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The Formation of Aroma Compounds During Fermentation in Relation to Yeast Nutrient Source in Sauvignon Blanc Wine

Zorica Lelova Temelkova [1,2], Helena Baša Česnik [3], Andreja Vanzo [3] and Klemen Lisjak [3,*]

[1] Tikves Winery AD, 8mi Septemvri, 1430 Kavadarci, North Macedonia; zorica.lelova@tikves.com.mk
[2] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva ulica 101, 1000 Ljubljana, Slovenia
[3] Department of Fruit Growing, Viticulture, and Oenology and Central Laboratories, Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia; andreja.vanzo@kis.si (A.V.)
* Correspondence: klemen.lisjak@kis.si; Tel.: +386-12805170

Abstract

This study aimed to determine the effects of diammonium phosphate (DAP) and yeast autolysates (organic nutrients) added during alcoholic fermentation on the content and profile of aroma compounds in Sauvignon Blanc wines. Sequential additions of either DAP or organic nutrients were applied mainly during the first half of fermentation, increasing yeast assimilable nitrogen (YAN) from an initial 124 mg N/L to final concentrations of 208 and 209 mg N/L for DAP and yeast autolysates, respectively. Control musts were fermented without nutrient supplementation. All treatments were fermented using commercial yeast strain. Varietal thiols, ethyl and acetate esters, higher alcohols, glutathione (GSH), and YAN were monitored at early, mid, and late stages of fermentation, as well as in the final wines. Varietal thiols were formed at early stages of fermentation in all treatments; however, concentrations of both 4-methyl-4-sulfanylpentan-2-one (4MSP) and 3-sulfanylhexan-1-ol (3SH) were higher in wines supplemented with organic nutrients comparing to DAP and control. Compared to the control, DAP and organic nutrient supplementation increased ethyl ester concentrations in wine by 40.2% and 26.9%, respectively. Both nutrient treatments also resulted in higher acetate ester concentrations, while total higher alcohols were reduced by 19.1% with DAP and 12.1% with organic nutrients. No significant differences in GSH concentrations were observed among treatments. Sensory analysis revealed that wines supplemented with DAP achieved the highest scores for tropical aroma, varietal aroma, and overall quality. Overall, sequential supplementation with either inorganic or organic nitrogen positively influenced fermentation kinetics and aroma compound composition, resulting in improved varietal expression of Sauvignon Blanc wines. However, in low-YAN musts, DAP had a greater impact than organic nitrogen sources and should therefore be considered a key strategy for ensuring an adequate yeast nitrogen status.

Keywords: fermentation; yeast nutrients; varietal thiols; esters; higher alcohols; wine

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1. Introduction

Nitrogen is an essential nutrient for yeasts during alcoholic fermentation [1]. Under oenological conditions, a low concentration of yeast assimilable nitrogen (YAN; <150 mg N/L) in grape must is widely recognized as a major cause of slow or problematic fermentations, as it limits yeast growth and reduces fermentation rate [2,3]. YAN concentration directly affects not only fermentation kinetics and duration but also yeast metabolism and the formation of volatile aroma compounds [4]. To ensure reliable fermentation, Bisson and

Butzke (2000) [5] recommended the addition of approximately 25 mg N/L per 1 °Brix of soluble solids. However, several studies have demonstrated that complete fermentation can be achieved in high-sugar musts with substantially lower nitrogen levels. Wang et al. [6] reported that 60 mg N/L YAN was sufficient to complete fermentation in synthetic juice containing 240 g/L sugars (~22 °Brix).

YAN in grape must consists primarily of free amino nitrogen (FAN) and ammonium ions (NH_4^+). Ammonium represents the most readily assimilable nitrogen source for yeasts; however, its presence can suppress the uptake of amino acids, including glutathione (GSH) precursors such as cysteine, glutamate, glycine, and serine, potentially limiting intracellular GSH synthesis [7]. Yeasts regulate nitrogen uptake through nitrogen catabolite repression (NCR), a mechanism that prioritizes preferred nitrogen sources [3]. The strength of NCR depends on nitrogen source quality, with asparagine and glutamine acting as the strongest repressors, followed by ammonium, glutamate, urea, γ-aminobutyric acid, and finally proline [8]. YAN concentrations in must are influenced by multiple factors, including grape variety, vineyard practices, and growing region [3].

In many viticultural regions worldwide, YAN levels have been reported as suboptimal. A median YAN concentration of 187 mg N/L was reported in Australian grape and juice samples sampled between 2006 and 2019 (n = 1390) [9]. Similarly, 36% of Greek musts (n = 274) exhibited YAN concentrations below 140 mg N/L [10], while 58.3% of Italian must samples (n = 586) were below the classical nitrogen deficiency threshold [11]. Despite low YAN levels, several studies have demonstrated successful completion of fermentation in musts containing 22–24 °Brix with YAN concentrations below 100 mg N/L [6,12], far lower than the traditionally recommended range of 225–275 mg N/L [5]. Other studies, however, have shown that nitrogen supplementation does not always prevent sluggish or stuck fermentations, particularly at higher sugar concentrations [13]. For instance, among four yeast strains in fermenting media containing 240 g/L sugar, one completed fermentation with 120 mg N/L YAN, two required 190 mg N/L, and one failed to complete fermentation even at 290 mg N/L [14]. These findings highlight the complexity of nitrogen requirements and the strain-dependent nature of yeast nitrogen metabolism.

Nitrogen metabolism in yeast, particularly amino acid, plays a key role in the formation of fermentation-derived aroma compounds, including higher alcohols and their acetate esters. Ethyl esters and higher alcohols contribute significantly to the fruity and floral sensory attributes of wines. In addition to nitrogen, grape solids after pressing provide essential nutrients such as phytosterols and fatty acids. Excessive must clarification can remove an important source of lipids, negatively affecting yeast growth and fermentation rate, even when YAN levels are available [15]. Studies examining the impact of nitrogen supplementation on volatile compound formation have showed contradictory results, mainly related to differences in nitrogen source (organic versus inorganic) and timing of addition. Most of previous research has focused on inorganic nitrogen additions, particularly ammonium salts applied at the beginning of fermentation [16]. However, increasing evidence indicates that both the composition and timing of nitrogen supplementation strongly influence aroma compound production [17,18]. Moreover, yeast genetic background significantly affects nitrogen requirements, with reported nitrogen consumption ranging from 0.62 to more than 0.91 mg N per g of sugar consumed among different strains [14]. In all cases, higher nitrogen availability led to increased nitrogen uptake which means that more abundant is the yeast assimilable nitrogen, more the yeast consumes it. The nitrogen that yeast assimilable nitrogen can also be stored intracellularly in vacuoles. Yeasts could use reserves of nitrogen during the stationary phase for new protein synthesis following protein turnover as suggested [19].

Varietal thiols are key contributors to the characteristic of grapefruit, citrus, and tropical fruit aromas of Sauvignon Blanc wines [20]. These compounds are released during fermentation from non-volatile cysteinylated and glutathionylated precursors through yeast β-lyase activity [21,22]. The concentration of thiols in grapevine is related to the concentration of their pre-cursors. However, only 1–4% of these precursors release the aromatic thiol during fermentation [23]. The effect of diammonium phosphate (DAP) supplementation on thiol release remains unclear, with studies reporting both increased [24] and decreased [25] thiol formation. Ammonium-driven catabolic repression can reduce the synthesis of amino acid transporters, limiting the uptake of cysteinylated thiol precursors and their subsequent intracellular conversion [26]. In addition to nitrogen, lipid availability in must plays a crucial role in thiol metabolism: lipid supplementation has been shown to enhance the formation of 4-methyl-4-sulfanylpentan-2-one (4MSP) and 3-sulfanylhexan-1-ol (3SH) [27,28], while increased lipid levels may reduce 3-sulfanylhexyl acetate (3SHA) formation [28].

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) is a tripeptide with antioxidant properties that protects wines against oxidative degradation and preserves aroma compounds such as esters, terpenes, and varietal thiols [29]. Herbst et al. [30] showed that the addition of sulphur dioxide and glutathione reduced the decrease in 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in Sauvignon Blanc wine over a four-week period. In grape must, glutathione interferes with the enzymatic oxidation mechanism by trapping the caftaric acid quinones produced during oxidation, forming 2-S-glutathionyl caftaric acid, also known as the grape reaction product (GRP) [31]. However, the influence of nitrogen supply on GSH formation during fermentation remains unresolved. While some studies reported no significant effect of nitrogen concentration on GSH levels [32], others observed increased GSH concentrations following nitrogen supplementation, depending on grape variety and fermentation conditions [33].

