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Research Article

Impact of Leaf Removal on Berry Amino Acid Content of Sequentially Harvested Cabernet Sauvignon and Shiraz in Stellenbosch

E. Blancquaert[1]*, G. Gutiérrez-Gamboa[2,3]

(1) South African Grape and Wine Research Institute, Department of Viticulture and Oenology, Stellenbosch University, 7599, South Africa
(2) Instituto de Investigaciones Agropecuarias, INIA Carillanca, km 10 Camino Cajón-Vilcún s/n, Temuco P.O. Box 929, Chile
(3) Escuela de Agronomía, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Temuco P.O. Box 54-D, Chile

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***Corresponding author eMail**
ewitbooi@sun.ac.za

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ABSTRACT

The amino acid composition at harvest is a key determinant of the final wine quality. The composition of the grape must at harvest is strongly influenced by the grape variety, environmental conditions and the viticultural practices. These compounds play a key role in fermentation dynamics and flavour development, being precursors of volatile compounds. This study aimed to investigate how leaf removal and harvest timing influence amino acid content and physicochemical parameters in Cabernet Sauvignon and Shiraz grapevines. The results show that the Shiraz grapes had higher concentrations of sugars, amino acids and anthocyanins compared to Cabernet Sauvignon, whereas leaf removal increased key amino acids such as glutamine, phenylalanine and glycine, improving the nitrogen content essential for alcoholic fermentation. The optimal harvest time for maximising amino acid content was found to be at E-L 38 + 18 days close to 25°Brix. Moreover, the content of proline, an amino acid with osmo-protective properties, was positively correlated with glucose, fructose and total sugar content, suggesting its role in mitigating stress and promoting sugar accumulation. This study shows the significance of canopy management and precise harvest timing for improving grape composition and optimising wine quality.

Keywords: Arginine; defoliation; grape; osmoregulation; proline; optimal harvest

INTRODUCTION

Grape ripening includes different physical and biochemical modifications in the seeds, skin and pulp (Van Leeuwen *et al*., 2023). Optimal berry ripeness depends on the wine style goal defined by the winemakers (Gutiérrez-Gamboa *et al*., 2021). The sensory characteristics of the finished wine, and thus its quality, are strongly dependent on the perception of the primary and secondary metabolites, including the alcohol level (Blancquaert *et al*., 2019). Optimal grape maturity is determined mostly by sugar content, acidity, berry size, phenolic content and taste, which have a direct impact on the type of wine that the winemaker wants to achieve (Le Moigne *et al*., 2008).

Ripening tools have been developed to determine berry maturity objectively and accurately at harvest. Berry maturity indices include (i) total soluble solids (TSS), (ii) titratable acidity (TA), (iii) pH and (iv) combinations thereof (maturity indexes) (Boulton *et al*., 1999). Some authors identify three levels of grape maturity: (i) technological maturity, which corresponds to maximum sugar accumulation/ concentration and low acidity, (ii) phenolic maturity, defined as the level and concentrations of phenolics in the skins and seeds and (iii) aromatic maturity, associated with the decrease in vegetal notes and the evolution of wine volatile profile (Schneider *et al*., 2002; Blancquaert *et al*., 2019).

An in-depth knowledge of grape compositional factors, grape cultivar, climate, topography, seasonal weather conditions and vineyard management practices is required to reach the "optimal ripeness" decision to achieve a targeted wine style (Bindon *et al*., 2013). One of these management practices is leaf removal, which directly influences light exposure and berry temperature, thereby affecting the sink-to-source relationship (Song *et al*., 2018). By enhancing light penetration and improving air circulation around the clusters, leaf removal can optimise berry temperature conditions and increase the effectiveness of biostimulants and fungicide formulations, ultimately reducing the incidence of grapevine diseases (Ivanišević *et al*., 2020). Timing of harvest maximise grape quality in terms of its amino acid composition under different viticultural practice however research on this topic has been minimal (Garde-Cerdán *et al*., 2018; Gutiérrez-Gamboa *et al*., 2020). Leaf removal alters the berry microclimate, resulting in significant changes in the concentration and composition of amino acids, with responses differing between cultivars and across harvest dates used by producers.

Berry nitrogen composition is a key factor of must composition (Gutiérrez-Gamboa *et al*., 2020). Must nitrogen composition is essential for yeast growth, since it affects the fermentation kinetics and flavour metabolism of wines (Verdenal *et al*., 2021). A low nitrogen content in the must usually leads to stuck or sluggish fermentations, affecting the operational logistic of the wine cellar (Bisson & Butzke, 2000). Some of the amino acids contained in the musts are precursors of the higher alcohols, esters, carbonyl compounds, volatile fatty acids and sulphur compounds in wines, which are formed from the enzymatic metabolism of yeast and bacteria through alcoholic and malolactic fermentation (Bell & Henschke, 2005). A strong relationship between the amino acid composition of the must and some volatile compounds in wines has been reported by several studies (Bisson & Butzke, 2000; Bell & Henscke, 2005; Garde-Cerdán *et al*., 2018; Verdenal *et al*., 2021).
Leaf removal increases the composition of primary and secondary metabolites, as well as the air flow in the fruit zone; however, it can result in an increase in sun exposure, which can have a negative impact on fruit chemistry. This paper is the first to study the post-harvest amino acid profile and one of the first to characterise the amino acid composition of berries in vineyards from Stellenbosch.

MATERIALS AND METHODS

Study site and plant material

The study was conducted in Shiraz and Cabernet Sauvignon vineyards located in the Stellenbosch Wine of Origin district during the 2018/2019 season. The Shiraz clone SH21K vineyard was oriented northwest to southeast at planting, whereas the Cabernet Sauvignon clone CS 20 vineyard was oriented north to south. The vineyards were grafted on 101-14 Mgt (Vitis riparia × V. rupestris). The vines were trained on a six-wire vertical trellis system, and the blocks were subjected to drip irrigation as required throughout the season.

