CENTENARY OF THE OIV
45th WORLD CONGRESS OF VINE AND WINE
14-18 OCTOBER 2024 FRANCE-DIJON
OIV
Available on IVES Conference Series vine & wine
DOI : https://doi.org/10.58233/l46ZEPAC

# Is complex nutrition more advantageous than mineral nitrogen for the fermentative capacities of *S. cerevisiae*?

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**Abstract.** During alcoholic fermentation (AF), nitrogen is one of essential nutrient for yeast. The main issue of a lack in yeast assimilable nitrogen (YAN) is sluggish or stuck fermentations leading to economic losses. However, correcting this deficiency is sometimes not enough to restore proper fermentation performance. This suggests the existence of other nutritional shortages. The aim of this work was to study the impact of the timing and the nature of nutrient addition (mineral nitrogen, 100 % yeast derivative or mixed) on AF. First, 16 commercial strains were inoculated in Sauvignon blanc grape must deficient in YAN and with high reducing sugars concentration. Fermentation kinetics of strains were then classified in 3 groups: stuck, sluggish or complete AF. New experiments were carried on in the same grape must supplemented in YAN with ammonium or yeast derivatives products. Nutrient additions were made at the beginning of AF or in two additions. Our results show that supplementation with mixed nutrients is more beneficial for fermentation performance than mineral nitrogen alone. Fractionated addition also seems to have a greater impact than single addition. This study highlights the need to think about yeast nutrition from a holistic perspective (nitrogen and lipid addition, timing of addition).

## 1. Introduction

During alcoholic fermentation (AF), assimilable nitrogen is one of the essential nutrients for yeast. It plays a key role in sugar transport, protein synthesis and the biosynthesis/biotransformation of aromatic compounds of interest in winemaking, such as thiols and esters. A lack of assimilable nitrogen (<140 mg/L) generally leads to sluggish or even stuck fermentations, resulting in a reduction in wine quality. However, correcting this nitrogen deficiency is sometimes not enough to restore good fermentation performance due to the existence of other nutritional deficiencies.

To compensate for nitrogen deficiencies only, nitrogen can be added to the must in mineral (ammonium) or organic (amino acids and small peptides) form. While there are simple nutrients based on mineral nitrogen and/or thiamine, organic nitrogen can only be supplemented via yeast derivatives. And while the effects of mineral nitrogen on the behaviour of *S. cerevisiae* during AF have been studied in detail [1, 2], few studies have been carried out on the contribution of yeast derivatives such as yeast hulls, inactivated yeast and yeast autolysates, these complex products also containing other nutritional sources (lipids, minerals, vitamins, etc.).

The aim of this work was therefore to study the impact of the timing and nature of the addition of different nutrients: based on mineral nitrogen, yeast derivatives (organic nitrogen + other nutrients) or mixed (mineral nitrogen + yeast derivatives) on the AF produced by *S. cerevisiae* strains previously characterised according to their resistance to difficult fermentation conditions.

## 2. Methodology

### 2.1. Classification of *S. cerevisiae* strains

The fermentation behaviour of 16 commercial strains of *S. cerevisiae* was compared under difficult fermentation conditions, in this case on a Sauvignon blanc must (whose initial composition is shown in Table 1), deficient in assimilable nitrogen at 110 mg/L, with a high concentration of reducing sugars adjusted to 240 g/L, and with a low turbidity level, adjusted to 90 NTU by centrifugation (indicating a probable lipid deficiency, [3, 4]). The different strains of *S. cerevisiae* were inoculated at 20 g/hL and fermentations took place at 20°C with moderate orbital agitation, in 12 mL fermenters in triplicate. The fermenters were weighed regularly to monitor the loss of mass due to the $CO_2$ released and thus the progress of fermentation. At the end of the fermentations, two parameters were calculated:

– the maximum amount of $CO_2$ released, to determine whether all the sugars had been consumed,
– the duration of AF, to assess the fermentation kinetics of the strains.