Ammonium-based supplements are widely used in winemaking due to their rapid assimilation by yeast, however, organic nutrient additions have also been shown to positively affect wine aroma composition. Despite their widespread practical use, the combined effects of nitrogen source and sequential addition strategy on aroma development and sensory outcomes in wines remain insufficiently characterized. Therefore, this study hypothesized that sequential supplementation with inorganic versus organic nitrogen sources would differentially modulate fermentation performance, aroma compound formation, and sensory profiles of Sauvignon Blanc wines under low YAN conditions.

2. Materials and Methods

2.1. Chemicals and Reagents

HPLC-grade ethanol was obtained from J.T. Baker (Deventer, The Netherlands). Sodium chloride (p.a.), ethyl acetate (p.a.), hydrochloric acid (37% *v*/*v*), and anhydrous sodium sulfate were purchased from Carlo Erba (Val de Reuil, France). HPLC-grade methanol, reduced glutathione, cysteine hydrochloride hydrate, dichloromethane, *p*-hydroxymercuribenzoate (p-HMB), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), Dowex ion-exchange resin, formic acid, and sulfuric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was produced using a Milli-Q purification system (Millipore, Bedford, MA, USA).

Aroma reference standards, with the highest available purity (≥98%), were purchased from Merck (Rahway, NJ, USA), Sigma-Aldrich, Fluka (Seelze, Germany), and SAFC (St. Louis, MO, USA), except for 4-methyl-4-sulfanylpentan-2-one (4MSP) and 3-sulfanylhexyl acetate (3SHA), which were obtained from Oxford Chemicals (Hartlepool, UK), and 3-sulfanylhexanol (3SH) and 4-methoxy-2-methyl-2-sulfanylbutane (4MMB),

which were purchased from Penta Manufacturing Company (Livingston, MT, USA). Deuterated [$^2H_2$]-3-sulfanylhexan-1-ol (d5-3SH) was obtained from Eptes (Vevey, Switzerland). Reduced glutathione (GSH) and oxidized glutathione (GSSG) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sauvignon Blanc Grapes

Sauvignon Blanc grapes 2019 from the Tikveš winegrowing region (North Macedonia) were used in the experiments. The grapes were hand-harvested into 20 kg boxes, cooled overnight to 10–12 °C, and subsequently destemmed, crushed, and pressed using an inert press (Bucher Vaslin, Chalonnes-sur-Loire, France). The system is based on an inert gas atmosphere (in our case carbon dioxide) to reduce oxygen exposure during pressing. At pressing, potassium metabisulfite ($K_2S_2O_5$; 8 g/100 kg of grapes) and a commercial pectolytic enzyme (Vinozym Vintage FCE G, Lamothe Abiet, Canéjan, France; 2 g/100 kg of grapes) were added.

After sedimentation, basic must analyses were performed. The grape must exhibited the following parameters: 23.5 °Brix, total acidity of 6.8 g/L (expressed as tartaric acid), pH 3.24, yeast-assimilable nitrogen (YAN) of 124 mg N/L (60 mg N/L as NH_4^+ and 64 mg N/L as free amino nitrogen, FAN), and sulfur dioxide contents of 14 mg/L free SO_2 and 43 mg/L total SO_2. Before yeast and yeast nutrient addition, turbidity values of the grape juice ranged from 93 to 101 NTU across all samples. Total soluble solids (°Brix) were determined using a Digital Refractometer ATAGO RJ-100 (Atago Co., Ltd., Tokyo, Japan), and turbidity was analyzed using a turbidimeter 2100N (Hach Company, Loveland, CO, USA). The basic methods of analysis for must and wine are described in Section 2.4.

2.3. Fermentation and Yeast Nutrient Additions

Fermentations were conducted in 1 L glass vessels in triplicate for each treatment. The commercial yeast Zymaflore® VL3 (Laffort, Floirac, France) was inoculated at 0.2 g/L after rehydration in water at 38 °C for 30 min. During alcoholic fermentation, the temperature was maintained between 14 and 18 °C using a water bath.

Diammonium phosphate (DAP) and organic yeast nutrients were added on the third day of fermentation (average sugar concentration: 180 g/L from an initial 225 g/L), on the fifth day (144 g/L), and on the seventh day (96 g/L) (Table 1). A final nutrient addition was performed at a late fermentation stage (day 13, at approximately 20 g/L residual sugars) to ensure completion of fermentation (Table 1). The total DAP addition corresponded to 84 mg N/L of yeast-assimilable nitrogen (YAN), with 72% supplied during the first half of fermentation (Table 1).

Table 1. Timeline of nutrient additions (mg/L) and sampling for secondary metabolites during and at the end of fermentation.

Days of Fermentation	Day 3	Day 5	Day 7	Day 10	Day 13	Day 18
Nutrient additions (mg/L)						
Control	0	0	0	–	0	–
DAP	+200	+50	+50	–	+30	–
Organic N	+200	+200	+200	–	+125	–
Sampling for secondary metabolites	✔	–	✔	✔	–	✔

The organic yeast nutrient Lysopol (Bioenologia 2.0, Oderzo, Italy) consisted of a pure yeast lysate in the form of a commercial autolysate produced from a mixture of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, characterized by a high content of B vitamins. An addition rate of 20 g/hL released 20 mg N/L of YAN equivalent. The total

organic nutrient addition resulted in 83 mg N/L of YAN equivalent, comparable to the DAP treatment.

Samples for secondary metabolite analysis were collected on day 3 (6 h after nutrient addition), and on days 7 and 10 of fermentation (Table 1). Following sampling, 50 mg/L of SO_2 was added, and samples were stored at −20 °C until analysis (within 3 months). At the end of fermentation (day 18), 50 mg/L of SO_2 was added, wines were racked off the lees, and samples were collected for basic physicochemical analyses and secondary metabolite analysis. For the analysis of basic parameters, aliquots from each replicate were pooled and analysed as a representative sample. Wines were stored at 12 °C until sensory evaluation, which was performed four months later.

2.4. Basic Analyses

Acetic acid and glucose/fructose concentrations were determined enzymatically using a Diatron 400, an automated clinical chemistry analyzer (Diatron, Budapest, Hungary), suitable for enzymatic analysis of grape must and wine, with UV/VIS spectrophotometric detection according to the procedures of the International Organization of Vine and Wine (OIV) [34]. Total and free SO_2, titratable acidity, and pH were measured using a Metrohm titrator (Herisau, Switzerland). Alcohol content was determined with an alcohol analyzer (Alcolyzer Wine M, Graz, Austria) according to standard methods established by the European Economic Community (1990) [35]. Due to the limited volume of wine available from each fermentation, standard physicochemical analyses could not be performed in triplicate. Therefore, equal volumes from each biological fermentation were pooled to obtain a composite sample, which was analyzed once. The reliability of the laboratory's routine physicochemical analytical methods is ensured through daily internal quality control using certified reference standards (Titrivin BTA) and monthly verification through participation in BIPEA proficiency testing schemes.

2.5. Analysis of Glutathione, Varietal Thiols, Esters, Higher Alcohols, Other Volatile Compounds, and Yeast Assimilable Nitrogen (YAN)

2.5.1. Glutathione and Oxidized Glutathione

Reduced (GSH) and oxidized glutathione (GSSG) were analyzed using UHPLC-MS/MS, as previously described [36]. Ferment and wine samples were diluted in 50% (*v*/*v*) aqueous methanol, filtered through a 0.22 µm PVDF membrane filter, and directly injected for analysis.