Treatments and harvest

The treatments consisted of modifying the bunch microclimate, as follows – i) control: no lateral shoot or leaf removal was applied in the bunch zone and ii) leaf removal: defoliation of the leaves located on the eastern/morning side of the bunch zone (Supplementary Fig. 1). 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 system (Lorenz *et al*., 1995). Leaves were removed on the eastern side of the canopy (morning sun) at the fruit zone level (approximately 35 cm to 40 cm above the cordon), as done previously by Coombe (1995). Each treatment consisted of five replicates, and each replicate comprised two panels (six vines between poles), accounting for a total of 120 vines (60 for the control and 60 for the treatment) in each of the vineyards.

Grapes were harvested sequentially on three different dates according to the Eichhorn and Lorenz system (Lorenz *et al*., 1995) (Supplementary Table S1): i) E-L

38: the berries were ripe for the commercial harvest; E-L 38 + 18 days: the berries were harvested 18 days after the commercial harvest; E-L 38 + 34 days: the berries were harvested 34 days after the commercial harvest. Four vines from each of the replicates were harvested on each harvest date. The harvesting was conducted between 06:00 and 10:00. Thirty berries from each of the treatment replicates (n = 5) were sampled on each of the harvest dates. Each berry was weighed individually (n = 150) using a Mettler Toledo AG204 Delta Range (Columbus, Ohio).

Technological parameters of grapes and wines

The determination of soluble solids (°Brix), pH and titratable acidity (g L^{-1} of tartaric acid) in the grapes and the alcohol level (% v/v) of the wines was performed according to OIV (2003) methods.

Organic acids and sugars determination

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

Amino acid analysis

The amino acids in the grape juice were derivatised, separated and quantified on an HPLC equipped with a fluorescence detector, using a Poroshell HPH-C18 column (4.6 × 150 mm, 2.7 µm) and UHPLC Guard 3PK Poroshell HPH-C18 guard column (4.6 × 5 mm, 2.7 µm). The analytical method was adapted from those published in the report of Šuklje *et al*. (2019).

Colour and phenolic measurements

Fifty berries from the Shiraz and Cabernet Sauvignon were sampled at each harvest date and frozen for further analysis. The grapes were thawed for 30 min and homogenised with an IKA Ultra Turrax T18 basic blender for 4 min. The colour density (CD), modified colour density (MCD), co-pigment content, SO_2-resistant pigments, total red pigments (TRP) and total phenolics (TP) of the grape pulp were measured using Boulton indices by spectrophotometric analysis (Boulton, 2001). The tannin levels were determined by the methyl cellulose precipitation (MCP) method and the results were expressed in catechin equivalents (Sarneckis *et al*., 2006). All measurements were performed in triplicate (n = 3).

Small-scale winemaking

Standard winemaking procedures of the experimental cellar of the Department of Viticulture and Oenology, Stellenbosch University were followed. The grapes from each harvest date and replicate were vinified individually. The grapes were crushed and destemmed into 20 L plastic drums and 30 mg L^{-1} SO_2 was added. Must samples for pH, titratable acidity and soluble solids were taken before the SO_2 addition. The crushed grapes were inoculated with 30 g hL^{-1} Saccharomyces cerevisiae (Lalvin ICV D21 ®) for Shiraz and Cabernet Sauvignon. Thus, 30 g hL^{-1} Go Ferm Protect (Lallemand) was added to the rehydration water. Fermentation took place at 25°C in Shiraz and Cabernet Sauvignon, and punch-downs were done three times a day. The rate of fermentation was measured daily with a hydrometer. After this, 0.25 g L^{-1} Fermaid K (Lallemand) was added. The fermentation took about five days, after which the skins were pressed at 1 bar and moved to 20°C to ensure that the fermentation process was complete. Following the alcoholic fermentation, the wines were racked off the lees and 50 mg L^{-1} of SO_2 was added. The wines underwent cold stabilisation for three weeks at -4 °C before adjusting the 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 one day after bottling.

Statistical analysis

The variables were analysed considering a completely randomised design with factorial arrangement, accounting for two treatments (control and leaf removal), two varieties (Shiraz and Cabernet Sauvignon) and three harvest dates (E-L 38, E-L 38 + 18 days and E-L 38 + 34 days). The variables were subjected to an analysis of variance (ANOVA). The significance of the differences was determined by Duncan's test ($p \leq 0.05$). In addition, a multivariate analysis was performed by principal component analysis (PCA) to determine the relationships among the variables according to factors. Both analyses were performed using the InfoStat software (InfoStat, www.infostat.com.ar).

RESULTS

Grape physicochemical parameters

The grape physicochemical parameters of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different dates for harvest are outlined in Table 1. The grape variety affected berry weight, soluble solids, pH, titratable acidity, malic acid, succinic acid, glucose, total sugars, anthocyanins and the colour density of grapes, whereas the treatment factor statistically affected titratable acidity, citric acid and succinic acid (Table 1). Shiraz grapes presented higher berry weight, soluble solids, pH, malic acid, succinic acid, glucose, total sugars, anthocyanins and colour density and lower titratable acidity than Cabernet Sauvignon (Table 1). Leaf removal increased citric acid and decreased the titratable acidity and succinic acid content in grapes compared to the control (Table 1; Fig. 1). The harvest date statistically affected berry weight, soluble solids, pH, titratable acidity, citric acid, succinic acid, glucose, anthocyanins, tannins, total phenol index (TPI) and

colour density (Table 1). Grapes harvested at E-L 38 + 34 days showed the highest soluble solids, pH and succinic acid content, as well as the lowest berry weight, titratable acidity and glucose (Table 1). Grapes harvested at the E-L 38 date showed the highest levels of titratable acidity, citric acid, anthocyanins and colour density (Table 1; Fig. 1).

