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# Unconventional methods to delve deeper into the influence of temperature and nutrition on Chardonnay wine profiles

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**Abstract.** Temperature and yeast nutrition profoundly impact wine quality and sensory attributes by modulating yeast aroma production and release during fermentation. While temperature and nitrogen's individual effects are well-studied, their combined influence, including nutrient type and addition timing, remains underexplored. Hence, this study aimed to investigate the simultaneous effects of these factors on fermentation kinetics, aroma production and sensory profile, particularly in a Chardonnay wine production selected as a quite aromatically neutral base. Using a Box-Behnken design of experiment, we selected 14 conditions, including triplicates of the central point. We examined temperatures ranging from 12 to 20°C and varied final Yeast Available Nitrogen/Sugar ratios to achieve nitrogen-limited and excess levels. Two types of nutrients, organic or mineral nitrogen-rich, have been administered at pitching or 30% fermentation advancement. Fermentations occurred in 20L temperature-controlled vessels using Chardonnay must from France, inoculated with 20g/hL of yeast, and malolactic fermentation was prevented. Fermentation kinetics were monitored and classical fermentative aromas were analyzed post-yeast implantation verification. Sensory analyses, employing the Pivot© profile method with wine experts, facilitated the comparison of each model point to the central one, determining the combined effects of the studied parameters on the wine's sensory profile. In summary, our study offers insights into optimizing Chardonnay wine production by examining the interplay of temperature and nutrition on fermentation and sensory outcomes. Through advanced experimental designs and rigorous sensory analysis, we highlighted the critical role of yeast in shaping wine quality, providing practical implications to improve winemaking practices..

## 1. Introduction

Winemaking is a complex process in which *Saccharomyces cerevisiae* plays a crucial role metabolizing sugars into ethanol and carbon dioxide during the alcoholic fermentation (ALF). Beyond its role in alcohol production, *S. cerevisiae* also produce secondary metabolites such as aromatic volatile compounds contributing to the complexity and nuance of wine profiles.

### 1.1. Nitrogen nutrition and temperature can influence yeast behaviour

Monitoring and managing Yeast Assimilable Nitrogen (YAN) during fermentation is essential for achieving desired wine quality outcomes [1]. Low nitrogen levels can lead to sluggish fermentations, while excessive nitrogen may cause microbial instability [1, 2]. Optimal nitrogen levels contribute to a balanced wine aroma profile, with esters, higher alcohols, and organic acids playing significant roles in the fermentation bouquet [1, 3 ,4]. On the other side, low YAN maximizes the production of certain volatile sulphur compounds [5]. Both the form, amount of YAN and timing of addition have significant implications for wine [1, 6]. The most historic and common addition product is diammonium phosphate (DAP) i.e mineral nitrogen [1, 2], however nutrition preparations based on inactivated yeast or yeasts products have been more and more used as organic nitrogen sources [7, 8]. These organic sources are considered as complex, bringing not only nitrogen but also other nutrients such as lipids [1, 7, 8].

Fermentation temperature plays a crucial role in winemaking, significantly influencing yeast metabolism, aroma compound production, and phenolic extraction [4, 9, 10, 11]. Studies have shown that optimal fermentation temperatures vary for different concern. For example higher temperatures generally accelerate fermentation and increase the production of certain compounds like glycerol, acetate esters and higher alcohols [9, 11]. Low fermentation temperatures improve wine aroma profiles, benefiting to ethyl esters and yeast viability but extend fermentation duration [6, 11].

Understanding these factors allows winemakers to modulate yeast behaviour and optimize wine quality by adjusting fermentation temperatures and nitrogen supplementation strategies. However, few studies have explored the combination of these main fermentation parameters, in the aim of identifying optimum cases.