2.5.2. Varietal Thiols

Varietal thiols were determined four months after bottling using a modified method as described previously [20,37]. Two internal standards—4-methoxy-2-methyl-2-sulfanylbutane (4MSB) and [$5H_2$]-3-sulfanylhexan-1-ol (d_5-3SH)—were added to 50 mL of wine. Sample preparation followed an extraction procedure using Dowex columns (ThermoFisher, Fair Lawn, NJ, USA), as described previously [38]. The separated organic phases were evaporated under reduced pressure (250 mbar) to approximately 0.5 mL and transferred to dark 1.5 mL vials. The Soxhlet flask was subsequently rinsed with 0.5 mL of dichloromethane and placed in an ultrasonic bath for 1 min. The combined extracts were collected in dark 1.5 mL vials and further concentrated to approximately 30 µL under reduced pressure (100 mbar). Identification and quantification were carried out using gas chromatography–mass spectrometry (GC–MS) with a gas chromatograph (Agilent Technologies 7890A) equipped with an automatic sampler (MPS 2, Gerstel, Mülheim an der Ruhr, Germany) and coupled to a mass spectrometric detector (Agilent Technologies 5975C, upgraded with a Triple Axis detector). The chromatographic oven was programmed as follows: 50 °C for 5 min; from 50 °C to 115 °C at a rate of 3 °C/min; from 115 °C to 150 °C at a rate of

40 °C/min; and held at 150 °C for 3 min. Separation was performed on an HP-INNOWAX column (60 m × 0.25 mm i.d., 0.25 µm film thickness). The temperatures of the injector, ion source, auxiliary, and quadrupole were 240 °C, 230 °C, 250 °C, and 150 °C, respectively. Analyses were conducted in SIM mode. Selected ions (m/z) were 134 and 75 for 4MSB, 139 and 105 for d5-3SH, 132 and 75 for 4MSP, 116 and 101 for 3SHA, and 134 and 100 for 3SH.

2.5.3. Analysis of Esters, C6 Compounds, Aldehydes, and Lactones

Esters and other volatile compounds were determined by liquid–liquid extraction with dichloromethane, followed by gas chromatography–mass spectrometry (GC–MS) analysis. Analyses were performed using a gas chromatograph (HP 6890 GC; Hewlett Packard, Waldbronn, Germany) coupled to a mass spectrometer (HP 5973 MS; Hewlett Packard, Palo Alto, CA, USA). Separation was achieved using a capillary column (CP-WAX 57CB, 50 m × 0.25 mm i.d., 0.20 µm film thickness; Varian, Lake Forest, CA, USA) coupled to a fused-silica-deactivated guard column (2 m × 0.25 mm i.d.; Agilent Technologies, Palo Alto, CA, USA). Chromatographic conditions were applied as previously described [39,40]. Quantification was performed using a calibration solution containing all analyzed compounds dissolved in dichloromethane. Compound identification was based on retention times and mass spectra acquired in selective ion monitoring (SIM) mode. For quantification, the peak area of each analyte in the sample was multiplied by its concentration in the calibration solution and divided by the corresponding peak area obtained from the calibration solution. The calibration solution was analyzed after every six samples. Final results were corrected for the concentration factor and the recovery of the internal standard, 4-nonanol.

2.5.4. Higher Alcohols

Higher alcohols were analyzed using gas chromatography with flame ionization detection (GC–FID) on a gas chromatograph (HP 6890 GC–FID, Hewlett Packard, Böblingen, Germany), following previously described methods [39,40]. Separation was achieved using a capillary column (CP-WAX 57CB; Varian, 50 m × 0.25 mm i.d., 0.20 µm film thickness) and a split/splitless liner (Agilent Technologies; part no. 5183-4647).

For the analysis of higher alcohols, a wine distillate was prepared according to the method described in the International Organisation of Vine and Wine standard OIV-MA-AS312-01 [41]. A measured volume of wine was transferred to a distillation flask, alkalinised with a calcium hydroxide suspension to prevent the carry-over of volatile acids, and distilled using a standard distillation apparatus. To 5 mL of wine distillate, 50 µL of the internal standard 4-methyl-2-pentanol (Sigma-Aldrich; 2.78 g dissolved in 100 mL of absolute ethanol) was added, and the sample was injected directly into the GC–FID under the conditions described above. One-point calibration was performed using a single concentration level containing all analyzed compounds diluted in 12% (v/v) absolute ethanol. The calibration solution was injected after every five samples and was used for both identification and quantification of the analyzed compounds.

2.5.5. Yeast Assimilable Nitrogen Analysis

Yeast assimilable nitrogen (YAN), composed of ammonium nitrogen (NH_4^+) and free amino acid nitrogen (FAN), was analyzed enzymatically and colorimetrically using commercial assay kits (Oenolab Diagnostics, Hendaye, France). Commercial enzymatic kits used were supplied in liquid-stable form and are designed as enzyme-coupled UV spectrophotometric assays adapted for automated chemical analyzers. Each parameter is determined using a specific kit that includes reagents, cofactors, and calibration solutions with a standard of known concentration. Ammonium nitrogen was determined by measuring the absorbance of NADH at 340 nm, whereas FAN was quantified by measuring

absorbance at 340 nm following derivatization with *o*-phthaldialdehyde in the presence of N-acetyl-L-cysteine, as previously described [42].

YAN equivalents in diammonium phosphate (DAP) and organic yeast nutrients were verified by formol titration [43,44].

2.6. Sensory Analysis

Sensory characterization was performed on 4-month-old wines by an expert panel of eight assessors. The assessors were professional oenologists from a winery, all male, aged between 30 and 50 years. The panel demonstrated the ability to discriminate among sensory attributes, was familiar with wine evaluation, and was motivated to participate in the sensory analysis. Three replicates of each sample were presented to the panellists in three-digit-coded OIV standard wine glasses at 22–24 °C under daylight conditions. The intensity of four sensory attributes—tropical aroma, varietal aroma, wine body, and overall quality—was evaluated using an unstructured 10 cm line scale ranging from 0 (low intensity) to 10 (high intensity). Prior to the evaluation, the panel attended a training session aimed at defining and discriminating the selected sensory attributes. Training included the assessment of various commercial white wines exhibiting different intensities of the target attributes. During training, panellists were asked to identify and describe perceived aromas and sensations. For the formal evaluation, 30 mL of wine was served per sample, with a 30 s rest period between tastings. Analysis of variance (ANOVA) was performed using Statgraphics 16 (Statgraphics Technologies Inc., The Plains, VA, USA).

2.7. Statistical Analysis

Statistical analyses were performed using Statgraphics Centurion (Version 18.1.16; Manugistics Inc., Rockville, MD, USA). Data were analyzed by one-way analysis of variance (ANOVA). Differences among means were evaluated using Duncan's multiple range test, with statistical significance established at $p < 0.05$ (95% confidence level).

3. Results and Discussion

3.1. Fermentation and Basic Wine Parameters

Fermentation kinetics were monitored by measuring sugar concentrations (glucose + fructose) throughout alcoholic fermentation. Sugar depletion over time was modelled in R using an exponential decay function $y(t) = Ae^{-kt} + B$ (Figure 1). The coefficient A = 319.18; coefficient B = −77.25 while fitted rate constants (k) and goodness of fit (R^2) for each treatment are: Control: k = 0.0866, R^2 = 0.969, DAP: k = 0.0913, R^2 = 0.977, and Organic N: k = 0.0853, R^2 = 0.977 (Figure 1). Nonlinear regression was used to compare the decay behaviour of the three samples (Supplementary Materials). Pairwise comparisons of decay coefficients were performed within Model 2 using Wald tests. The decay parameter differed significantly between DAP and Organic nitrogen treatments after adjustment ($p = 0.025$). The difference between Control and DAP did not remain significant after correction ($p = 0.075$), and no difference was observed between Control and Organic nitrogen treatments ($p = 0.54$) (Supplementary Materials). Overall, DAP exhibited the fastest decay, whereas Control and Organic treatments showed similar decay behaviors.

