapes to solar radiation and higher temperatures, which in turn reduced acidity and increased the total soluble solids in the must. Succinic acid synthesis regulated by succinyl-coenzyme A ligase (SUCLA) increased in response to abiotic stress (Wu et al., 2025) and γ-aminobutyric acid was rapidly metabolized into succinic acid enhancing plant tolerance to environmental stresses (Hijaz and Killiny, 2019). Succinate levels increase in grapevines under drought stress and have been associated with enhanced stomatal regulation, elevated proline accumulation, and increased antioxidant activity (Haider et al., 2017). These mechanisms contribute to osmotic adjustment, protection of cellular structures, and overall stress resilience (Haider et al., 2017). Similar to this, Miliordos et al. (2025) showed that the content of malic acid decreased significantly as maturity, whereas succinic acid increased, affecting acidity in grapes.

Table 1. Effect of leaf removal and harvest date on physicochemical parameters of 'Chenin blanc' grapes. Distinct letters in the row indicate significant differences according to Duncan's test (*p*-value ≤ 0.05). Red color indicates significant difference. E-L 38: Berries ripening for commercial harvest according to Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995); E-L 38 + 18 d: berries harvested 18 d after commercial harvest.

	Berry weight	°Brix	pH	Total acidity	Citric acid	Tartaric acid	Succinic acid	Total acids
Treatment (T)								
Control	1.76^{a}	21.50^{a}	3.60^{a}	7.56^{b}	0.21^{a}	6.55^{a}	1.72^{b}	15.33^{a}
Leaf removal	1.83^{a}	23.28^{b}	3.69^{a}	4.85^{a}	0.20^{a}	6.73^{a}	1.55^{a}	14.78^{a}
p-value	0.8729	0.0369	0.1211	0.0030	0.3925	0.4676	0.0398	0.3862
Harvest date (HD)								
E-L 38	1.81^{a}	21.15^{a}	3.55^{a}	6.53^{a}	0.24^{b}	6.27^{a}	0.83^{a}	14.15^{a}
E-L 38 + 18 d	1.78^{a}	23.63^{b}	3.74^{b}	5.88^{a}	0.18^{a}	7.01^{b}	2.44^{b}	15.95^{b}
p-value	0.9461	0.0127	0.0108	0.1940	0.0060	0.0264	0.0000	0.0333
Factor interaction								
T × HD	0.9910	0.9675	0.4643	0.0677	0.4958	0.8188	0.3619	0.9729

The content of neutral amino acids was significantly affected by the factors (Table 2). Leaf removal increased the content of isoleucine (Ile), leucine (Leu), threonine (Thr), tyrosine (Tyr), alanine (Ala), phenylalanine (Phe) and tryptophan (Trp) and decreased the content of asparagine (Asn) in 'Chenin blanc' grapes compared to control. Yue et al. (2019) reported that basal leaf removal applied 72 d after flowering of 'Sauvignon blanc' grapevines promoted the synthesis of some amino acids such as Ala, arginine (Arg), aspartic acid (Asp), γ-aminobutyric acid (GABA), proline (Pro), serine (Ser) and total amino acids in grapes. In addition, these authors reported that the content of cysteine (Cys), glycine (Gly), lysine (Lys), methionine (Met) and Tyr in grapes were not affected by the timing or severity of basal defoliation in grapevines. These results may be explained by the fact that Cys, Gly, Lys, Met and Tyr are either involved in primary metabolism with relatively stable biosynthetic pathways or are possibly less sensitive to source-sink alterations induced by defoliation (Gutiérrez-Gamboa et al., 2020). Met and Cys are S-containing amino acids whose synthesis is tightly linked to S assimilation pathways and redox regulation (de Bont et al., 2022), often more influenced by nutrient status than canopy microclimate or source-sink dynamics. Gly and Lys are essential amino acids related to the strict metabolic control to maintain protein synthesis and photorespiration (Kishor et al., 2020), and probably less responsive to short-term viticultural interventions such as defoliation. Tyr is involved in the biosynthesis of aroma and phenolic compounds and appears to be homeostatically regulated during berry maturation (Vogt, 2010). In contrast, amino acids such as Pro, Arg, and GABA are more sensitive to environmental cues and C-N balance, often serving as osmoprotectants or stress indicators (Gutiérrez-Gamboa et al., 2020).