### 1.2. Unconventional methods to explore combinations of parameters

#### 1.2.1. Box-Behnken: an experimental design to optimize the fermentation

Box-Behnken Design (BBD) is a statistical technique used to optimize process parameters and explore combined effects with minimal experiments. BBD allows to maximize the obtained information while minimizing the number of modalities, enabling to envisage comparative methods for sensory analysis. It has been applied in various fields, including food science [12, 13]. The design allows for the evaluation of individual and interactive effects of multiple factors on response variables [14]. It also allows to identify optimal parameter combinations and can go up to develop predictive models with high correlation coefficients [15, 16]. Studies have shown that BBD can effectively determine the most influential factors and their interactions on the desired outcome, such as phytic acid content in bread [13] or grafting yield in antimicrobial film development [17]. Recently, BBD has been used to study combined effect of wine fermentation parameters [11].

#### 1.2.2. Pivot profile©: from professional expertise to quantitative sensory results

The Pivot© Profile (PP) is a recentrapid descriptive method to highlight main sensory differences discriminating the products by selecting a reference, the pivot, to which all products are compared [18]. This pivot can be a reference product or a central blend of all the products to be tested. This method relies entirely on the terms elicited by the tasters during the tasting [19]. PP's performance varies depending on the within-set product similarity, with optimal results observed for medium within-set variation [19, 20]. This method has already been used on wine by showing good results and advantages over conventional descriptive analysis [21, 22].

To sum up, few studies consider the combined influence of both temperature and nitrogen, including nutrient type and addition timing. Hence, this study explored temperatures (12, 16 and 20°C) with three nitrogen nutrition regimes, combining nature (mineral and organic) and moment of addition (beginning and 40% of ALF density wise). This study focused on fermentation kinetics, aromatic compounds production and sensory profile of wines. The trials have been performed on Chardonnay grapes. Chardonnay is considered as the most used variety for the white still wine [12]. This variety is defined as a neutral aromatic grape cultivar, which the expression can be modulated by several factors and producing a wine that is not defined by a specific set of aroma compounds [23, 24]. In the Beaujolais region, 2023 vintage has been considered as quite generous harvest, with some lack of acidity for white varieties [25]. This study was performed with a specific yeast strain selected by Fermentis for its medium YAN requirement and its ability to produce esters. It aims to understand the impact of fermentation parameters on the behaviour of this strain, and eventually identify an optimum protocol.

## 2. Materials and methods

The goal of this trial was to explore the combined effect of three factors on the fermentation and final wine profile: temperature, YANeq/Sugar ratio and nitrogen nutrition.

### 2.1. The design of experiment (DOE)

This design is based on three parameters, as shown on the Figure 1.

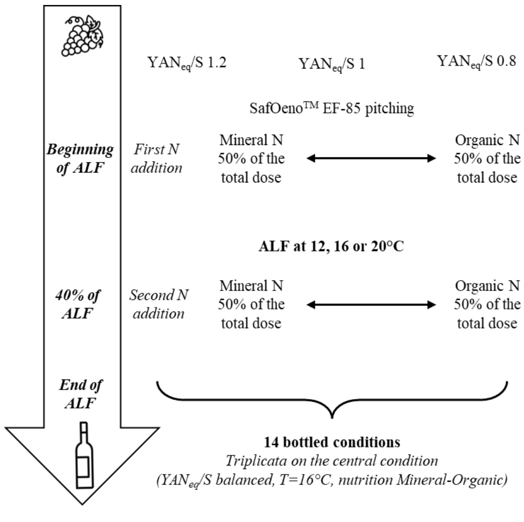


**Figure 1.** Experimental design diagram of the study.

The temperatures range is: 12 – 16 or 20°C for the fermentation. YANeq/Sugar ratio illustrates cases of deficit (0.8) but also of slight excess of nitrogen (1.2). The YANeq/Sugar ratio is modified by addition of nutrients and based on the fermentative power of each product. Nutrition is provided by organic (yeast autolysate) and mineral (DAP) nitrogen sources, following three possible regimes: mineral then organic, organic then mineral, organic then organic. Nutrition regime is defined with two additions, the first at the yeast inoculation, the second around 40% of fermentation progress, with an aeration of the must.

The experimental design follows a Box-Behnken design, resulting in 14 single modalities and one triplicate at the central point, as shown in table 1.