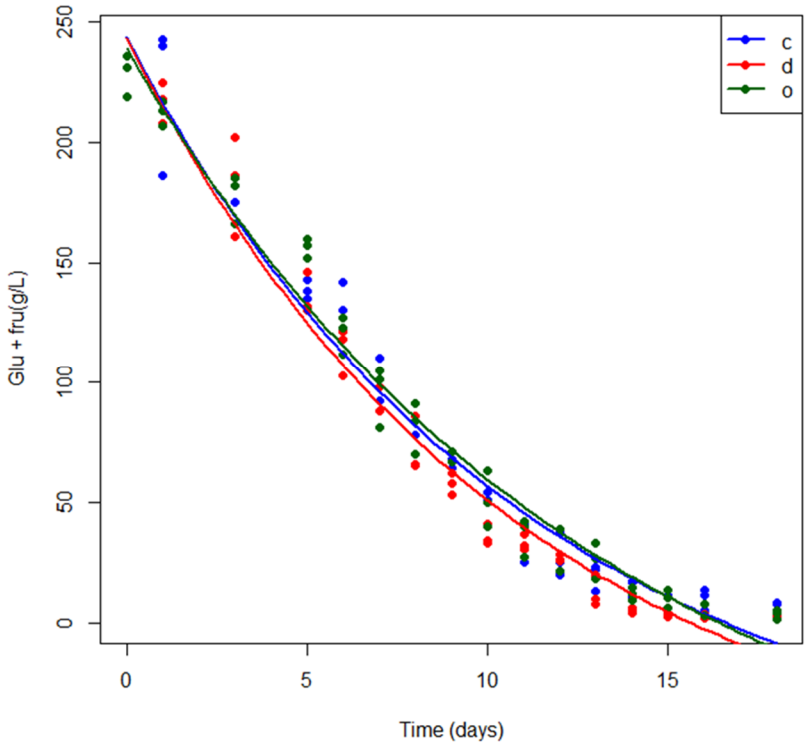

Figure 1. Fermentation kinetics of Sauvignon Blanc must under different nitrogen treatments. Data were fitted using an exponential decay function $y(t) = Ae^{-kt} + B$; c = Control (k = 0.0866, R^2 = 0.96970), d = DAP (k = 0.0913, R^2 = 0.977), and o = Organic N (k = 0.0853, R^2 = 0.9779). The coefficient A = 319.18; B = −77.25.

The intra-day comparison (Table S1) showed that DAP consistently exhibited lower predicted values over time, reflecting a faster sugar degradation rate. The differences between DAP and Organic N were statistically significant, and although the differences between DAP and the Control were systematic, they were not statistically significant. The Control and Organic treatments followed similar trajectories and did not differ significantly at any timepoint (Table S1).

After 18 days of fermentation, the Control and Organic nitrogen treatments showed residual sugar concentrations of 6.0 ± 3.5 g/L and 3.5 ± 2.1 g/L, respectively, indicating a tendency toward sluggish fermentation. In contrast, the addition of diammonium phosphate (DAP) resulted in a lower residual sugar concentration (1.8 ± 0.5 g/L) on day 18 (Table S1). Seguinot et al. [17] reported that nitrogen addition during the stationary phase reduced fermentation duration compared with supplementation at the beginning of fermentation, which may be attributed to improved yeast resistance to ethanol toward the end of fermentation [25]. Similarly, Taillandier et al. [14] demonstrated that glucose and fructose were not completely consumed by three out of four yeast strains at a low nitrogen concentration (120 mg N/L), whereas higher nitrogen levels (190 and 290 mg N/L), supplied mainly as ammonium in a synthetic medium, resulted in complete sugar utilization. Moreover, the average sugar consumption rate depends on the yeast strain, with some strains fermenting more rapidly regardless of nitrogen availability [45]. According to the manufacturer, the yeast strain used in the present study exhibits relatively high nitrogen requirements, underlining the necessity of nutrient supplementation in low-YAN musts when using nitrogen-demanding yeast strains. Fermentation temperature also strongly influences glucose and fructose consumption; in the present study, it was maintained between 14 and 18 °C, as higher temperatures generally accelerate yeast metabolic activity and sugar

uptake, shortening the time required for glucose and fructose depletion (Tronchoni et al., 2009 [46]).

The standard physicochemical parameters of the wines after fermentation, including free and total SO_2, are presented in Table S2. Acetic acid, a low-molecular-weight fatty acid produced in small amounts by yeast during fermentation, differed among treatments. The highest acetic acid concentration was observed in the control wine (0.37 g/L), whereas the DAP-treated wine exhibited the lowest concentration (0.21 g/L). This finding is consistent with the results of Hernández-Orte et al. [45], who reported that ammonia supplementation in a synthetic medium reduced volatile acidity compared with the control across three different yeast strains.

3.2. Yeast Assimilable Nitrogen

The concentration of yeast assimilable nitrogen (YAN) in the grape must before fermentation was 124 mg N/L, comprising 60 mg N/L of ammonium (NH_4^+) and 64 mg N/L of free amino nitrogen (FAN). In all treatments, NH_4^+ was rapidly consumed, reaching concentrations between 0.7 and 1.3 mg N/L by the mid-stage of fermentation (Table 2). After this point, yeasts primarily utilized FAN as the main nitrogen source due to the depletion of NH_4^+, except in the DAP treatment, where NH_4^+ was supplied during fermentation, as shown in Table 1.

Table 2. Concentrations of ammonium (NH_4^+) and free amino nitrogen (FAN) in fermenting musts and Sauvignon Blanc wines at different stages of fermentation under control, DAP, and organic treatments.

Stage of Fermentation	**NH_4^+ (mg N/L)**			**FAN (mg N/L)**		
	Control	DAP	Organic N	Control	DAP	Organic N
Early stage	25.7 ± 7.1 a	60.3 ± 12.7 b	26.0 ± 5.6 a	68.3 ± 13.9 a	66.3 ± 4.9 a	60.3 ± 12.7 a
Mid stage	0.7 ± 0.6 a	0.7 ± 0.6 a	1.3 ± 0.6 a	49.3 ± 36.9 a	27.7 ± 5.7 a	20.7 ± 1.2 a
Late stage	1.0 ± 0.0 a	12.3 ± 0.6 b	1.0 ± 0.0 a	16.7 ± 1.2 a	25.3 ± 2.5 b	20.3 ± 2.5 ab
After fermentation	2.0 ± 0.0 b	8.7 ± 0.6 c	1.0 ± 0.0 a	31.7 ± 0.6 a	35.3 ± 0.6 b	31.3 ± 1.2 c

Values represent mean ± standard deviation (n = 3). Different letters indicate significant differences among treatments within each fermentation stage (Duncan's test, $p \leq 0.05$).

In the late stage of fermentation (after approximately 80% sugar consumption), only 16.7 mg N/L of FAN and 1.0 mg N/L of NH_4^+ remained in the control treatment, corresponding to 14.3% of the initial YAN concentration. This low nitrogen availability could impact fermentation performance during the final stage, resulting in sluggish fermentation and a residual sugar concentration of 6.0 ± 3.5 g/L in the control wine. Sluggish fermentation occurs when yeast struggles to ferment and may ultimately lead to stuck fermentation, as described by Blateyron [47]. In the present study, the initial YAN concentration of 124 mg N/L represents a high-risk level for normal fermentation progression.

The sequential utilization of nitrogen sources observed in this study is consistent with nitrogen catabolite repression, whereby preferred nitrogen sources such as NH_4^+ are consumed first, followed by the activation of metabolic pathways enabling the uptake of less-preferred nitrogen sources, including FAN [47]. Christofi et al. [48] reported that approximately 70% of amino acids were consumed within the first two days of fermentation by three *Saccharomyces cerevisiae* strains. Similarly, in the control treatment of the present study, where no nitrogen supplementation was applied, approximately 60% of the initial YAN was consumed during the first half of fermentation.

At the late fermentation stage (80% sugar consumption), FAN concentrations were 16.7, 25.3, and 20.3 mg N/L in the control, organic, and DAP treatments, respectively, while NH_4^+ concentrations were 1.0, 12.4, and 2.0 mg N/L, respectively (Table 2). Considering the low YAN concentrations in both the control (17.7 mg N/L) and organic (21.3 mg N/L)

treatments, assimilable nitrogen levels may have been insufficient to complete fermentation. In contrast, the DAP treatment retained a higher YAN concentration (37.6 mg N/L) during the late fermentation stage, enabling successful fermentation and a final residual sugar concentration of 1.8 g/L.

An increase in FAN concentration toward the end of fermentation was observed, which may be attributed to the release of amino acids from yeast cells, as previously reported by Taillandier et al. [14].

3.3. Glutathione

Glutathione (GSH) is a potent antioxidant naturally present in grape berries and also synthesized by yeasts during alcoholic fermentation [49]. In the early stage of fermentation, GSH concentrations did not differ significantly among treatments (Table 3). However, at the mid stage of fermentation, the highest GSH concentration was observed in the DAP treatment (6.8 ± 0.6 mg/L), followed by the control (5.9 ± 0.2 mg/L) and organic (5.4 ± 0.2 mg/L) treatments. From the early to the mid stage of fermentation, a slight increase in GSH concentration was detected in all treatments. After mid fermentation, GSH concentrations decreased, reaching final values of 1.8 ± 0.1 mg/L, 1.2 ± 0.1 mg/L, and 1.4 ± 0.1 mg/L in the Control, DAP, and Organic N treatments, respectively.