The variety and harvest interaction affected a higher number of physicochemical parameters in the grapes than the rest of the resultant interactions. The variety and treatment interaction affected berry weight and succinic acid content, whereas the variety and harvest date interaction affected berry weight, citric acid, tartaric acid, succinic acid, glucose, fructose, total sugars and anthocyanins. The treatment and harvest date interaction affected berry weight, citric acid, succinic acid, glucose, fructose and total sugars, whereas the variety, treatment and harvest date interaction affected citric acid, succinic acid and tannins.

Non-polar and neutral amino acids

Table 2 shows the content of non-polar and neutral amino acid in the grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates. Grape variety affected the content of the studied non-polar and neutral amino acids in the grapes, whereas the treatment factor affected the glycine (Gly), isoleucine (Ile), methionine (Met), serine (Ser), glutamine (Gln), threonine (Thr), tyrosine (Tyr), alanine (Ala) and phenylalanine (Phe) content in the grapes (Table 2). Shiraz grapes presented a higher content of the studied non-polar and neutral amino acids than Cabernet Sauvignon grapes. Leaf removal increased the content of Gly, Ile, Met, Ser, Gln, Thr, Tyr, Ala and Phe in the grapes compared to the control (Table 2). As in the case of grape variety, harvest date affected the content of the studied non-polar and neutral amino acids in the grapes. Grapes harvested at E-L 38 + 18 days showed the highest content of Gly, valine (Val), leucine (Leu), asparagine (Asn), Ser, Gln, Tyr and Phe. Harvest at the E-L 38 stage resulted in grapes with the lowest content of Ile and Met, whereas harvest at E-L 38 + 34 days resulted in grapes with the lowest content of Ala.

The variety and treatment interaction statistically affected the content of the studied non-polar and neutral amino acids in the grapes except for Met, whereas the variety and harvest date interaction affected the grape content of Gly, Ile, Leu, Ser, Gln, Thr, Tyr, Ala and Phe. The treatment and harvest date interaction affected only the Asn, Gln, Thr and Ala content in grapes, whereas the interaction of variety x treatment harvest date factors affected the Gln, Thr and Ala content of the grapes.

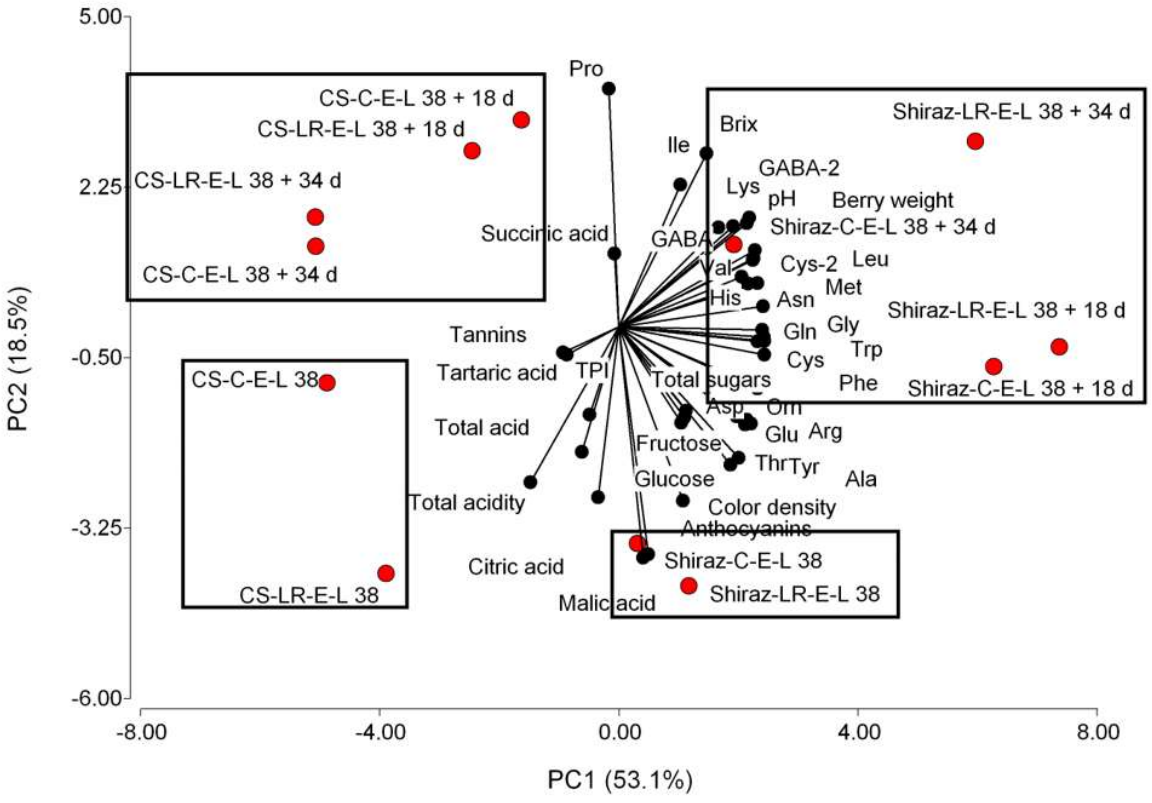

FIGURE 1 Principal component analysis (PCA) of physicochemical parameters and amino acids measured in grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates (i: E-L 38; ii: E-L 38 + 18 days; and iii: E-L 38 + 34).