Grapes harvested at E-L 38 + 18 d showed higher content of most of the studied neutral amino acids than the grapes harvested at E-L 38, except for glutamine (Gln) and Thr. These results matched to those reported by Garde-Cerdán et al. (2018), where the content of some amino acids such as Phe, Trp, Arg, histidine (His), Leu, Ile, valine (Val), Met and Pro showed an increase from 10 to 25 °Brix and towards the 30 °Brix, it was considerably decreased. The accumulation of amino acids in grape berries is a dynamic process closely linked to berry development and ripening, influenced by both primary metabolism and environmental factors. Several studies have demonstrated that many amino acids, such as Phe, Trp, Arg, Leu, Ile, Val, Met and Pro increased during ripening up to approximately 25 °Brix, suggesting this sugar concentration coincides with the peak of nitrogenous or amino acid maturity (Garde-Cerdán et al., 2018). At this stage, the grape reaches a physiological balance between sugar accumulation, acid degradation, and nitrogenous compound synthesis. Beyond 25 °Brix, the concentration of amino acids often declines due to metabolic shifts, including enhanced catabolism, dilution effects from increased berry size, or the reallocation of nitrogen for secondary metabolism and senescence-related processes (Stines et al., 1999). Therefore, harvesting at around 25 °Brix may represent the optimal compromise for both sugar and nitrogenous ripeness, ensuring desirable berry composition for wine quality. These findings suggest that the harvest timing, specifically when the grape reaches sugar maturity at 25 °Brix, might coincided to the optimal nitrogenous maturity for winemaking proposes (Gutiérrez-Gamboa et al., 2020).

Table 2. Effect of leaf removal and harvest date on neutral amino acids of 'Chenin blanc' grapes. Distinct letters in the row indicate significant differences according to Duncan's test (*p*-value ≤ 0.05). Red color indicates significant difference. Gly: Glycine; Val: Valine; Ile: Isoleucine; Leu: Leucine; Met: Methionine; Asn: Asparagine; Gln: Glutamine; Thr: Threonine; Tyr: Tyrosine; Ala: Alanine; Phe: Phenylalanine; Trp: Tryptophan; E-L 38: berries ripening for commercial harvest according to Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995); E-L 38 + 18 d: berries harvested 18 d after commercial harvest.

	Gly	Val	Ile	Leu	Met	Asn	Gln	Thr	Tyr	Ala	Phe	Trp
Treatment (T)												
Control	3.93^{a}	29.59^{a}	4.53^{a}	10.51^{a}	8.12^{a}	6.12^{b}	46.93^{a}	38.98^{a}	3.92^{a}	46.69^{a}	10.92^{a}	3.74^{a}
Leaf removal	3.88^{a}	31.78^{a}	7.92^{b}	17.43^{b}	19.45^{a}	4.36^{a}	43.49^{a}	48.54^{b}	4.79^{b}	51.62^{b}	14.02^{b}	3.88^{b}
p-value	0.9613	0.0514	0.0001	0.0000	0.0512	0.0024	0.0571	0.0013	0.0008	0.0000	0.0011	0.0469
Harvest date (HD)												
E-L 38	1.05^{a}	21.67^{a}	5.14^{a}	10.16^{a}	5.11^{a}	4.06^{a}	43.98^{a}	43.20^{a}	3.63^{a}	48.73^{a}	10.46^{a}	3.11^{a}
E-L 38 + 18 d	6.76^{b}	39.70^{b}	7.30^{b}	17.78^{b}	22.47^{b}	6.43^{b}	46.44^{a}	44.32^{a}	5.07^{b}	49.58^{b}	14.47^{b}	4.50^{b}
p-value	0.0043	0.0000	0.0003	0.0000	0.0135	0.0008	0.1319	0.4046	0.0001	0.0038	0.0004	0.0000
Factor interaction												
T × HD	0.1093	0.4094	0.0002	0.0000	0.4018	0.7379	0.0002	0.0002	0.0013	0.0000	0.0015	0.0001

Most of the studied amino acids, except Gly, Val, Met and Asn were affected by the interaction of the treatment and harvest factors. The grapevines subjected to leaf removal showed higher contents of Ile, Leu, Thr, Tyr, Ala, Phe and Trp in grapes harvested at E-L 38 + 18 d than the control grapevines harvested at E-L 38 stage (Figure 2). The interaction between leaf removal and harvest timing plays a significant role in the final composition of the grape must (Gutiérrez-Gamboa et al., 2020). Leaf removal early in the season can increase the rate of sugar accumulation, influencing when the grapes reach 25 °Brix (Table 1). Leaf removal may improve temperature and vine photosynthetic efficiency, leading to fast berry ripening. This could cause the grapes to reach the desired sugar maturity (25 °Brix) earlier in the growing season, that can influence the timing of amino acid peak levels (Garde-Cerdán et al., 2018).