Table 3. Concentrations of reduced glutathione (GSH) and oxidized glutathione (GSSG) in fermenting musts at different fermentation stages and in young Sauvignon Blanc wines under control, DAP, and organic treatments.

Compound	Treatment	Early Stage	Mid Stage	Late Stage	Young Wine
GSH (mg/L)	Control	5.3 ± 1.1 a	5.9 ± 0.2 a	3.4 ± 0.1 b	1.8 ± 0.1 a
	DAP	5.8 ± 0.5 a	6.8 ± 0.6 b	3.3 ± 0.1 b	1.2 ± 0.1 a
	Organic N	4.7 ± 0.7 a	5.4 ± 0.2 a	2.7 ± 0.3 a	1.4 ± 0.1 a
GSSG (mg/L)	Control	0.9 ± 0.0 a	0.9 ± 0.0 a	0.9 ± 0.0 a	0.7 ± 0.0 a
	DAP	0.9 ± 0.0 a	0.9 ± 0.0 a	1.0 ± 0.1 a	0.7 ± 0.0 a
	Organic N	0.9 ± 0.0 a	0.9 ± 0.0 a	0.9 ± 0.0 a	0.7 ± 0.0 a

Values are expressed as mean ± standard deviation (n = 3). Different letters within the same fermentation stage indicate significant differences among treatments according to Duncan's multiple range test ($p \leq 0.05$). GSH: reduced glutathione; GSSG: oxidized glutathione.

Nutrient addition had no apparent effect on oxidized glutathione (GSSG), whose concentration remained ≤1 mg/L throughout fermentation and in young wines across all treatments (Table 3). It has been reported that Saccharomyces cerevisiae reaches maximum intracellular GSH levels at the end of the exponential growth phase, followed by GSH secretion as cells enter the stationary phase [29]. This observation is consistent with the present results, where peak GSH concentrations were detected during mid fermentation.

Dragojlović et al. [33] investigated the effects of different nitrogen sources (organic and inorganic) on glutathione content during and after alcoholic fermentation and reported a positive influence of nitrogen supplementation on reduced GSH concentrations. In contrast, Kritzinger et al. [32] observed no significant effect of nitrogen addition on wine GSH concentrations, suggesting that yeast strain–specific nitrogen requirements may influence GSH synthesis and retention after fermentation.

Furthermore, Kritzinger et al. [32] evaluated GSH and GSSG levels in grape juice fermentations supplemented with glutathione-enriched inactivated dry yeast (GSH-IDY) at one-third and two-thirds of fermentation. Early supplementation resulted in increased wine GSH concentrations. More recently, Bekker et al. [50] demonstrated that increasing yeast assimilable nitrogen (YAN) levels in defined media minimized, but did not completely prevent, GSH depletion during fermentation. Their results showed that GSH concentrations

decreased during fermentation regardless of the initial YAN or GSH concentration, or the yeast strain employed.

3.4. Influence of Yeast Nutrients on the Aromatic Composition of Wine

3.4.1. Effect on Varietal Thiols

The concentrations of varietal thiols are presented in Table 4. Varietal thiols were already formed at the early stages of fermentation, which is consistent with the previous findings [51], reporting that thiol formation occurs predominantly during the initial phases of alcoholic fermentation. Among the treatments, the addition of organic nutrients resulted in the highest concentrations of 4MSP and 3SH at the early fermentation stage compared with the control.

Table 4. Concentrations of varietal thiols measured at different stages of fermentation and in the resulting Sauvignon Blanc wines under control, DAP, and organic nitrogen treatments.

Compound	Treatment	Early Stage	Mid Stage	Late Stage	Young Wine
4MSP (ng/L)	Control	14.3 ± 0.5 a	14.2 ± 2.5 a	13.6 ± 0.7 b	12.9 ± 0.2 a
	DAP	16.5 ± 2.6 ab	13.1 ± 2.0 a	11.2 ± 1.4 a	14.7 ± 0.2 a
	Organic N	21.4 ± 4.9 b	16.0 ± 1.9 a	11.9 ± 1.2 ab	20.1 ± 1.5 b
3SH (ng/L)	Control	429 ± 16.5 a	306 ± 54.5 a	445 ± 59.6 b	530 ± 34.6 a
	DAP	370 ± 59 a	297 ± 13.1 a	265 ± 43.9 a	491 ± 80.8 a
	Organic N	517 ± 126.3 a	353 ± 9.3 a	587 ± 6.7 c	647 ± 1.4 b
3SHA (ng/L)	Control	32 ± 3.7 a	33 ± 3.0 a	43 ± 2.5 b	47 ± 5.5 a
	DAP	31 ± 0.4 a	33 ± 2.5 a	38 ± 1.5 ab	49 ± 3.7 a
	Organic N	32 ± 2.6 a	31 ± 0.8 a	35 ± 2.9 a	43 ± 7.0 a

Data present the mean of three replicates ± standard deviation. Different letters indicate significant differences among treatments within the same fermentation stage, as determined by Duncan's multiple range test ($p \leq 0.05$). 4MSP: 4-methyl-4-sulfanylpentan-2-one; 3SH: 3-sulfanylhexan-1-ol; 3SHA: 3-sulfanylhexyl acetate.

As fermentation progressed, only the concentration of 3SHA increased, whereas the concentrations of 4MSP and 3SH remained relatively stable during the mid and late stages of fermentation. The formation of 3SHA during fermentation occurs via the esterification of 3SH, catalyzed by alcohol acetyltransferases [52]. The observed increase in acetate ester formation in response to nitrogen supplementation has been associated with enhanced expression of genes encoding alcohol acyltransferase enzymes [53,54].

In the final wines, the concentrations of 4MSP and 3SH were highest in the organic nutrient treatment compared with the diammonium phosphate (DAP) and control treatments. DAP supplementation resulted in the lowest concentrations of 3SH throughout the mid and late stages of fermentation. Excessive addition of inorganic ammonium at the beginning of alcoholic fermentation has previously been shown to limit the release of varietal thiols by yeast. Thibon et al. [26] and Subileau et al. [25] demonstrated that nitrogen catabolite repression (NCR) suppresses thiol release in synthetic media supplemented with S-cysteine–conjugated thiol precursors, and that DAP supplementation leads to a decrease in 3SH production. In contrast, Duc et al. (2020) [55] reported no significant differences in varietal thiol concentrations when comparing peptide-based and inorganic nitrogen additions at the onset of fermentation.

3.4.2. Effects on Esters, Higher Alcohols, and Other Volatile Compounds

Most odorous wine esters are formed during alcoholic fermentation through enzymatic or non-enzymatic esterification of carboxylic acids [56]. These compounds, which impart pleasant fruity and floral aromas, are largely derived from yeast metabolism of sugars and amino acids [57]. The two main classes of esters contributing to wine aroma are ethyl esters and acetate esters.

Effect on Ethyl Esters

Ethyl esters are formed via enzymatically catalyzed reactions between ethanol and activated medium- and long-chain fatty acids [11], whereas acetate esters are synthesized by the condensation of acetyl-CoA with alcohols through the action of alcohol acetyltransferases encoded by ATF1 and ATF2 [53]. Ethyl ester formation is closely associated with fatty acid synthesis, as these compounds result from the addition of ethanol to fatty acids acyltransferases [58].

The concentrations of aromatic compounds analyzed at different fermentation stages under the various treatments are shown in Table 5. Ester concentrations increased as fermentation progressed, in contrast to varietal thiols, which were formed primarily during the early stages of fermentation (Table 5). At the early fermentation stage, ethyl ester concentrations were only a few μg/L, whereas higher concentrations were observed during the mid and late stages, increasing toward the end of fermentation. Mouret et al. [59] reported that ethyl hexanoate and ethyl octanoate were mainly produced during the stationary phase, with concentrations at early fermentation stages (after 20% sugar consumption) representing only a small part of the final levels. Their concentrations increased progressively toward the end of fermentation.