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TABLE 1 Grape physicochemical parameters of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different dates for harvest (E-L 38 stage, E-L 38 stage + 18 days and E-L 38 stage + 34 days).

	Berry weight	°Brix	pH	Total acidity	Citric acid	Tartaric acid	Malic acid	Succinic acid	Glucose	Fructose	Total acids	Total sugars	Antho-cyanins	Tannins	Total phenols index	Colour density
Variety (V)																
Cabernet Sauvignon	1.465a	24.06a	3.76a	5.29b	0.29a	5.13a	4.93a	2.14a	84.29a	82.84a	12.60a	167.13a	0.97a	3.16a	12.36a	9.72a
Shiraz	1.525b	25.18b	4.06b	4.42a	0.28a	4.23a	5.83b	2.25b	90.14b	88.32a	12.68a	178.46b	1.17b	2.89a	12.42a	11.67b
p-value	0.0000	0.0127	0.0000	0.0000	0.1084	0.1636	0.0086	0.0170	0.0211	0.0581	0.9015	0.0361	0.0107	0.2989	0.9446	0.0098
Treatment (T)																
Control	1.536a	24.27a	3.89a	4.96b	0.20a	4.13a	5.39a	2.32b	87.62a	86.42a	12.06a	174.04a	1.04a	2.99a	12.23a	10.46a
Leaf removal	1.568b	24.97a	3.94a	4.74a	0.36b	5.22a	5.37a	2.07a	86.81a	84.75a	13.21a	171.55a	1.10a	3.06a	12.55a	10.93a
p-value	0.3318	0.0914	0.1141	0.0276	0.0000	0.0967	0.9473	0.0000	0.7181	0.5350	0.0972	0.6141	0.3283	0.7752	0.7292	0.4722
Harvest date (HD)																
E-L 38 stage	1.563b	23.11a	3.73a	5.90c	0.41c	4.98a	5.80a	2.12b	89.24b	87.09a	13.49a	176.33a	1.27b	3.37b	14.07b	12.81b
E-L 38 + 18 days	1.624c	24.56b	3.95b	4.54b	0.21a	5.04a	5.10a	1.51a	89.97b	87.22a	11.94a	177.20a	0.93a	2.48a	10.41a	9.28a
E-L 38 + 34 days	1.469a	26.18c	4.06c	4.11a	0.23b	4.02a	5.25a	2.95c	82.43a	82.43a	12.48a	164.87a	1.01a	3.22b	12.68ab	10.00a
p-value	0.0000	0.0001	0.0000	0.0000	0.0000	0.3351	0.1595	0.0000	0.0304	0.2723	0.1766	0.1046	0.0024	0.0330	0.0225	0.0017
Factor interaction																
V × T	0.0132	0.6395	0.6167	0.2052	0.3439	0.1896	0.2653	0.0000	0.2475	0.1402	0.3867	0.1814	0.5290	0.4424	0.6384	0.5539
V × HD	0.0000	0.8899	0.2673	0.2884	0.0011	0.0360	0.0746	0.0050	0.0015	0.0106	0.1665	0.0044	0.0265	0.1350	0.2430	0.0167
T × HD	0.0000	0.3536	0.6247	0.4264	0.0000	0.9120	0.1050	0.0000	0.0230	0.0383	0.2832	0.0297	0.4411	0.6447	0.7594	0.4632
V × T × HD	0.4210	0.0741	0.5771	0.3394	0.0022	0.5630	0.5019	0.0000	0.8667	0.8546	0.3545	0.8703	0.3012	0.0221	0.1065	0.2726

TABLE 2 Non-polar and neutral amino acid (mg L^{-1}) in grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates (E-L 38 stage, E-L 38 stage + 18 days and E-L 38 stage + 34 days).

	Gly	Val	Ile	Leu	Met	Asn	Ser	Gln	Thr	Tyr	Ala	Phe
Variety (V)												
Cabernet Sauvignon	3.44a	20.53a	9.35a	14.60a	19.26a	4.03a	15.09a	34.96a	15.77a	4.09a	16.70a	7.07a
Shiraz	6.76b	37.46b	10.36b	22.02b	32.74b	8.16b	36.62b	113.19b	47.94b	8.33b	45.16b	10.85b
p-value	0.0000	0.0000	0.0164	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Treatment (T)												
Control	4.78a	28.39a	9.06a	16.83a	22.61a	5.93a	24.41a	68.10a	29.64a	5.76a	28.94a	8.59a
Leaf removal	5.43b	29.60a	10.65b	19.79b	29.38b	6.26a	27.30b	80.05b	34.07b	6.66b	32.92b	9.33b
p-value	0.0037	0.6186	0.0009	0.0051	0.0120	0.1748	0.0137	0.0000	0.0014	0.0042	0.0000	0.0104
Harvest date (HD)												
E-L 38 stage	4.48a	19.88a	7.24a	14.47a	20.62a	5.02a	24.42b	58.47a	34.04b	6.31b	35.66b	8.44a
E-L 38 + 18 days	6.16b	40.82c	11.57b	22.22c	28.57b	7.35c	32.12c	89.93c	37.00c	7.94c	34.17b	10.09b
E-L 38 + 34 days	4.66a	26.28b	10.75b	18.25b	28.81b	5.92b	21.03a	73.83b	24.52a	4.37a	22.95a	8.35a
p-value	0.0000	0.0000	0.0000	0.0000	0.0199	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
Factor interaction												
V × T	0.0049	0.0304	0.0333	0.0187	0.4431	0.0180	0.0395	0.0000	0.0031	0.0425	0.0042	0.0377
V × HD	0.0062	0.1009	0.0003	0.0004	0.0505	0.1429	0.0018	0.0000	0.0024	0.0010	0.0000	0.0205
T × HD	0.2502	0.1419	0.3713	0.2183	0.5282	0.0029	0.0609	0.0000	0.0100	0.2719	0.0037	0.0994
V × T × HD	0.1966	0.3615	0.2012	0.1660	0.3383	0.1242	0.1273	0.0000	0.0133	0.0753	0.0010	0.2837