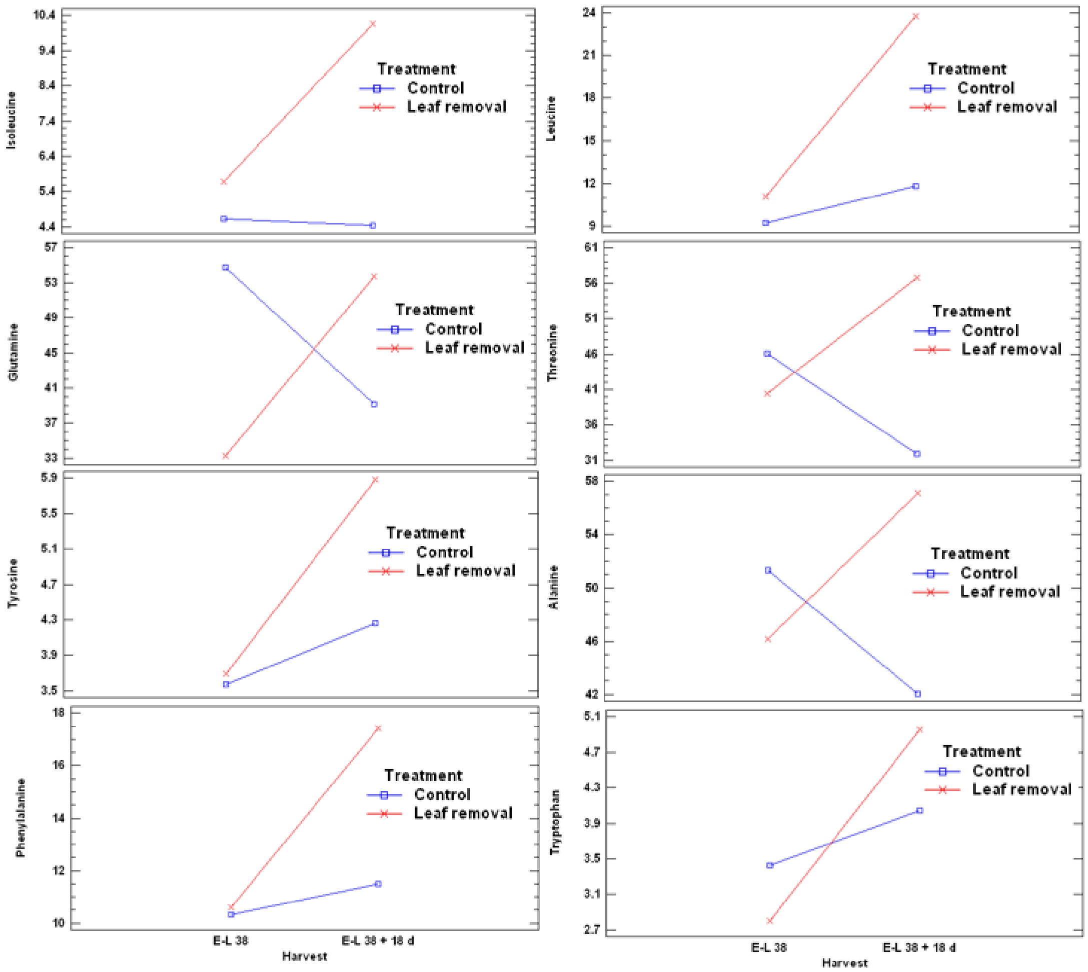

Figure 2. Interaction between treatment and harvest date in the content of neutral amino acids from grapevines subjected to leaf removal and sequent harvests in ‘Chenin blanc’. E-L 38: Berries ripening for commercial harvest according to Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995); E-L 38 + 18 d: berries harvested 18 d after commercial harvest.