**Table 1.** Initial composition of Sauvignon blanc and Gamay musts.

| | Sugars (g/L) | TAP (% vol.) | Total acidity (g/L $H_2SO_4$) | pH | Malic acid (g/L) | YAN (mg/L) | Organic N (mg/L) | Mineral N (mg/L) | Free $SO_2$ (mg/L) | Total $SO_2$ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sauvignon blanc | 181.8 | 11.5 | 3.75 | 3.37 | 4.5 | 110 | 83 | 28 | 9 | 24 |
| Gamay | 195.2 | 11.6 | 3.32 | 3.29 | 4.8 | 115 | 98 | 17 | 10 | 27 |

The strains were then classified into three categories:

– complete and rapid AF: depletion of sugars in less than 15 days,
– complete but slow AF (sluggish): depletion of sugars in more than 21 days,
– incomplete AF (stuck): presence of residual sugars when weight loss stopped.

### 2.2. Alcoholic fermentations in supplemented musts

AF were carried out in the same Sauvignon blanc must as above and in a red Gamay must (Table 1).

For both musts, reducing sugars were adjusted to 240 g/L and turbidity to 90 and/or 210 NTU. Different nutritional supplementation conditions were tested. The musts, initially very close in assimilable nitrogen (~110 mg/L) were adjusted identically to ~200 mg/L assimilable nitrogen via three types of nutrient: Mineral (DiAmmonium Phosphate- DAP + Thiamine), Organic (Yeast Autolysate - YA) or Mixed (SpringFerm™ Equilibre, composed of yeast derivatives and DAP). Supplementation was either fractional (50% at the start of AF then 50% at mid-AF) or non-fractional (100% at the start of AF). Figure 1 shows a summary of all modalities.

The AF were carried out under the same conditions as before, except for those carried out on the Gamay must, where the AF temperature was set at 24°C. Calculation of the maximum amount of $CO_2$ released and the duration of AF for each of the strains under the different supplementation conditions made it possible to compare the efficiency of the different nutrition programs.

## 3. Results

### 3.1. Alcoholic fermentations under difficult conditions: classification of *S. cerevisiae* strains

The AF kinetics of the 16 *S. cerevisiae* strains are shown in Figure 2.

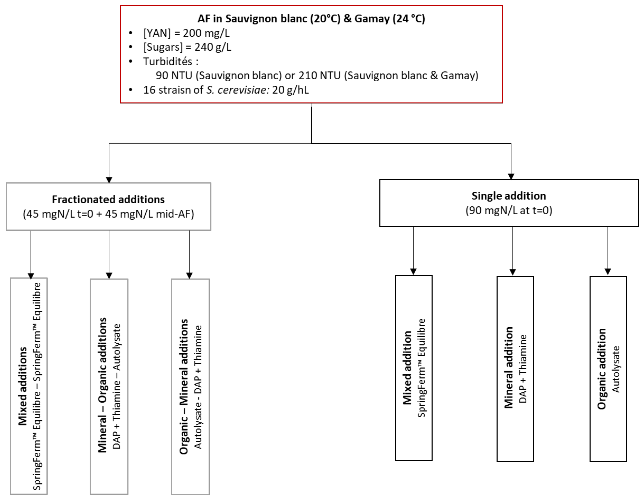


**Figure 1.** Experimental design.

For an initial sugar concentration of 240 g/L, the maximum $CO_2$ released should be 105 g/L (no residual sugars). The strains were classified into three groups:

– strains capable of complete and rapid AF (< 15 days): A, B, C, D, E, F, G and H,
– strains capable of producing complete but slow AF: I, J, K, L and M,
– strains causing incomplete AF: N, O and P (achieving 95%, 86% and 82% of AF respectively).

### 3.2. Alcoholic fermentations with additions: fermentative power of the three types of nutrient

#### 3.2.1. Strains experiencing stuck fermentations

The use of strains N, O and P resulted in incomplete fermentations (AF achieved at 97%, 86% and 82% respectively). Figure 3 shows the maximum quantities of $CO_2$ released for strains O and P as a function of the different nutrition protocols on Sauvignon blanc. For these two strains, we observed that some of the nutrition methods enabled AF to be completed, compared with the deficient method.