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Positively and negatively charged amino acids

Table 3 shows the content of positively and negatively charged amino acids in the grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates. The grape variety affected the grape content of the positively and negatively charged amino acids that were studied, whereas the treatment factor affected the content of cysteine (Cys), serine (Ser), arginine (Arg), ornithine (Orn) and lysine (Lys) in the grapes. Shiraz grapes exhibited higher concentrations of both positively and negatively charged amino acids compared to Cabernet Sauvignon, except for proline (Pro), which was found at lower levels in Shiraz grapes. Leaf removal increased the content of Cys, Ser, Arg, Orn and Lys in the grapes. Harvest date affected the content of most of the positively and negatively charged amino acids in the grapes, except for cysteinylcysteine (Cys-2). Grapes harvested at E-L 38 + 34 days showed the lowest content of aspartic acid (Asp), glutamic acid (Glu), Ser and Arg, whereas the E-L 38 harvest date resulted in the lowest content of Cys, γ-aminobutyric acid (GABA), GABA-2, Lys, hydroxyproline (HoPro) and Pro. Grapes harvested at E-L 38 + 18 days showed the highest content of Glu, Ser, histidine (His), GABA, GABA-2, Orn, Lys and HoPro.

The variety and treatment interaction affected most of the charged amino acid in the grapes except for Glu, whereas the variety and harvest date factors affected most of the amino acids in the grapes, except for His and HoPro. The variety and harvest date interaction, including the interaction of the studied factors, affected the content of Asp, Glu, Cys, Arg, Orn and Lys.

Wine physicochemical parameters

Table 4 shows the content of physicochemical parameters of wines produced from the grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates. The grape variety affected ethanol wine content, whereas the treatment did not affect any of the determined parameters. Shiraz wines presented a higher ethanol content than the Cabernet Sauvignon wines. The harvest date factor affected the studied wine physicochemical parameters. Wines derived from grapes harvested at the E-L 38 stage presented a lower glucose, fructose, glycerol and ethanol content than the wines produced from grapes harvested at the E-L 38 stage + 34 days.

The variety and treatment interaction affected the ethanol content of wines, whereas the variety and harvest date interaction affected the content of glycerol in the wines. The treatment and harvest date interaction did not affect the physicochemical parameters of wines, whereas the interaction of the studied factors affected the ethanol and glycerol content of the wines.

Matrix corrections and principal component analysis

The correlations among the physicochemical parameters and amino acids measured in the grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates are outlined in Table 5. Berry weight was strongly correlated with pH (+0.87), total acidity (-0.70), colour density (-0.80) and most of the studied amino acids (> +0.91) except for Pro. Soluble solids (°Brix) was correlated with total acidity (-0.82) and total tannins (+0.92). pH was correlated with total acidity (-0.94), glucose (+0.78), total sugars (+0.73) and most of the amino acids except for Pro (> +0.91), whereas total acidity was correlated with most of the amino acids except for Pro (> -0.78). Citric acid was correlated with malic acid (-0.83), succinic acid (+0.83), glucose (-0.78), fructose (-0.85), total sugars (-0.81), anthocyanins (-0.99) and Pro (-0.91). Tartaric acid was correlated with malic acid (-0.70), total acids (+0.79) and the total phenolic index (TPI) (+0.85), whereas malic acid was correlated with succinic acid (+0.98), fructose (+0.99), total sugars (+0.99), anthocyanins (+0.86) and Pro (+0.94). Succinic acid was correlated with anthocyanins (-0.81) and colour density (-0.96). Glucose was correlated with fructose (+0.99), total sugars (+0.99), anthocyanins (+0.80), Glu (+0.74), Gly (+0.70), GABA (+0.77), Cys-2 (+0.75), Val (+0.71), GABA-2 (+0.75), HoPro (+0.81) and Pro (+0.95), whereas fructose was correlated with total sugars (+0.99), anthocyanins (+0.87) and Pro (+0.95). Total sugars were correlated with anthocyanins (+0.84), GABA-1 (+0.71), GABA-2 (+0.70), HoPro (+0.71) and Pro (+0.95). Anthocyanins were correlated with Pro (+0.95), whereas tannins were correlated with TPI (+0.88). Colour density was correlated with most of the amino acids (> -0.70) except for Glu, GABA, Cys-2, Val, GABA-2, Orn, HoPro, Pro and total amino acids.

Principal component analysis of the physicochemical parameters and amino acids measured in the grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal was done at three different harvest dates. The first principal component (PC1) accounted 53.1% of the variance, whereas the second principal component (PC1) achieved 18.5% of the variance, corresponding to a total variance of 71.6%. PC1 was strongly correlated with most of the amino acids (> 0.80), except for Asp, Ile and Pro, whereas PC2 was strongly correlated with anthocyanins, colour density and Pro (> 0.80). Shiraz grapes harvested at E-L 38 + 18 days and at E-L 38 + 34 days from the control and leaf removal treatment were positively correlated with most of the amino acids and negatively with tannins, tartaric acid, total phenolic index (TPI) and total amino acids, being located in an opposite direction to Cabernet Sauvignon grapes harvested at E-L 38

TABLE 3 Positively and negatively charged amino acid (mg L^{-1}) in grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates (E-L 38 stage, E-L 38 stage + 18 days and E-L 38 stage + 34 days).