An early harvest with leaf removal may result in musts with both high sugar content and enhanced amino acid concentrations, which are ideal for the alcoholic fermentation (Gutiérrez-Gamboa et al., 2018). On the other hand, delayed harvest to obtain an adequate phenolic maturation, the higher temperatures caused by leaf removal might also result in a more rapid degradation of some amino acids (Gutiérrez-Gamboa et al., 2018). This can impact the fermentation process and the final wine quality. Therefore, the timing of both leaf removal and harvest needs to be carefully managed to optimize both sugar and N level.

The contents of non-polar amino acids were significantly affected by the factors (Table 3). Leaf removal increased the content of Cys, Ser, GABA, hydroxyproline (HoPro) and decreased the content of Arg and Lys compared to control. Some amino acids such as Arg and ornithine are involved in N storage and transport within the plant (Gutiérrez-Gamboa et al., 2020). Leaf removal can also trigger a stress response, leading to the production of compounds that help mitigate this stress, such as polyamines and stress-associated proteins. Putrescine can be both the direct product of an enzymatically catalyzed reaction with Arg as a substrate or arise from non-enzymatic decarboxylation of ornithine (Liebsch et al., 2022). Under these conditions, the metabolic shift induced by leaf removal may lead to a reallocation of N, prioritizing amino acids like Pro (Wei et al., 2022), which plays a key role in stress tolerance, at the expense of Arg.

Table 3. Effect of leaf removal and harvest date on non-polar amino acids of 'Chenin blanc' grapes. Distinct letters in the row indicate significant differences according to Duncan's test (*p*-value ≤ 0.05). Red color indicates significant difference. Asp: Aspartic acid; Glu: Glutamic acid; Cys: Cysteine; Ser: Serine; His: Histidine; Arg: Arginine; GABA: Gamma-aminobutyric acid; Orn: Ornithine; Lys: Lysine; HoPro: Hydroxyproline; Pro: Proline; E-L 38: berries ripening for commercial harvest according to Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995); E-L 38 + 18 d: berries harvested 18 d after commercial harvest.

	Asp	Glu	Cys	Ser	His	Arg	GABA	Orn	Lys	HoPro	Pro
Treatment (T)											
Control	8.70^{a}	21.48^{a}	2.11^{a}	22.22^{a}	17.71^{a}	217.05^{b}	97.80^{a}	8.67^{a}	7.24^{b}	17.01^{a}	43.20^{a}
Leaf removal	8.89^{a}	20.43^{a}	2.24^{b}	27.30^{b}	19.46^{a}	165.50^{a}	114.07^{b}	8.17^{a}	4.33^{a}	22.79^{b}	45.42^{a}
p-value	0.7292	0.2617	0.0373	0.0017	0.2560	0.0045	0.0097	0.4955	0.0001	0.0009	0.0605
Harvest date (HD)											
E-L 38	10.30^{b}	21.38^{a}	2.19^{a}	23.11^{a}	12.65^{a}	175.58^{a}	75.38^{a}	6.47^{a}	3.93^{a}	17.46^{a}	23.88^{a}
E-L 38 + 18 d	7.30^{a}	20.54^{a}	2.16^{a}	26.41^{b}	24.52^{b}	206.97^{b}	136.49^{b}	10.38^{b}	7.64^{b}	22.34^{b}	64.74^{b}
p-value	0.0043	0.3546	0.4937	0.0084	0.0008	0.0246	0.0001	0.0041	0.0001	0.0017	0.0000
Factor interaction											
T × HD	0.0004	0.0066	0.2169	0.0002	0.3677	0.0333	0.0025	0.1498	0.0007	0.0609	0.0010

Grapes harvested at E-L 38 + 18 d showed higher content of most of the studied neutral amino acids than the grapes harvested at E-L 38, except for Asp, Glu and Cys. Some non-polar amino acids such as Asp, Glu, Ser, Arg, GABA, Lys and Pro were affected by the interaction of the treatment and harvest factors (Figure 3). The Glu and Asp showed the highest concentration when were harvested at E-L 38 in control vines, like Ser, GABA and Pro from grapes harvested at E-L 38 + 18 d in treated vines. The Arg and Lys content in grapes was higher in control than in the treated vines at both harvested days. Glutamate is the primary N compound transported in grapevines, and aminotransferase enzymes convert grape Gln and aspartate into other amino acids, among these, Arg, and Pro together account for 60% to 80% of the free amino acids present in ripe grapes (Gutiérrez-Gamboa et al., 2020). Most of the Pro accumulation in grapes occurs toward the end of ripening, approximately 4 wk after veraison (Wei et al., 2022). In contrast, Arg accumulation begins before veraison and continues until full maturity, except in grapevine varieties that are Pro accumulators (Gutiérrez-Gamboa et al., 2020). Proline is synthesized in plant tissues to protect cells from osmotic stress (Canoura et al., 2018). Therefore, the accumulation of Pro during ripening is likely linked to the osmotic pressure induced by the accumulation of hexose sugars (Chun et al., 2018). Furthermore, Pro accumulation may also contribute to an increase in must pH, as its production from glutamate can release hydroxide ions (Gutiérrez-Gamboa et al., 2020).