**Table 1.** Description of modalities according to Box-Behnken design. Modalities 7, 8 and 9 corresponds to the central point in triplicate.

| Modality | Temperature | $YAN_{eq}/S$ | Nutrient 1 | Nutrient 2 |
|---|---|---|---|---|
| M1 | 12°C | 0.8 | Organic | Mineral |
| M2 | 12°C | 1 | Mineral | Organic |
| M3 | 12°C | 1.2 | Organic | Organic |
| M4 | 16°C | 0.8 | Organic | Organic |
| M5 | 16°C | 0.8 | Mineral | Organic |
| M6 | 16°C | 1 | Organic | Organic |
| M7 | 16°C | 1 | Mineral | Organic |
| M8 | 16°C | 1 | Mineral | Organic |
| M9 | 16°C | 1 | Mineral | Organic |
| M10 | 16°C | 1 | Organic | Mineral |
| M11 | 16°C | 1.2 | Mineral | Organic |
| M12 | 16°C | 1.2 | Organic | Mineral |
| M13 | 20°C | 0.8 | Organic | Mineral |
| M14 | 20°C | 1 | Organic | Organic |
| M15 | 20°C | 1 | Mineral | Organic |
| M16 | 20°C | 1.2 | Organic | Organic |
| M17 | 20°C | 1.2 | Organic | Mineral |

## 2.2. Winemaking process

### 2.2.1. The must characteristics

The targeted must was a Chardonnay from the Beaujolais region, France, with a slight nitrogen deficiency to fit the DOE. The harvest took place end of August 2023. The must oenological characteristics are described in Table 2.

**Table 2.** Must characteristics.

| | |
|---|---|
| Sugars (g/L) | 208.7 |
| YAN (mg/L) | 188 |
| Turbidity (NTU) | 400 |
| Total acidity (g H2SO4/L) | 3.25 |
| pH | 3.42 |
| Malic acid (g/L) | 2.3 |
| Tartaric acid (g/L) | 4.5 |
| Volatile acidity (g H2SO4/L) | 0 |
| Free SO2 (mg/L) | <10 |
| Total SO2 (mg/L) | 0 |

### 2.2.2. Fermentation

This study was performed with the strain SafOeno™ EF-85 selected by Fermentis to produce white wines with complex and balanced fruity aromas, respecting varietal aromas [26].

Yeast were rehydrated before inoculation following supplier advices (water around 37°C, 20 minutes then cooled down to reach must temperature) [26].

Fermentations occurred in 25L vessels placed in three different temperature-controlled chambers programmed to reach the fermentation temperature asked (12°C, 16°C and 20°C). Vessels were inoculated with 20g/hL of yeast, and malolactic fermentation was prevented.

### 2.2.3. Stabilization and bottling

After the fermentation, vessels were racked into a 20L vessels and 4g/hl of SO2 were added. For tartaric stabilisation, the vessels were placed in a temperature-controlled chambers at 3°C during 15 daysand racked before filtration and bottling. Filtration was made with a cartridge filter with 3, 1,254 and 0,65 µm filters and bottling in 0,75cl glass bottle nitrogen-inerted and corked with Diam 5 corks.

## 2.3. Analysis

### 2.3.1. Classical oenological analysis

Fermentation kinetics were followed by daily density measurements. Classical oenological analyses were carried out at the end of the fermentation and on bottled wines: glucose/fructose, total and volatile acidity, malic and lactic acids, available nitrogen and SO2 (free and total) were analysed with Y15 multiparametric analyser (BioSystems, Barcelona, Spain), pH was measured with a pH meter and alcoholic degree was analysed with an infrascan.

Strain implantation checks were carried out on several modalities to ensure that the pitched yeast is properly implanted.

#### 2.3.2. Volatile compounds analysis

Volatiles compounds was analysed at the end of the fermentation, before bottling, to get as close as possible to the yeast expression. Ethyl esters and ethyl acetate were analysed as well as higher alcohols.

Volatiles compounds were analysed by Nyséos as follows: first, a liquid-liquid extraction with a solvent and internal isotope dilution calibration was carried out, the extract obtained was then injected into a gas chromatograph coupled to a mass spectrometer detector.