	Asp	Glu	Cys	Ser	His	Arg	GABA-1	CyS-2	GABA-2	Orn	Lys	HoPro	Pro
Variety (V)													
Cabernet Sauvignon	1.78a	7.42a	2.19a	15.09a	19.13a	61.88a	117.12a	7.41a	85.47a	5.36a	3.10a	18.34a	177.25b
Shiraz	6.36b	26.24b	3.35b	36.62b	34.30b	306.02b	176.20b	12.61b	132.13b	12.89	5.40b	34.13b	140.88a
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Treatment (T)													
Control	4.07a	17.05a	2.69a	24.41a	25.61a	168.40a	145.81a	9.43a	106.05a	8.59a	3.87a	25.78a	162.93a
Leaf removal	4.08a	16.61a	2.84b	27.30b	27.82b	199.50b	147.51a	10.59a	111.54a	9.65b	4.62b	26.69a	155.21a
p-value	0.9262	0.3645	0.0004	0.0137	0.2379	0.0001	0.7922	0.0992	0.3345	0.0002	0.0000	0.6771	0.1813
Harvest date (HD)													
E-L 38 stage	4.86b	15.98b	2.61a	24.42b	20.64a	202.55b	119.44a	8.75a	78.49a	8.86a	3.64a	15.54a	105.54a
E-L 38 + 18 days	4.94b	25.94c	2.85b	32.12c	35.18b	206.23b	174.68c	10.85b	134.54c	10.03b	4.73c	35.55c	184.47b
E-L 38 + 34 days	2.41a	8.57a	2.84b	21.03a	24.32a	143.07a	145.87b	10.43ab	113.35b	8.47a	4.38b	27.62b	187.20b
p-value	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0520	0.0000	0.0001	0.0000	0.0000	0.0000
Factor interaction													
V × T	0.0156	0.1127	0.0034	0.0395	0.0108	0.0068	0.0290	0.1515	0.0308	0.0007	0.0080	0.0463	0.4610
V × HD	0.0000	0.0000	0.0002	0.0018	0.2625	0.0000	0.0017	0.0013	0.0012	0.0194	0.0001	0.0839	0.0000
T × HD	0.0001	0.0127	0.0001	0.0609	0.0864	0.0002	0.1625	0.6168	0.3188	0.0165	0.0072	0.3394	0.5254
V × T × HD	0.0001	0.0327	0.0000	0.1273	0.2125	0.0010	0.4360	0.8404	0.4799	0.0369	0.0028	0.1547	0.1461

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TABLE 4 PPhysicochemical parameters of wines produced from grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates (E-L 38 stage, E-L 38 stage + 18 days and E-L 38 stage + 34 days).

	Glucose	Fructose	Ethanol (% v/v)	Glycerol
Variety (V)				
Cabernet Sauvignon	1.88a	0.82a	13.00a	12.76a
Shiraz	3.00a	0.74a	14.35b	12.41a
p-value	0.1028	0.4382	0.0000	0.2907
Treatment (T)				
Control	2.24a	0.69a	13.65a	12.25a
Leaf removal	2.64a	0.88a	13.69a	12.92a
p-value	0.5334	0.0115	0.8208	0.0562
Harvest date (HD)				
E-L 38 stage	1.04a	0.56a	12.39a	11.35a
E-L 38 + 18 days	2.64ab	0.78ab	14.16b	12.21b
E-L 38 + 34 days	3.63b	1.02b	14.47b	14.20c
p-value	0.0185	0.0115	0.0000	0.0000
Factor interaction				
V × T	0.4083	0.3387	0.0340	0.0728
V × HD	0.4907	0.3497	0.3350	0.0126
T × HD	0.4956	0.8968	0.6224	0.0509
V × T × HD	0.2574	0.6401	0.0384	0.0094

from the control and leaf removal treatment. Shiraz grapes harvested at E-L 38 from the control and leaf removal treatment were positively correlated with glucose, fructose, anthocyanins and colour density, and negatively correlated with Pro and succinic acid, being located in an opposite direction to Cabernet Sauvignon grapes harvested at E-L 38 +18 days and at E-L 38 + 24 days from the control and leaf removal treatment.

DISCUSSION

Shiraz exhibited higher berry weight, pH, soluble solids (SS), sugars, glucose, anthocyanins and colour density, while Cabernet Sauvignon had higher titratable acidity and tartaric acid (Table 1). Similarly, Sadras and Petrie (2011) showed that the rate of increase in total SS during ripening was more significant for Shiraz than for Cabernet Sauvignon. The onset of ripening for both varieties was positively correlated with temperature, but Shiraz exhibited a higher sensitivity to temperature changes compared to Cabernet Sauvignon; warmer conditions led to earlier ripening for Shiraz, while Cabernet Sauvignon showed a more stable onset (Sadras & Petrie, 2011). This suggests that Shiraz ripens faster, accumulating sugars more quickly compared to Cabernet Sauvignon, which has a slower rate of sugar accumulation. The differences in grape physicochemical parameters influenced the sensory characteristics of the wines, since Shiraz wines had a higher ethanol content than those from Cabernet Sauvignon, and, as expected, the harvest date also affected ethanol and glycerol concentrations. Grapes harvested at later phenological stages (E-L 38 + 34 days) produced wines with a higher ethanol content, which is consistent with the increase in soluble solids at this harvest point. Leaf removal increased citric acid content and decreased titratable acidity in the grapes, altering the overall profile of the resultant wines.