The treatment factors scarcely affected the physicochemical parameters of the wines (Table 4). The fructose content in wines was significantly higher in both the leaf removal treatment and the later harvest (E-L 38 + 18 d), which could suggest that both factors contribute to enhancing grape sugar composition, potentially providing more fermentable sugar for wine production. This is particularly relevant as fructose, along with glucose, is a key sugar involved in the fermentation process and can impact the final alcohol content in wine (Jordão et al., 2015). However, the absence of significant effects on glucose and ethanol concentrations indicates that these factors might not influence fermentation efficiency. Glycerol production in wines was higher in the produced from grapes harvested at E-L 38 + 18 d. Glycerol is often associated with wine body and mouthfeel, and its increased concentration in grapes harvested later could suggest that the extended ripening period enhances glycerol production (Goold et al., 2017). The lack of significant interactions between leaf removal and harvest date in wine determinations suggests that the effects of these factors are independent. Overall, the results support the idea that harvest timing has a more pronounced effect on wine sugar composition and secondary metabolites like glycerol, while leaf removal primarily influences fructose content, which can impact the wine's fermentation dynamics and aroma profile. Further studies could explore how these practices interact with other environmental and winemaking factors to optimize grape and wine quality.

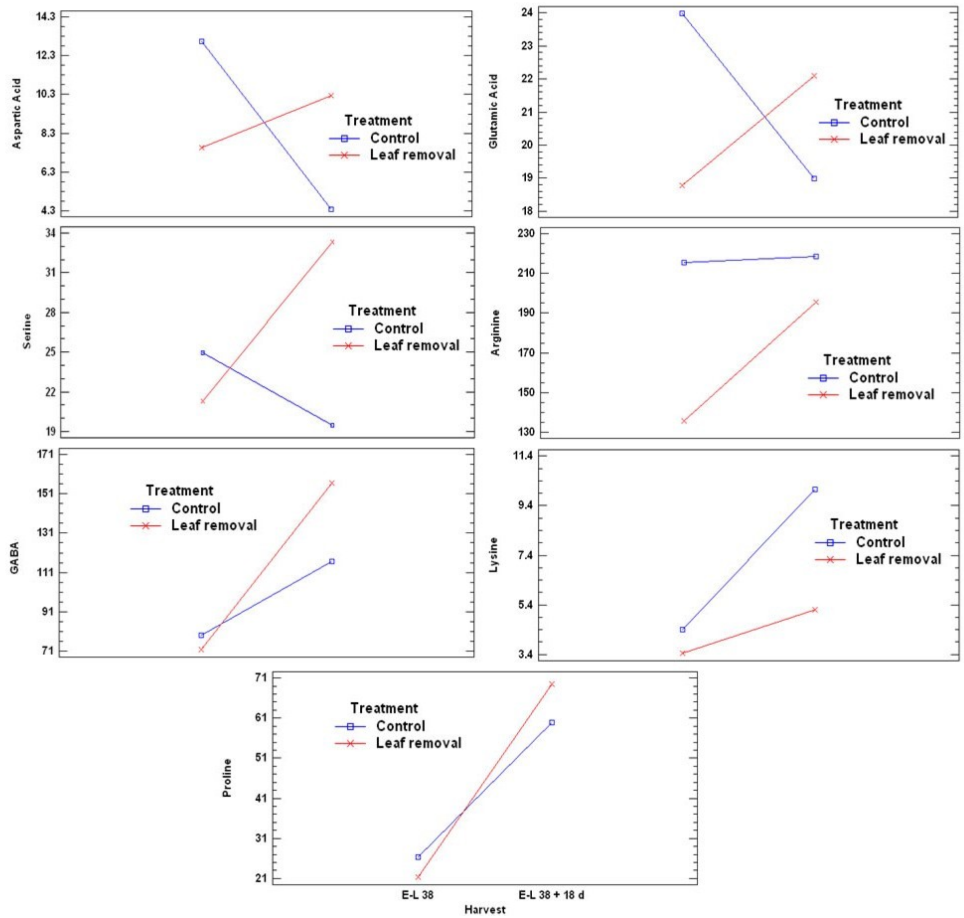

Figure 3. Interaction between treatment and harvest date in the content of non-polar amino acids from grapevines subjected to leaf removal and sequent harvests in 'Chenin blanc'. E-L 38: Berries ripening for commercial harvest according to Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995); E-L 38 + 18 d: berries harvested 18 d after commercial harvest; GABA: gamma-aminobutyric acid.

Table 4. Effect of leaf removal and harvest date on non-polar amino acids of 'Chenin blanc' wines. Distinct letters in the row indicate significant differences according to Duncan's test (p-value ≤ 0.05). Red color indicates significant difference. E-L 38: Berries ripening for commercial harvest according to Eichhorn and Lorenz (E-L) system (Lorenz et al., 1995); E-L 38 + 18 d: berries harvested 18 d after commercial harvest.

	Glucose	Fructose	Ethanol (% v/v)	Glycerol