#### 2.3.3. Sensory analysis

Pivot profile © (PP) was conducted with a panel of 14 French wine professionals (10 men, 4 women, mean age: 50,1) in a single session to highlight the main differences between the modalities from a sensory point of view [18, 21]. The PP is a descriptive method for which each sample of wine is directly compared to a reference, called the 'pivot'. In this study, the pivot was composed of a blend of the three central point modalities (7, 8 and 9 in Table 2). All wines were served at 50 mL at 10°C in clear glasses with a 3-digit code in a randomised order. The pivot was served in the same way with the possibility of refilling at any time during the tasting session. After a familiarisation step with the pivot wine, each participant had to freely describe in their own words all the sensory characteristics that were perceived as less and/or more intense in each wine when compared to the pivot wine. They had to write only positive words, without hedonic judgements or sentences. Data were collected on digital tablets using the TopDegust software (IFV, France).

#### 2.3.4. Data analysis

Sensory data were analysed using R [27].

PP data was first lemmatized and categorized to group the elicited words by synonymy. This was done independently by two researchers to limit personal bias. After that, a contingency table was realized by following Thuillier et al, 2015 [18]. and then analysed by Correspondence Analysis (CA) performed by using the free software R version 4.3.2 with the additional R packages *FactoMineR* [28], *factoextra [29]*, and *corrplot* [30].

## 3. Results

### 3.1. Kinetics

All fermentations were completed, however, the trial parameters impacted on fermentation time as shown in Figure 2.

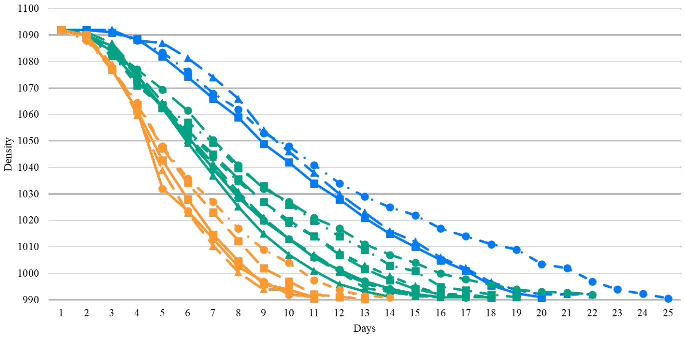


**Figure 2.** Kinetics curves for all the modalities.

In orange the higher temperature, in green the middle one and in blue the cold one. Square shape represents full organic nutrients regime, round one the organic-mineral regime, triangle one the mineral-organic regime. Dotted lines are for YANeq/S=0.8, solid lines for YANeq/S=1.2.

The three identical modalities (7, 8 and 9) have the same kinetic curve, showing the repeatability of this trial, at least regarding fermentation kinetics.

Kinetic curves showed that the most impacting parameter on kinetics was the temperature. The higher the temperature, the faster the fermentation profile (11 days), while the lower the temperature, the slower the fermentation process (over 20 days).

The YANeq/S had much less impact on kinetics but higher ratio speeded up the kinetics, especially for the medium and lower temperature. This means that nutrition can help compensate for low temperature kinetics, no matter the type of nitrogen source.

### 3.2. Aromatic compounds

For aromatic compounds, Figure 3.a and Figure 3.b show that there is a variability of acetate and ethyl esters production according to the level of temperature, YAN, and nutrients regime.

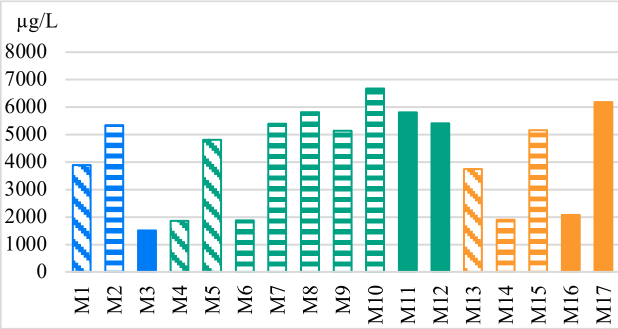


**Figure 3.a.** Sum of acetate esters for all the studied modalities, described in Table 1.

In orange the higher temperature, in green the middle one and in blue the cold one. Diagonal hatched bars are for YANeq/S=0.8, horizontal hatched bare for YANeq/S=1 and solid bars for YANeq/S=1.2.