Some factor interactions, such as variety and harvest date and treatment and harvest date, considerably affected the content of the studied amino acids (Tables 2 and 3). This study shows that grapes harvested at E-L 38 + 34 days (~26.2°Brix) had a low content of several amino acids, whereas those harvested at E-L 38 + 18 days (~24.6°Brix) exhibited the highest amino acid levels. This finding suggests that this might be the optimal harvesting window for enhancing amino acid content, which is crucial for yeast fermentation and subsequent wine flavour (Gutiérrez-Gamboa *et al*., 2020). These findings are in accordance with Garde-Cerdán *et al*. (2018), who reported that the content of specific amino acids,

TABLE 5 Correlations physicochemical parameters and amino acids measured in grapes of Cabernet Sauvignon and Shiraz grapevines subjected to leaf removal at three different harvest dates (E-L 38 stage, E-L 38 stage + 18 days and E-L 38 stage + 34 days).

	Berry weigh	ºBrix	pH	Total acidity	Citric acid	Tartaric acid	Malic acid	Succinic acid	Glucose	Fructose	Total acids	Total sugars	Antho-cyanins	Tannins	TPI	Color density
Berry weight	1															
ºBrix	0.26	1														
pH	0.87	0.59	1													
Total acidity	0.70	0.82	0.94	1												
Citric acid	0.12	0.22	0.31	0.34	1											
Tartaric acid	0.69	0.36	0.54	0.24	0.22	1										
Malic acid	0.40	0.11	0.65	0.53	0.83	0.70	1									
Succinic acid	0.61	0.16	0.27	0.22	0.83	0.06	0.48	1								
Glucose	0.52	0.25	0.78	0.67	0.78	0.69	0.98	0.34	1							
Fructose	0.39	0.15	0.66	0.55	0.85	0.66	0.99	0.49	0.99	1						
Total acids	0.11	0.64	0.07	0.19	0.49	0.79	0.69	0.49	0.57	0.66	1					
Total sugars	0.46	0.21	0.73	0.62	0.81	0.68	0.99	0.41	0.99	0.99	0.61	1				
Anthocyanins	0.08	0.21	0.34	0.36	0.99	0.26	0.86	0.81	0.80	0.87	0.51	0.84	1			
Tannins	0.13	0.92	0.30	0.59	0.39	0.58	0.07	0.18	0.16	0.12	0.61	0.14	0.37	1		
TPI	0.57	0.64	0.19	0.14	0.29	0.85	0.21	0.36	0.19	0.17	0.63	0.18	0.25	0.88	1	
Color density	0.80	0.29	0.52	0.46	0.65	0.23	0.23	0.96	0.08	0.23	0.39	0.15	0.62	0.10	0.40	1
Aspartic acid	0.96	0.47	0.97	0.87	0.09	0.61	0.52	0.47	0.66	0.53	0.06	0.60	0.13	0.12	0.37	0.70
Glutamic acid	0.92	0.51	0.99	0.90	0.22	0.61	0.61	0.36	0.74	0.62	0.10	0.69	0.25	0.18	0.30	0.60
Cysteine	0.97	0.44	0.96	0.84	0.05	0.62	0.50	0.50	0.64	0.50	0.07	0.58	0.09	0.08	0.40	0.72
Asparagine	0.97	0.48	0.96	0.86	0.05	0.59	0.49	0.51	0.63	0.49	0.02	0.57	0.09	0.12	0.36	0.72
Serine	0.97	0.47	0.95	0.85	0.02	0.58	0.46	0.54	0.60	0.46	0.01	0.54	0.05	0.10	0.38	0.75
Glutamine	0.99	0.42	0.91	0.80	0.08	0.58	0.39	0.61	0.53	0.39	0.01	0.47	0.04	0.04	0.43	0.80
Histidine	0.97	0.48	0.96	0.86	0.03	0.58	0.47	0.52	0.61	0.47	0.01	0.55	0.07	0.12	0.36	0.74
Glycine	0.94	0.52	0.99	0.90	0.16	0.59	0.57	0.41	0.70	0.57	0.06	0.64	0.20	0.18	0.30	0.64
Threonine	0.98	0.44	0.95	0.84	0.01	0.61	0.47	0.53	0.61	0.47	0.04	0.55	0.05	0.07	0.41	0.74

TABLE 5 (CONTINUED)

	Berry weigh	°Brix	pH	Total acidity	Citric acid	Tartaric acid	Malic acid	Succinic acid	Glucose	Fructose	Total acids	Total sugars	Antho-cyanins	Tannins	TPI	Color density
Arginine	0.97	0.49	0.95	0.86	0.02	0.56	0.45	0.54	0.60	0.46	0.01	0.53	0.05	0.13	0.35	0.75
Alanine	0.97	0.46	0.96	0.85	0.05	0.60	0.49	0.51	0.63	0.49	0.04	0.57	0.08	0.10	0.38	0.72
GABA1	0.91	0.51	0.99	0.91	0.25	0.61	0.64	0.32	0.77	0.65	0.12	0.71	0.29	0.20	0.29	0.57
Tyrosine	0.98	0.45	0.93	0.83	0.05	0.57	0.41	0.59	0.55	0.41	0.02	0.49	0.01	0.08	0.39	0.79
Cys2	0.91	0.52	0.99	0.91	0.23	0.60	0.62	0.34	0.75	0.63	0.10	0.69	0.27	0.21	0.28	0.59
Valine	0.94	0.5	0.98	0.89	0.16	0.60	0.57	0.41	0.71	0.58	0.08	0.65	0.20	0.16	0.33	0.64
Methionine	0.98	0.45	0.94	0.83	0.03	0.58	0.43	0.57	0.57	0.43	0.00	0.51	0.01	0.08	0.40	0.77
GABA2	0.92	0.5	0.99	0.90	0.23	0.61	0.63	0.34	0.75	0.63	0.11	0.70	0.26	0.19	0.30	0.59
Tryptophan	0.98	0.44	0.93	0.82	0.04	0.58	0.41	0.58	0.56	0.41	0.01	0.49	0.01	0.07	0.41	0.78
Phenylalanine	0.96	0.48	0.97	0.87	0.08	0.59	0.51	0.48	0.65	0.51	0.04	0.59	0.12	0.13	0.35	0.70
Isoleucine	0.97	0.44	0.88	0.78	0.18	0.51	0.28	0.69	0.44	0.28	0.11	0.37	0.14	0.05	0.40	0.86
Ornithine	0.95	0.47	0.98	0.87	0.13	0.62	0.56	0.43	0.69	0.56	0.09	0.63	0.17	0.13	0.36	0.66
Leucine	0.98	0.45	0.94	0.84	0.01	0.59	0.44	0.55	0.59	0.45	0.01	0.52	0.03	0.08	0.40	0.76
Lysine	0.98	0.46	0.93	0.83	0.05	0.56	0.40	0.59	0.55	0.40	0.03	0.48	0.01	0.08	0.39	0.79
HoPro	0.87	0.54	0.99	0.93	0.34	0.59	0.69	0.24	0.81	0.70	0.14	0.76	0.37	0.26	0.23	0.49
Proline	0.30	0.41	0.68	0.67	0.91	0.42	0.94	0.52	0.95	0.95	0.42	0.95	0.92	0.42	0.12	0.27
Total aas	0.95	0.49	0.98	0.88	0.11	0.60	0.54	0.45	0.67	0.54	0.05	0.61	0.15	0.14	0.34	0.68
Total aasPro	0.96	0.49	0.97	0.87	0.08	0.59	0.51	0.48	0.65	0.52	0.04	0.59	0.12	0.13	0.35	0.70