Treatment (T)				
Control	1.75^{a}	11.01^{a}	9.34^{a}	573.87^{a}
Leaf removal	4.86^{a}	12.44^{b}	8.22^{a}	530.44^{a}
p-value	0.1428	0.0162	0.3659	0.6327
Harvest date (HD)				
E-L 38	2.05^{a}	10.79^{a}	8.31^{a}	275.69^{a}
E-L 38 + 18 d	4.56^{a}	12.67^{b}	9.26^{a}	828.62^{b}
p-value	0.2157	0.0063	0.4383	0.0028
Factor interaction				
T × HD	0.9004	0.2861	0.3382	0.2358

Figure 4 shows the matrix of correlation for the grape amino acids and wine composition. Glucose was strongly correlated to Ser (+0.50), GABA (+0.57), Tyr (+0.71), Val (+0.57), Met (+0.51), Trp (+0.50), Phe (+0.71), HoPro (+0.69) and Pro (+0.52), whereas fructose was strongly correlated to His (+0.86), Gly (+0.76), GABA (+0.77), Tyr (+0.77), Val (+0.80), Met (+0.88), Trp (+0.62), Phe (+0.73), Ile (+0.76), Orn (+0.72), Leu (+0.76), HoPro (+0.88) and Pro (+0.73). These patterns suggest fructose and glucose may be particularly linked to amino acids involved in osmoregulation, sulfur metabolism, and secondary metabolism. The observed correlation between sugars and amino acids supports the hypothesis of coordinated biosynthetic regulation during grape ripening, as previously reported by Gutiérrez-Gamboa et al. (2018). Moreover, the high correlations with hydrophobic and branched-chain amino acids further imply their role in later stages of berry development. In this fashion, Hattori et al. (2019) reported that exogenous Ile applications to grapevines interact with abscisic acid-mediated anthocyanin accumulation in grape skins. Ethanol content in wines was strongly correlated to Thr (-0.52), Arg (-0.51) and Lys (+0.60). Arginine serves as a major N source and is rapidly assimilated by yeast during the early stages of fermentation, supporting biomass production and fermentation kinetics (Bell and Henschke, 2005). Threonine is also utilized by yeast and contributes to the synthesis of higher alcohols such as 1-propanol, whereas lysine is generally poorly assimilated under anaerobic conditions, often remaining unmetabolized during alcoholic fermentation (Bell and Henschke, 2005). Glycerol was strongly correlated to Asn (+0.76), Val (+0.92), Ile (+0.96), Orn (+0.85), Lys (+0.80) and Pro (+0.89). Glycerol, a major byproduct of yeast metabolism, contributes to the body and mouthfeel of wine (Ivit et al., 2020). Probably, the metabolism of these amino acids can affect the redox balance within yeast cells, potentially leading to increased glycerol synthesis as a mechanism to maintain this balance.