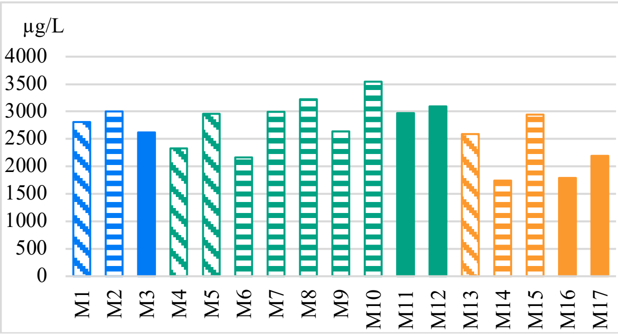


**Figure 3.b.** Sum of ethyl esters for all the studied modalities, described in Table 1.

In orange the higher temperature, in green the middle one and in blue the cold one. Diagonal hatched bars are for YANeq/S=0.8, horizontal hatched bare for YANeq/S=1 and solid bars for YANeq/S=1.2.

Whatever the modality, aroma production seemed to be maximized by medium temperature (16°C). However higher YANeq/S could enhance aroma production for higher temperatures, especially regarding production of acetate esters. The full organic nutrition leads to less acetate and ethyl esters production, especially lower production of acetate esters. This can be explained by a lower amount of available nitrogen (real YAN) but same YAN equivalent, in organic nutrition compared to that of DAP. However, increasing the YANeq/S ratio by using organic nutrition could help to increase the production of ethyl esters at lower temperature.

According to these results, the maximum of production for both acetate esters and ethyl esters would be around YANeq/S=1 with a combined nitrogen nutrition (mineral and organic, whatever the order). However, the acetate esters are promoted by higher temperatures (16 to 20°C), while ethyl esters seem to be less affected by temperature but are still promoted by lower temperatures (12 to 16°C).

### 3.3. Sensory analysis: Pivot profil©

After lemmatization and categorization, a final list of 16 descriptors remained. Figure 4 shows the projection of the modalities and the descriptors on the first two dimensions of the CA, which explain 58,5% of the variance. The first dimension opposes the fruity characteristics with the spicy, reduced, and lactic ones. The second dimension opposes the bold character with the spicy one. In comparison with the pivot, Figure 4 shows an effect of the temperature level: M1, M2 and M3 are described as bolder and fruitier while M14, M16, and M17 are described as less fruity and less bold but more lactic, reduced and spicy. This is consistent with the low acetate and ethyl esters production (Figure 3). For the full organic nutrition, Figure 4 also shows a combined effect with temperature, as M3 is described as fruitier while M4, M14, and M16 are described as less fruity and more lactic, reduced and spicy than the pivot wine. So, it seems that the higher the temperature, the stronger the lactic, reduced, and spicy aromas. In addition, the YANeq/S seems to enhance the fruity aromas when it is higher according to the temperature level.

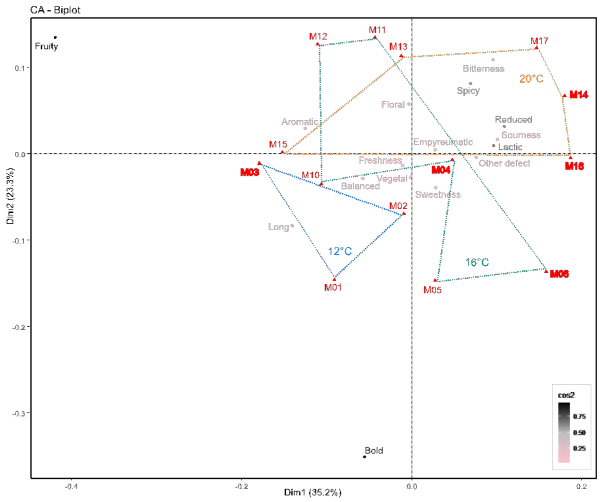


**Figure 4.** Projection of the modalities on the first two dimensions of the Correspondence Analysis.

The quality of representation of the descriptors are coloured in black for a $cos^2$ close to 1, in grey for a $cos^2$ around 0,5, and in light pink for a $cos^2$ close to 0. The temperature is represented by dotted lines in blue for 12°C, in green for 16°C, and in orange for 20°C modalities. Full organic nutrients regime is represented in **bold.**

## 4. Discussion and conclusion

Through advanced experimental designs and rigorous sensory analysis, we highlighted the critical role of yeast and fermentation conditions in shaping wine characteristics in Chardonnay.