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such as phenylalanine, tryptophan, arginine, histidine, leucine, isoleucine, valine, methionine and proline, showed a maximum content at 25°Brix and decreased considerably towards 30°Brix. The findings suggest that the harvest timing, specifically when the grape reaches sugar maturity at 25°Brix, might coincided with the optimal nitrogenous maturity, which is crucial for balancing the fermentation needs for proline-accumulator varieties like Cabernet Sauvignon (Table 3) (Gutiérrez-Gamboa *et al.*, 2020). Shiraz had generally higher amino acid concentrations than Cabernet Sauvignon, except that proline and leaf removal notably increased the levels of several amino acids. Proline is not metabolised by yeasts, and stuck and sluggish fermentations can occur when the content of yeast assimilable nitrogen is low, as in the case of Cabernet Sauvignon (Bell & Henscke, 2005).

Several amino acids in grapes were significantly affected by the interactions between the variety and treatment factors, especially in relation to neutral and non-polar amino acids, and leaf removal seemed to amplify varietal differences, with Shiraz responding more significantly than Cabernet Sauvignon (Tables 2 and 3). Shiraz presented higher water uptake and stomatal conductance, and it can reach an optimum technological maturity at low leaf-to-fruit ratios compared to Cabernet Sauvignon (Hochberg *et al.*, 2013; Gutiérrez-Gamboa *et al.*, 2019). This supports the suggestions that Shiraz is characterised by being a more vigorous and sensitive variety to changes in the canopy. In this regard, basal leaf removal applied 72 days after flowering in Sauvignon blanc grapevines promoted the synthesis of alanine, arginine, aspartic acid, proline and serine in grapes, and showed the highest content of total amino acids (Yue *et al.*, 2019). This increase in the grape amino acid content of Shiraz subjected to leaf removal could be attributed to enhanced sunlight exposure and improved photosynthetic activity, which in turn enhance nitrogen assimilation and amino acid synthesis (Yue *et al.*, 2019; Gutiérrez-Gamboa *et al.*, 2020).

This study reports that the glucose, fructose and total sugar content in the grapes was strongly correlated with proline (Table 5). Proline has been identified as a key molecule with significant osmoprotective potential (Gutiérrez-Gamboa *et al.*, 2020). The correlation of proline with glucose, fructose and total sugars in grapes provides insight into its possible role in protecting grapevines from environmental stress, particularly high temperatures and water scarcity, during berry development. Canoura *et al.* (2018) studied the effects of irrigation in Chardonnay and Shiraz varieties and reported that the proline concentration in water-stressed Shiraz vines was more than double that in the irrigated treatments. This contrasts with the Chardonnay grapes, where proline accumulation in the grapes of non-irrigated vines was like that of the three sets of irrigated vines, supporting the above-mentioned findings (Canoura *et al.*, 2018). During periods of water stress or heat stress, grapevine cells may experience osmotic stress, leading to the activation of various stress-related metabolic pathways, such as proline synthesis, which is upregulated under these conditions (Haider *et al.*, 2019; Wei *et al.*, 2022). The increased proline concentrations can influence the cell's osmotic potential, promoting the retention of water in the tissues (Wei *et al.*, 2022). This osmo-protective role of proline, particularly in water-deficit or high-temperature conditions, likely helps grapevines optimise sugar accumulation during berry ripening.

CONCLUSION

Leaf removal enhanced the levels of several key amino acids, thereby improving grape nitrogen content which is crucial for fermentation. In addition, the harvest date significantly influenced amino acid levels, with the optimal harvest occurring at E-L 38 + 18 days, providing the highest amino acid concentrations beneficial for fermentation. Proline, an amino acid with osmo-protective properties, was correlated with a higher sugar content, indicating its potential role in protecting grapevines under stress and aiding in sugar accumulation during ripening. Shiraz grapes had higher concentrations of sugars, amino acids and anthocyanins compared to Cabernet Sauvignon, confirming its faster ripening and greater sensitivity to environmental factors. The Shiraz wines had a higher ethanol content, consistent with the higher sugar concentrations found in the fruit. Overall, this study highlights the importance of canopy management and precise harvest timing to optimise grape composition, particularly amino acids, for improved fermentation efficiency and wine quality.

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