For strain O, 3 modalities led to complete AF:

- "Fractionated supplementation with SpringFerm™ Equilibre - SpringFerm™ Equilibre with an initial turbidity of 90 or 210 NTU",
- "Fractionated supplementation with Autolysate - DAP + Thiamine at 210 NTU".

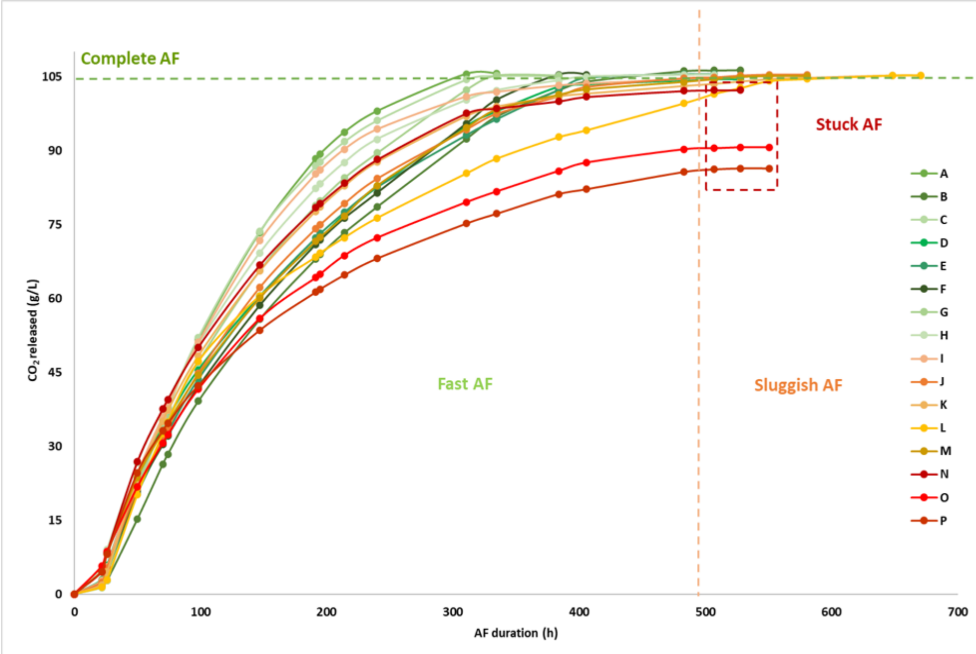


**Figure 2.** Fermentation kinectics of 16 strains of *S. cerevisiae* in Sauvignon blanc must without supplementation.

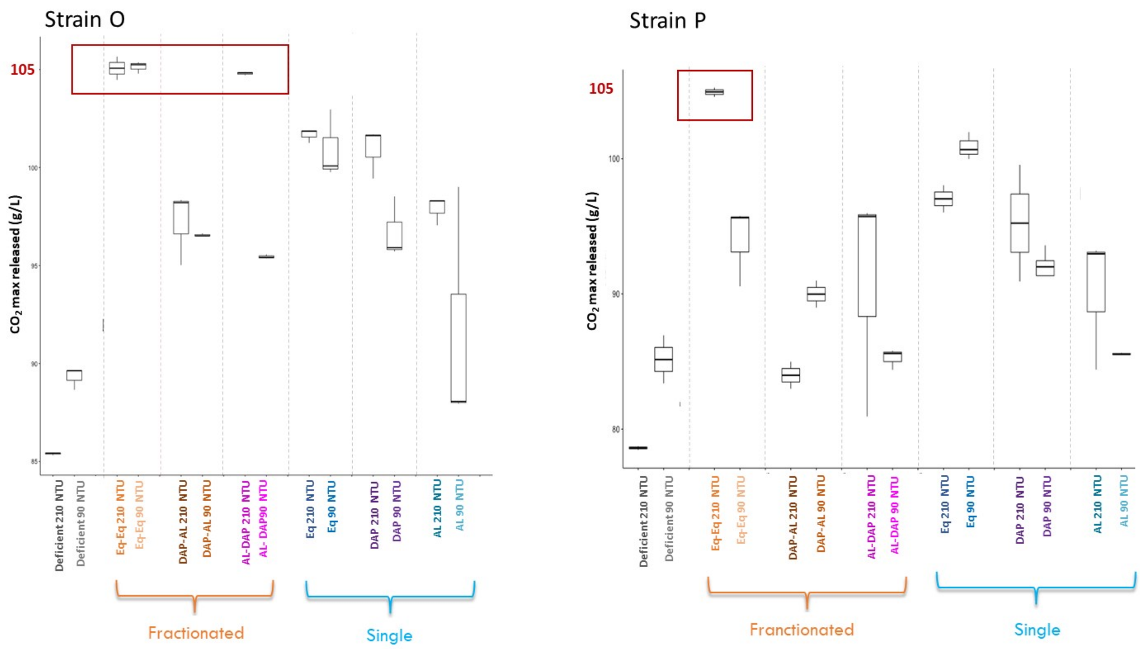


**Figure 3.** Maximum quantity of CO2 released by the strains O and P as a function of different nutrition modalities in Sauvignon blanc.

For strain P, only one condition completed AF:

- "Fractionated supplementation with SpringFerm™ Equilibre - SpringFerm™ Equilibre at 210 NTU".

We then looked at the fermentation duration associated with these four conditions to exclude those leading to sluggish AF (>21 days) (Figure 4).

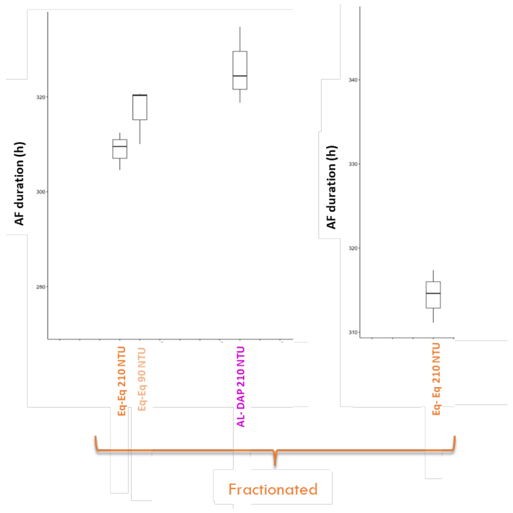


**Figure 4.** AF duration of strains O and P strains as a function to different nutrition in Sauvignon blanc (supplementation conditions resulting in complete AF).

Depending on the strain used and the nature of the supplementation, AF durations between 13 and 14 days were obtained. These conditions therefore enabled AF to be completed quickly without leading to sluggish fermentation.