Table 5. Concentrations of volatile compounds at different stages of fermentation and in young wines under control, DAP, and organic nitrogen treatments.

Compound	Treatment	Early Stage	Mid Stage	Late Stage	Young Wine
Ethyl esters (μg/L)					
Ethyl butanoate	Control	2 ± 0.8 a	95 ± 9 a	134 ± 16 a	111 ± 13 a
	DAP	2 ± 0.3 ab	181 ± 36 b	238 ± 33 b	170 ± 6 c
	Organic N	3 ± 0.2 b	110 ± 28 a	141 ± 8 a	137 ± 4 b
Ethyl hexanoate	Control	25 ± 6 a	455 ± 4 a	619 ± 77 a	630 ± 25 a
	DAP	20 ± 2 a	748 ± 102 b	779 ± 77 b	733 ± 25 b
	Organic N	20 ± 2 a	541 ± 117 a	575 ± 43 a	736 ± 17 b
Ethyl octanoate	Control	113 ± 38 a	463 ± 50 a	587 ± 25 a	1745 ± 6 a
	DAP	100 ± 26 a	653 ± 72 ab	762 ± 41 b	2084 ± 8 b
	Organic N	78 ± 2 a	708 ± 203 b	626 ± 37 a	1995 ± 4 b
Ethyl decanoate	Control	41 ± 0.3 a	97 ± 16 a	103 ± 11 a	361 ± 8 a
	DAP	40 ± 1 a	141 ± 25 a	158 ± 9 b	747 ± 64 c
	Organic N	39 ± 2 a	166 ± 75 a	141 ± 14 b	538 ± 2 b
Ethyl dodecanoate	Control	23 ± 16 a	43 ± 10 a	38 ± 14 a	31 ± 0.4 a
	DAP	21 ± 15 a	33 ± 4 a	34 ± 3 a	186 ± 36 c
	Organic N	14 ± 1 a	81 ± 55 a	40 ± 4 a	132 ± 3 b
Ethyl hexadecanoate	Control	17 ± 1 a	35 ± 8 a	10 ± 4 ab	21 ± 0.2 a
	DAP	14 ± 1 a	16 ± 4 a	6 ± 1 a	145 ± 21 b
	Organic N	15 ± 2 a	77 ± 61 a	13 ± 1 b	140 ± 20 b
Total ethyl esters	Control	221	1188	1491	2899
	DAP	197	1772	1977	4065
	Organic N	169	1683	1539	3678
Acetate esters					
Ethyl acetate (mg/L)	Control	n.d.	15 ± 6 a	44 ± 8 a	43 ± 1 b
	DAP	n.d.	31 ± 3 b	57 ± 2 ab	36 ± 1 a
	Organic N	n.d.	21 ± 4 ab	69 ± 4 b	50 ± 0.3 c
Isoamyl acetate (μg/L)	Control	15 ± 3 a	659 ± 34 a	782 ± 37 a	515 ± 38 a
	DAP	14 ± 1 a	1110 ± 241 b	1218 ± 167 b	767 ± 11 c
	Organic N	17 ± 2 a	632 ± 113 a	736 ± 75 a	578 ± 14 b
Ethyl lactate (mg/L)	Control	129 ± 44 b	2832 ± 352	5257 ± 126 a	4148 ± 44 a
	DAP	68 ± 12 a	–	9308 ± 156 b	4148 ± 44 a
	Organic N	105 ± 19 ab	4755 ± 1153	5165 ± 603 a	5513 ± 72 b
Hexyl acetate (μg/L)	Control	46 ± 4 b	196 ± 9 a	137 ± 20 a	119 ± 8 a
	DAP	36 ± 1 a	236 ± 37 b	184 ± 24 b	140 ± 3 b
	Organic N	39 ± 6 ab	190 ± 21 a	133 ± 13 a	142 ± 3 b

Table 5. *Cont.*

Compound	Treatment	Early Stage	Mid Stage	Late Stage	Young Wine
2-Phenylethyl acetate (μg/L)	Control	n.d.	49 ± 2 a	57 ± 2 ab	47 ± 1 ab
	DAP	n.d.	61 ± 10 a	62 ± 4 b	49 ± 2 b
	Organic N	n.d.	52 ± 9 a	53 ± 7 a	46 ± 0.5 a
Diethyl succinate (μg/L)	Control	n.d.	85 ± 18 a	288 ± 73 a	815 ± 18 b
	DAP	n.d.	185 ± 60 a	388 ± 48 a	763 ± 28 a
	Organic N	n.d.	140 ± 59 a	296 ± 83 a	906 ± 9 c
Total acetate esters	Control	79	989	1264	1496
	DAP	50	1592	1852	1719
	Organic N	106	1014	1218	1672
Higher alcohols (mg/L)					
1-Propanol	Control	n.d.	n.d.	9 ± 0.2 a	10 ± 0.3 a
	DAP	n.d.	n.d.	18 ± 1 b	16 ± 0.4 b
	Organic N	n.d.	n.d.	10 ± 0.3 a	9 ± 1.1 a
Isobutanol (2-methylpropanol)	Control	n.d.	12 ± 1 b	19 ± 1 a	19 ± 1 b
	DAP	n.d.	14 ± 2 b	20 ± 1 a	17 ± 0.4 a
	Organic N	n.d.	8 ± 0.3 a	20 ± 0.5 a	18 ± 1 a
2-Methyl + 3-methylbutanol	Control	12 ± 3 a	97 ± 28 a	170 ± 8 a	173 ± 2 c
	DAP	8 ± 8 a	120 ± 10 a	153 ± 13 a	140 ± 1 a
	Organic N	10 ± 3 a	90 ± 11 a	161 ± 2 a	152 ± 2 b
Total higher alcohols	Control	12	109	198	202
	DAP	8	134	191	173
	Organic N	10	98	191	179
Other compounds (μg/L)					
1-Hexanol	Control	1181 ± 156 a	1391 ± 87 a	1551 ± 59 b	1469 ± 79 a
	DAP	1314 ± 90 a	1405 ± 47 a	1581 ± 15 b	1559 ± 28 ab
	Organic N	1325 ± 90 a	1339 ± 153 a	1428 ± 32 a	1598 ± 23 b
cis-3-Hexen-1-ol	Control	128 ± 17 a	150 ± 11 a	160 ± 7 b	153 ± 7 a
	DAP	144 ± 11 ab	155 ± 6 a	163 ± 3 b	159 ± 3 a
	Organic N	151 ± 10 b	149 ± 17 a	149 ± 1 a	156 ± 3 a
Methanol (mg/L)	Control	35 ± 4	23 ± 13 a	38 ± 2 a	41 ± 0.3 b
	DAP	25.5	28 ± 7 a	42 ± 1 a	36 ± 1 a
	Organic N	27.2	25 ± 1 a	41 ± 1 a	38 ± 3 b
γ-Butyrolactone	Control	601 ± 84 a	1441 ± 107 a	2324 ± 286 a	3252 ± 123 a
	DAP	587 ± 56 a	1808 ± 194 a	2722 ± 108 b	3272 ± 92 a
	Organic N	611 ± 35 a	1827 ± 385 a	2836 ± 179 b	4121 ± 75 b
Benzyl alcohol	Control	29 ± 4 a	26 ± 2 a	37 ± 2 ab	78 ± 2 a
	DAP	28 ± 1 a	27 ± 1 a	39 ± 0.2 b	84 ± 3 b
	Organic N	25 ± 1 a	27 ± 2 a	36 ± 1 a	78 ± 1 a
Acetaldehyde	Control	29 ± 1 a	36 ± 11 a	24 ± 2 ab	24 ± 5 a
	DAP	21 ± 4 a	41 ± 2 a	19 ± 1 a	20 ± 4 a
	Organic N	25 ± 5 a	39 ± 6 a	31 ± 6 b	29 ± 8 b

Values represent the mean of three replicates ± standard deviation. Different letters indicate significant differences among treatments at the same fermentation stage according to Duncan's multiple range test ($p \leq 0.05$). n.d., not detected.

In the present study, yeast assimilable nitrogen (YAN) was increased from 124 mg N/L to 208 mg N/L by DAP or organic nutrient supplementation. Although this range represents low to moderate YAN levels, ethyl ester concentrations increased significantly compared to the control in both supplemented treatments. The highest concentrations of ethyl esters during the late fermentation stage and in young wines were observed in the DAP treatment, followed by the organic nutrient treatment. The most pronounced effect was observed for ethyl butanoate, for which the highest concentration following DAP addition was already detected at the mid-fermentation stage.