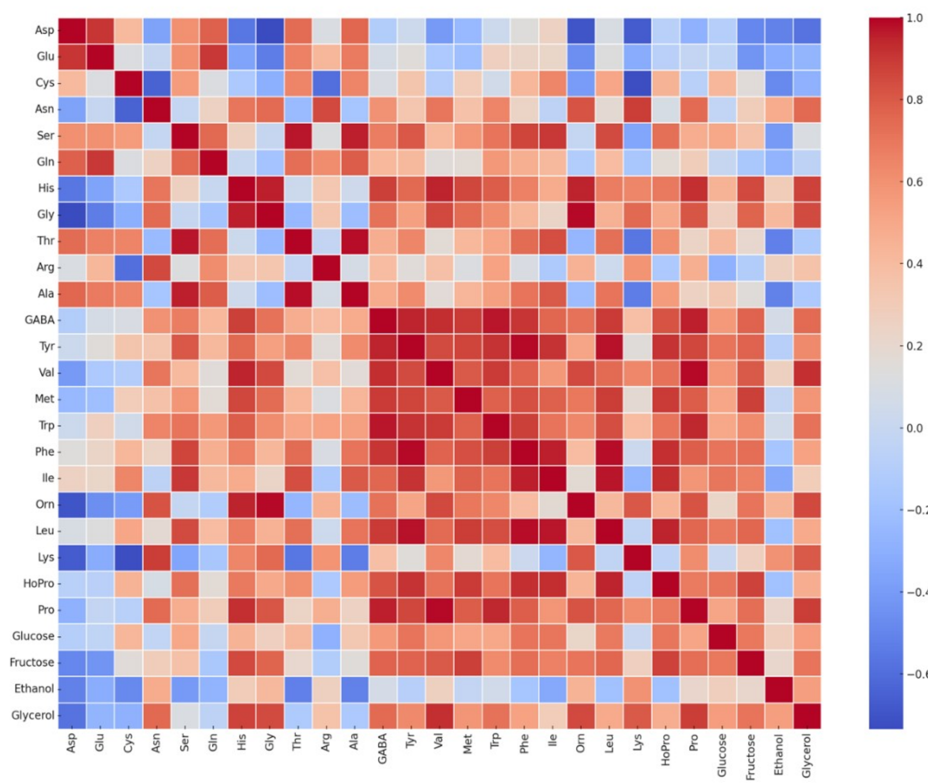

Figure 4. Correlation matrix of grape must amino acids and wine composition obtained from grapevines subjected to leaf removal and sequent harvests in 'Chenin blanc'. Asp: Aspartic acid; Glu: glutamic acid; Cys: cysteine; Asn: asparagine; Ser: serine; Gln: glutamine; His: histidine; Gly: glycine; Thr: threonine; Arg: arginine; Ala: alanine; GABA: gamma-aminobutyric acid; Tyr: tyrosine; Val: valine; Met: methionine; Trp: tryptophan; Phe: phenylalanine; Ile: isoleucine; Orn: ornithine; Leu: leucine; Lys: lysine; HoPro: hydroxyproline; Pro: proline.

CONCLUSIONS

Leaf removal significantly influenced the amino acid composition, increasing the content of cysteine, serine, tyrosine, isoleucine, γ-aminobutyric acid, leucine, threonine, tyrosine, alanine, phenylalanine, hydroxyproline and tryptophan compared to the control. 'Chenin blanc' grapes harvested at E-L 38 + 18 d showed higher levels of most amino acids, except for glutamic acid, cysteine, glutamine, and threonine. This suggests that the timing of harvest, particularly when grapes are close to 25 °Brix, optimizes amino acid concentrations. The interaction between leaf removal and harvest date significantly modified the amino acid content, in which ripen grapes under leaf removal enhanced concentrations of several amino acids, especially neutral type such as tyrosine, isoleucine, leucine, γ-aminobutyric acid, proline, phenylalanine and tryptophan. These findings highlight the importance of both leaf removal and harvest timing in optimizing amino acid profiles, which are crucial for fermentation and wine quality.

Author contribution

Conceptualization: E.B. Methodology: E.B. Software: G.G.G. Validation: E.B. Formal analysis: G.G.G. Investigation: E.B., G.G.G. Resources: E.B. Data curation: E.B. Writing-original draft: G.G.G. Writing-review & editing: E.B., G.G.G. Visualization: G.G.G. Supervision: E.B. Project administration: E.B. Funding acquisition: E.B. All co-authors reviewed the final version and approved the manuscript before submission.

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