The modalities that allowed the 105 g/L of CO2 released to be reached systematically corresponded to mixed additions (mineral nitrogen + yeast derivatives) split into 50% early AF and 50% mid-AF. The same observations were made on Gamay must for strain N (data not shown). For each strain, the fastest fermentations were obtained for the "Fractionated additions with SpringFerm™ Equilibre - SpringFerm™ Equilibre at 210 NTU" condition.

### 3.2.2. Strains leading to sluggish fermentations

Five strains lead to sluggish AF in Sauvignon blanc (I, J, K, L and M). The different supplementations all enabled AF to be completed (not shown). Figure 5 shows the fermentation durations obtained for strains L and M in Sauvignon blanc.

In Sauvignon blanc, 7 of the 18 nutrition modalities tested significantly reduced the duration of AF for strain L and 8 for strain M. Unlike the strains that initially showed stuck fermentations, and for which only fractional additions allowed AF to be completed, here non-fractionated additions also resulted in a significant reduction in the duration of AF between 190 and 310 h (i.e. from more than 21 days to between 8 and 13 days). The "Fractionated additions with SpringFerm™ Equilibre - SpringFerm™ Equilibre at 210 NTU" modality was the only condition common to both strains that allowed AF to be shortened below 200 h.

Figure 6 shows the fermentation durations obtained for strains I, J and K on Gamay must.