Seguinot et al. [17] investigated the effects of ammonium and amino acid nitrogen sources using two *Saccharomyces cerevisiae* strains in synthetic media. Nitrogen additions ranging from 100 to 170 mg N/L were applied either at the beginning of fermentation or at the start of the stationary phase (after consumption of 85 g/L of sugar). When nitrogen

was added at the beginning of fermentation, total ethyl hexanoate and ethyl octanoate production increased moderately (approximately +15%). However, nitrogen addition during the stationary phase resulted in distinct behaviours for individual esters: ethyl hexanoate concentrations increased to a similar extent as with early addition, whereas ethyl octanoate production was unaffected [17].

In the present study, DAP and organic nutrient additions resulted in 40.2% and 26.9% higher total ethyl ester concentrations in wines, respectively, compared to the control. Higher ethyl ester concentrations were also observed during the late fermentation stage (after 80% sugar consumption), with increases of 24.6% for DAP and 3.0% for organic nutrient treatments relative to the control. Volatile compounds were analyzed in the liquid phase as described in Section 2.5.3. Mouret et al. [59] demonstrated that substantial proportions of esters can be lost through fermentation off gas, potentially due to temperature effects. They further showed that temperature had no significant effect on total ethyl hexanoate and ethyl octanoate synthesis when total production (liquid plus gas phases) was considered, suggesting that temperature primarily influences ester accumulation in the liquid phase through evaporation.

Torrea et al. [60] examined nitrogen supplementation of a low-nitrogen Chardonnay must using ammonium or mixed amino acid–ammonium sources at low, moderate, and high concentrations (160, 320, and 480 mg N/L, respectively). In general, ethyl esters of medium-chain fatty acids increased with nitrogen supplementation. Ethyl octanoate and ethyl decanoate concentrations increased by up to three- to five-fold. At moderate nitrogen levels, nitrogen source had little effect on ester production except for ethyl butanoate, whereas at high nitrogen levels, amino nitrogen favoured the formation of longer-chain ethyl esters, particularly ethyl octanoate, ethyl decanoate, and ethyl dodecanoate. Similar trends were reported by Vilanova et al. [61] in Albariño musts supplemented with DAP to 350 and 450 mg N/L, where total ethyl ester concentrations were highest at these nitrogen levels.

Godillot et al. [62] showed that the ethyl ester-to-acid ratio was positively influenced by both the timing and level of nitrogen addition, indicating that acid-to-ester conversion is enhanced when nitrogen is supplied at higher concentrations during the second part of the stationary phase. However, the expression of EEB1, a key gene involved in fatty acid to ethyl ester bioconversion, was not affected by nitrogen addition, regardless of timing. These findings suggest that ethyl ester production is regulated primarily through allosteric mechanisms rather than through gene expression. Overall, the effect of nitrogen on ethyl ester synthesis remains complex, with no simple relationship between nitrogen source and ester production. Other factors, including yeast strain [17], yeast phytosterol content, temperature [63], and precursor availability (fatty acids), play critical roles [64]. Wang et al. [6] further demonstrated that pantothenic acid, a vitamin required for coenzyme A synthesis, plays a crucial role in ethyl ester formation, as its deficiency limits acetyl-CoA availability and reduces fatty acid synthesis.

Effect on Acetate Esters

Similarly to ethyl esters, acetate ester concentrations were low at the early fermentation stages and increased as fermentation progressed. Nitrogen supplementation positively influenced the production of all higher alcohol acetates, consistent with previous studies [59,60,65]. The highest isoamyl acetate concentrations during the mid and late fermentation stages and in young wines were observed in the DAP treatment, followed by the organic nutrient treatment, corresponding to increases of 48.9% and 12.2%, respectively, compared to the control. A similar trend was observed for hexyl acetate, with the highest concentrations detected in the organic and DAP treatments.

Ethyl acetate, the major ester produced by yeast, was not detected at the early fermentation stages but increased toward the later stages. Its concentration was lowest in the DAP treatment (36 ± 1 mg/L), followed by the control (43 ± 1 mg/L) and organic nutrient treatment (50 ± 1 mg/L). Ethyl acetate can impart undesirable vinegary aromas at concentrations exceeding 150 mg/L [66]. Garde-Cerdán et al. [65] evaluated amino acid supplementation as a source of assimilable nitrogen (45, 120, and 250 mg N/L) in nitrogen-deficient Mazuelo must. Isoamyl acetate and 2-phenylethyl acetate formation increased proportionally with amino acid nitrogen addition, whereas diethyl succinate formation decreased with increasing amino acid nitrogen concentration. In the present study, the lowest diethyl succinate concentrations were observed in the DAP treatment, while no significant effect of nutrient addition on 2-phenylethyl acetate was detected. Torrea et al. [60] reported a non-linear response of 2-phenylethyl acetate to nitrogen supplementation, with the highest concentrations observed at moderate nitrogen levels.

Acetate esters are synthesized via condensation of acetyl-CoA with higher alcohols catalyzed by alcohol acetyltransferases [54]. Godillot et al. [62] demonstrated that the conversion of higher alcohols to acetate esters was maximized when nitrogen was added at the beginning of the stationary phase, typically occurring after approximately one-third of sugar consumption. Seguinot et al. [17] further showed that the timing of nitrogen addition had a greater impact on aroma compound production than nitrogen composition, although organic nitrogen slightly enhanced acetate ester synthesis compared to ammonium supplementation.

Effect on Higher Alcohols and Other Volatile Compounds

Higher alcohols are produced by yeast as by-products of sugar metabolism and amino acid catabolism during alcoholic fermentation [56]. At concentrations below approximately 300 mg/L, they contribute positively to wine aroma by enhancing bouquet complexity, whereas concentrations above 400 mg/L may negatively affect aroma quality [67]. In the present study, the total concentration of higher alcohols in young wines was lowest in the DAP treatment (173 mg/L), followed by the organic nutrient treatment (179 mg/L), and was highest in the control wine (202 mg/L).

The concentration of isobutanol was also lowest in wines supplemented with DAP, followed by the organic nutrient and control treatments. This observation is consistent with previous studies showing that nitrogen supplementation of must generally results in lower concentrations of higher alcohols [60]. In particular, 2-methylbutanol, 3-methylbutanol, and 2-phenylethanol have been reported to occur at lower levels in must supplemented with ammonium nitrogen compared to those supplemented with amino nitrogen.

In contrast to most higher alcohols, the concentration of 1-propanol was positively affected by both DAP and organic nutrient additions, with the highest levels observed in the DAP treatment. Similar results were reported by Seguinot et al. [17], who observed increased 1-propanol production following organic nitrogen supplementation, while Mouret et al. [59] demonstrated that ammonium salt additions promoted greater 1-propanol formation than amino acid supplementation, particularly when nitrogen was added during the stationary phase. Rollero et al. [63] further reported a strong positive linear correlation between final 1-propanol concentration and initial assimilable nitrogen content. Consequently, overall, 1-propanol production has been proposed as a quantitative marker of the initial assimilable nitrogen concentration in must [59]. This behavior is consistent with the biochemical origin of 1-propanol, which is synthesized from α-ketobutyrate, a metabolic intermediate linked primarily to nitrogen rather than carbon metabolism [68].

3.4.3. Principal Component Analysis (PCA)

To assess the overall impact of yeast nutrient additions on aroma composition and nitrogen-related parameters (GSH, GSSG, YAN, FAN, and NH_4^+), a principal component analysis (PCA) was conducted on the final wines. The PCA biplot (Figure 2) illustrates the relationships between volatile compounds, nitrogen-related variables, and wine samples produced under control, diammonium phosphate (DAP), and organic nitrogen treatments. The first two principal components (PC1 and PC2) accounted for 100.0% of the total variance, with PC1 explaining 65.50% and PC2 explaining 34.50%.