One goal of a Box-Behnken design is usually to define optimal point. Here, optimal modality could be defined by a quite fast kinetics, with no stuck or sluggish fermentation, a maximized production of aromas (acetate esters and ethyl esters), and with no sensory defect detected, leading to a fruity, well-balanced Chardonnay wine.

Regarding kinetics, despite some slower fermentations, no major issues resulted from the DOE. The trial demonstrated the stronger impact of temperature and $YAN_{eq}/S$ on the time of fermentation. The relevance of the fermentation capacity used for the calculation of YANeq/S was also demonstrated. Finally, for kinetics, even if the nitrogen source has little effect, nutrients help to compensate for difficult fermentation conditions as cold temperatures.

Regarding volatile compounds, both acetate esters and ethyl esters had optimum (higher concentration) of production with medium temperature and medium $YAN_{eq}/S$ (=1). The strong impact of nutrition regime came mainly from the difference of provided nitrogen by mineral and organic nutrients when the dose is calculated with $YAN_{eq}$.

Despite a positive impact on the fermentation kinetic, high temperature (20 °C) of fermentation reduced the aroma production and leads to reductive, lactic and spicy Chardonnay wines. Even if these characteristics can be smoothed by increasing the $YAN_{eq}/S$ and by mixing a mineral and organic nutrition, the medium to lower temperatures resulted to more fruity or bolder wines. The order of addition between organic and mineral seems to have a minor impact, as long as both are used together, in comparison to full organic nutrition.

For this study, it was decided to use $YAN_{eq}$ to calculate nutrient additions, which may result in greater differences in real nitrogen addition between modalities than expected. If $YAN_{eq}$ was evaluated and is a relevant tool for kinetics, it showed limits when it comes to the need of nitrogen for the production aromatic volatiles compounds. In this trial, this choice may have biased the results of the full organic nutrition modality.

In this experiment, we have chosen to use a particular design of experiment, which allowed to analyze the combination of parameters. However, it can potentially lead to a lack of data for some extreme points [31, 32, 33], such as the modality 3 in the DOE for example. Mixing full organic nutrition with cold temperature, had led to slower fermentation and decreased production of acetate esters. Without a modality to compare with a common parameter at the same temperature, it seems difficult to conclude on the individual impact of these parameters at cold temperature. This first approach could be continued by a deeper understanding of the optimum shown in this work, exploring a smaller range of parameters. In this study, volatiles compounds have been analysed considering the two major families: acetate esters and ethyl esters. Some differences have been shown but a more in depth study, focusing on some molecules of interest (isoamyl acetate, 2-phenylethyl alcohol and its acetate etc..) could provide more detailed information on the optimisation of aroma production and the modulation of the yeast behaviour. Finally Pivot profile© showed good sensory results and allowed the main sensory characteristics of these samples to be explored, as a first step with a panel of wine professionals. This methodology made it possible to highlight the most striking sensory differences between close modalities with subtle levels of variation. This free vocabulary generating task with no predefined list of descriptors opens also some possibilities. For example, from an analytical point of view, the sensory descriptor "reduced" can be linked to sulphur compounds that were not measured here but could be in a next step. This methodology could also be used as a first step before further sensory analysis, using these initial results as a training base for tasters to explore more subtle differences between a few modalities and the optimum identified.

In conclusion, the combination of mineral and organic nitrogen nutrition, at a rather medium to cold fermentation temperature (12-16°C), seems to be the optimum for SafOeno™ EF-85 yeast for Chardonnay wines. The YAN seems to be the less impacting factor, but this yeast has a low requirement [26]. These parameters allow to ensure fermentation time around 20 days, to maximize the production of aromatic volatile compounds such as ethyl and acetate esters and to produce fruity and bold Chardonnay wines.

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