On this matrix, all the supplementation modalities significantly reduced the fermentation duration from more than 15 days to between 7 and 14 days, except for the "Autolysat - DAP + Thiamine fractionated supplementation" for strain J. For these 3 strains, the fastest fermentations were also obtained for the "Fractionated additions with SpringFerm™ Equilibre - SpringFerm™ Equilibre" modality (7 to 9 days).

### 3.2.3. Strains resulting in complete and rapid fermentation

Strains A, B, C, D, E, F, G and H produced complete and rapid AF on deficient must (AF duration of less than 15 days). Nonetheless, we were able to observe that certain nutrition conditions reduced the fermentation duration of these strains. Figure 7 shows the results obtained for strain A in Sauvignon blanc.

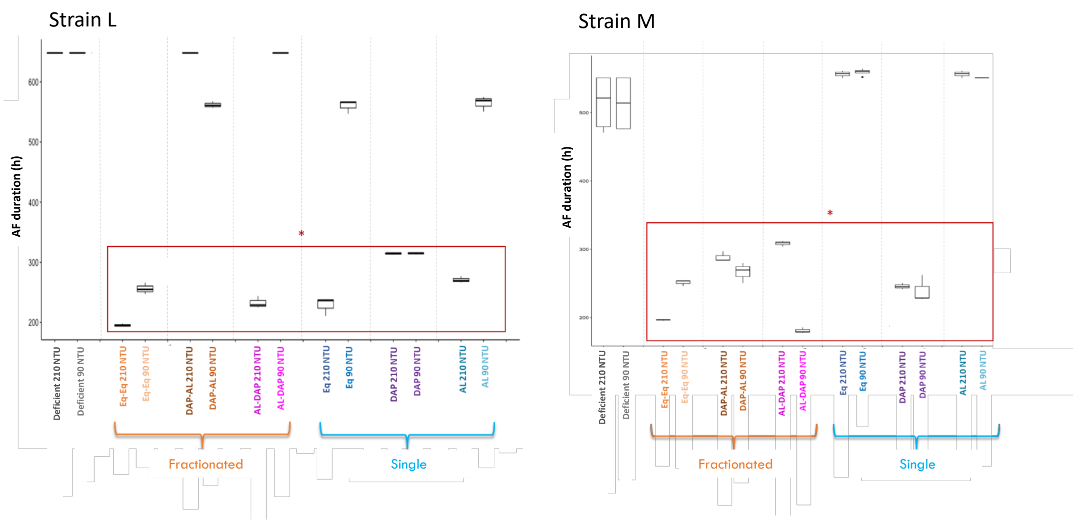


**Figure 5.** AF duration of L and M strains as a function of different supplementations in Sauvignon blanc.

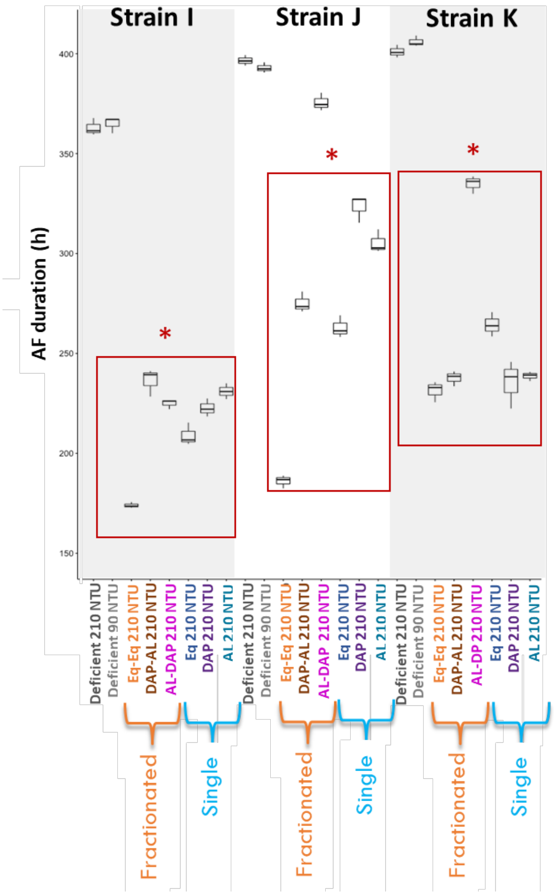


**Figure 6.** AF duration of strains I, J and K as a function of different supplementations on Gamay.

On deficient must, AF finished quickly in 255 h on average (10.5 days) whatever the initial turbidity of the medium. Nevertheless, nutrition generally reduced fermentation time by a few hours to 108 h for the "Fractionated additions with SpringFerm™ Equilibre - SpringFerm™ Equilibre at 210 NTU" modality, which completed AF in 6 days. This condition also resulted in the fastest fermentation, from 10 to 6 days on Gamay must.

## 4. Conclusion

In this study, the fermentative performances of 16 strains of *S. cerevisiae* were evaluated in an initial nitrogen-deficient must, at low turbidity and in response to various nutrition strategies. We found that fractionated additions of a mixed nutrient, SpringFerm™ Equilibre, consistently resulted in complete and rapid fermentations for all strains.