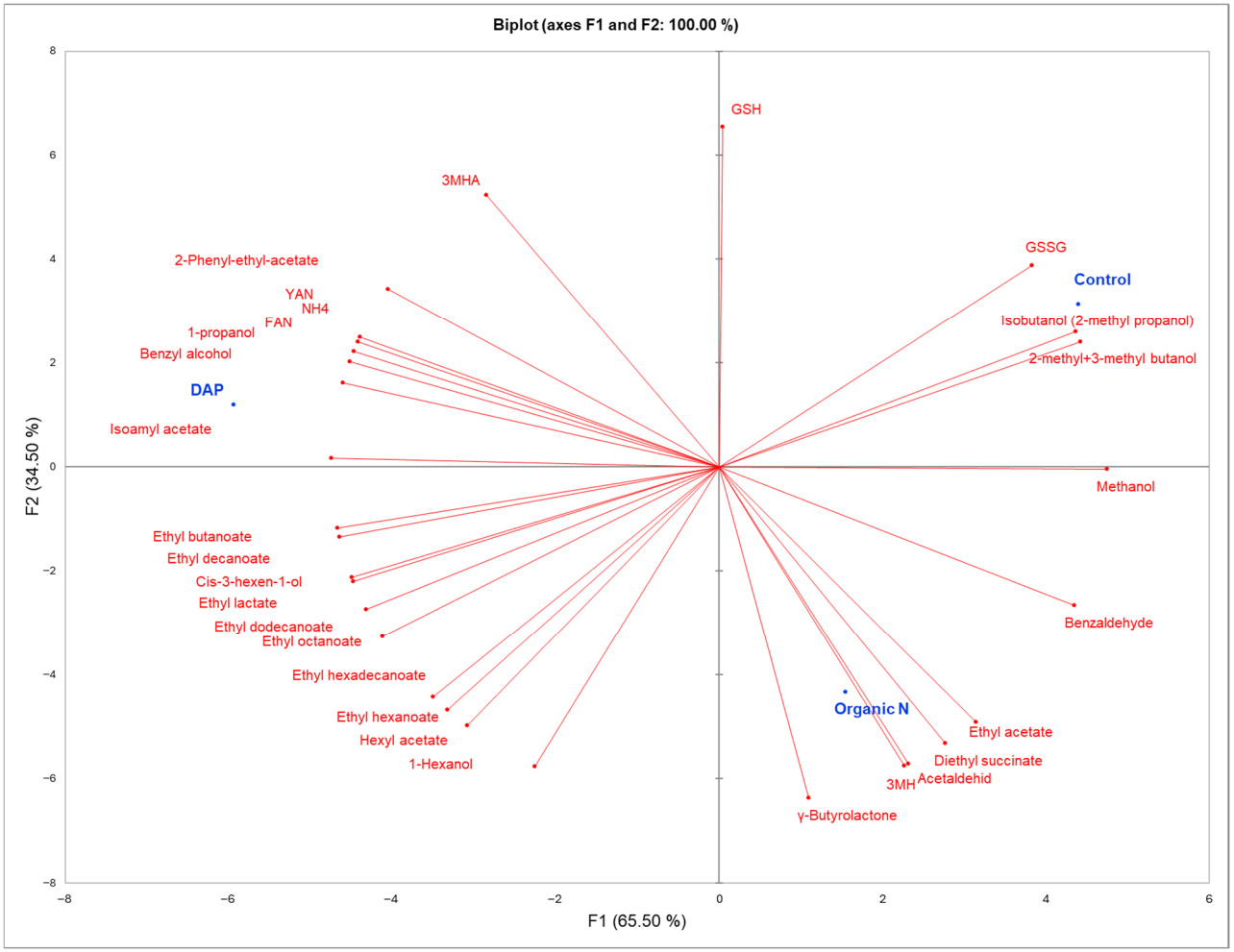

Figure 2. Principal component analysis (PCA) biplot showing the projection of wine samples and measured aroma compounds, glutathione (GSH), oxidized glutathione (GSSG), yeast assimilable nitrogen (YAN), free amino nitrogen (FAN), and ammonium (NH_4^+) on the first two principal components (PC1 and PC2) as affected by different nutrient additions.

Wines supplemented with DAP were positively associated with higher concentrations of acetate esters, particularly isoamyl acetate, ethyl esters, 1-propanol, and 3-mercaptohexyl acetate (3MHA). In contrast, control wines were primarily associated with higher alcohols, including isobutanol and 2- and 3-methylbutanol. These results indicate that nitrogen supplementation, particularly in the form of DAP, significantly influenced the aroma profile of the resulting wines.

3.5. Sensory Evaluation

Sensory characterization of the wines was conducted by evaluating selected parameters using a linear scale. The results (Table 6) revealed that wines supplemented with DAP

achieved the highest scores for tropical aroma, varietal aroma, and overall wine quality. Interestingly, despite the lowest concentration of 3SH being observed in the DAP treatment, tropical fruit aromas were the most pronounced. This observation suggests that esters play a major role in the perception of tropical aromas under these fermentation conditions. Similar findings have been reported by Benkwitz et al. [69], who demonstrated that β-damascenone, thiols, esters, and higher alcohols collectively contribute to Sauvignon blanc aroma, with esters enhancing tropical fruit perception even at low thiol concentrations. There were no significant differences in wine body between treatments, and the organic nutrient addition did not affect varietal aroma or overall wine quality. Previous studies indicate that low nitrogen levels reduce floral and fruity aroma intensity, whereas moderate nitrogen supplementation improves the balance of desirable attributes by increasing ester and medium-chain fatty acid concentrations while reducing higher alcohols [60,70]. Furthermore, Torrea et al. [60] demonstrated that supplementation of must from an initial nitrogen level of 160 mg N/L to 320 mg N/L, using either a mixture of amino acids and ammonium or ammonium alone, did not result in significant differences in any sensory attribute, suggesting that overall nitrogen availability, rather than its specific form, is the key driver of aroma development. At these moderate nitrogen levels, wines exhibited a more desirable sensory profile, with higher concentrations of esters and medium-chain fatty acids and lower concentrations of higher alcohols. Consistently, ammonium supplementation has been reported to enhance the sensory quality of wines by reducing higher alcohol production [70], increasing citrus-like aromas and decreasing sulphury notes [71], and improving overall sensory scores, particularly in comparison with wines produced from grape juices with low initial nitrogen content [72].

Table 6. Average score (n = 8 panellists) of evaluated sensory parameters in Sauvignon Blanc wines from control, DAP and organic treatment in triplicate.

	Control	DAP	Organic N
Tropical fruit	3.8 ± 2.1 B	5.7 ± 1.6 A	1.4 ± 0.9 C
Varietal aroma	2.6 ± 1.1 B	5.6 ± 1.8 A	2.6 ± 2.1 B
Wine body	3.0 ± 2.0 A	4.3 ± 2.8 A	3.3 ± 2.9 A
Overall wine quality	1.9 ± 1.5 B	5.9 ± 3.0 A	2.8 ± 1.9 B

Different letters indicate a significant difference between treatments in each fermentation stage with Fisher LSD test at $p \leq 0.05$.

4. Conclusions

The study demonstrates that in low-YAN Sauvignon Blanc must, sequential supplementation with inorganic (DAP) or organic nutrients differentially affects fermentation performance, aroma composition, and sensory perception. DAP addition (84 mg N/L YAN equivalents) ensured complete fermentation, reduced residual sugar, and maintained low volatile acidity, while strongly stimulating ethyl and acetate ester formation. Elevated ester levels enhanced the perception of varietal and tropical fruit aromas, highlighting the central role of esters in defining wine aroma. Organic nutrient supplementation promoted thiol (3SH and 4MSP) accumulation but was less effective at completing fermentation and enhancing varietal aroma. Both nutrient strategies lowered higher alcohols, such as active amyl alcohol and isoamyl alcohol, compared to the control.

These findings indicate that, in low-YAN, DAP supplementation is an effective strategy to ensure reliable fermentation while enhancing ester formation, thereby positively influencing wine sensory characteristics, including tropical and varietal aromas. Monitoring NH_4^+ and FAN during fermentation provides a practical and cost-effective approach to guide nutrient management and optimize wine sensory quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation12040183/s1, Table S1: Changes in total sugar concentration (glucose + fructose) during fermentation for Control, DAP, and Organic nitrogen treatments; Table S2: Standard physicochemical parameters of young Sauvignon Blanc wines after SO_2 addition in the control, DAP, and organic treatments and report about nonlinear regression analysis of sugar degradation kinetics and statistical comparisons of decay rates and time-specific differences.

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