Furthermore, although an increase in turbidity to 210 NTU seemed to favour fermentations, it is interesting to note that for some strains the fractioned addition of SpringFerm™ Equilibre presented the same efficiency at 90 NTU. This allowed us to highlight that this mixed product helped to meet the different requirements of the yeast when it was faced with a multi- deficient must, unlike purely mineral or organic products, and this in both the growth phase and the stationary phase.

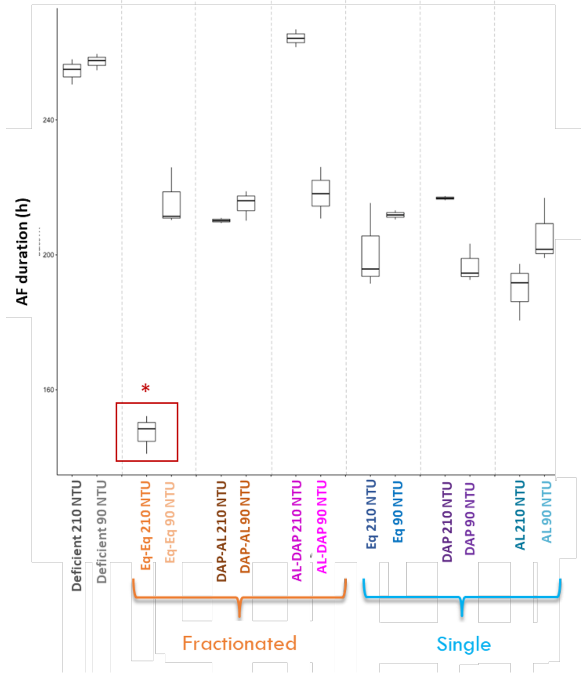


**Figure 7.** AF duration of strain A as a function of different nutrition conditions in Sauvignon blanc.

The composition of SpringFerm™ Equilibre may explain its high fermentation power. This mixed product provides both mineral and organic nitrogen essential for yeast growth, the main forms of lipids (fatty acids, sterols and phospholipids) necessary for their viability and a range of vitamins and minerals, present in very low concentrations but playing a vital role in yeast metabolism.

From a practical and only fermentative point of view, when the winemaker has to ferment a must with a low initial assimilable nitrogen content as well as low turbidity (< 150 NTU), it is preferable to use mixed products based on yeast derivatives, mineral nitrogen and vitamins such as SpringFerm™ Equilibre in order to provide complete nutrition to the yeast. In addition, its fractionated supply at the start and mid-AF generally optimises the fermentation performance of the yeast used.

## 5. References

1. L. Blateyron, J.M. Sablayrolles, J. Biosci. Bioeng. 91. (2001)
2. S.J. Bell, P.A. Henschke, Aust. J. Grape Wine Res. 11. (2005)
3. E. Casalta, M.F. Cervi, J.M. Salmon, J.M. Sablayrolles, Aust. J. Grape Wine Res. 19. (2013)
4. E. Casalta, A. Vernhet, J.M. Sablayrolles, C. Tesnières, J.M. Salmon, AM. J. Enol. Vitic. 67. (2016)