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The impact of yeast adaptation in the presence of bio-activators on wine alcoholic fermentation

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The first two steps of the PhD project are described. The present PhD project involved experimental conditions and conducting analyses. After rehydration, microbial concentration was determined through plate counts. Subsequently, grape musts were inoculated in the presence of bio-activators. Microbiological monitoring during the early stages of alcoholic fermentation was assessed through plate counts and flow cytometry (FC) assays.

Impatto dell'adattamento del lievito in presenza di bioattivi sulla fermentazione alcolica del vino

Nelle prime due fasi del progetto di dottorato, sono stati descritti gli aspetti iniziali. Il progetto di dottorato ha coinvolto la definizione delle condizioni sperimentali e l'esecuzione delle analisi. Dopo la reidratazione, la concentrazione microbica è stata determinata attraverso la conta in piastra. Successivamente, i mosti d'uva sono stati inoculati in presenza di bio-attivatori. Il monitoraggio microbiologico durante le prime fasi della fermentazione alcolica è stato valutato attraverso conte su piastra e analisi di citometria di flusso.

Key words: *Saccharomyces cerevisiae*, ADY rehydration, viability evaluation, flow cytometry, grape must fermentation

1. Introduction

In accordance with the PhD project plan, this poster reports the primary outcomes of initial research activities related to the following:

- (A1) determining and statistically analyzing the microbial concentration after rehydration using two different protocols;
- (A2) inoculating yeast strains into grape must in the presence of the bio-activators, assessing their microbial levels at various detection points post-inoculation, and evaluating their viability.

2. Materials and Methods

The yeast strain used in this study was active dry yeast (ADY) form. The initial step focused on rehydrating the strain. The microbiological analysis was conducted using plate counts as reported by Li et al. (2020). In detail, 10 g of ADY was rehydrated in two different solutions, as outlined by OIV (2023) and the manufacturer's protocol. Subsequently, serial decimal dilutions and plating were performed as described by Francesca et al. (2024). The second analysis involved monitoring yeast levels during the early hours of alcoholic fermentation. For this purpose, the inoculum was performed in the presence of two bio-activators to assess whether their inclusion at the inoculum stage would enhance fermentation performance. Two different modalities of grape must cv Catarratto were used: a standard grape must representing a regular environment and a challenging condition for yeast growth (pH 2.9). Monitoring was carried out by measuring weight losses, conducting plate counts, and using flow cytometry, following the method reported by Guzzon and Larcher (2015). Samples were taken at 14, 24 and 48 h after inoculation to evaluate microbial concentration and assess yeast viability using plate counts and flow cytometry (Chaney et al., 2006).

3. Results and Discussion

3.1 Evaluation of the rehydration process

The initial step in the winemaking process, where the primary microbial driver is *S. cerevisiae* in ADY form consists in rehydrating the strain before inoculating it into grape must. At this stage, achieving a sufficiently high yeast level is crucial for the overall success of the fermentation process. According to the OIV-OENO 602-2022 resolution, ADY should contain viable yeast cells equal to or exceeding 10^{10} CFU/g of dry matter. Therefore, the first task was to ensure an adequate level of viable yeast cells by applying both OIV and manufacturer rehydration protocols. The analyses (Figure 1) revealed that yeast levels exceeded the requirements set during rehydration. Specifically, plate counts

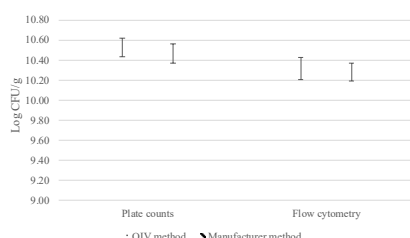


Figure 1 Plate counts and flow cytometry analyses of *S. cerevisiae* SH12 strain after two different rehydration processes. Green bars represent the OIV protocol; blue bars the manufacturer protocol.

yielded the highest cell densities, although the difference between the two methods was not significant. Similarly, flow cytometry analysis showed slightly lower levels compared to the previous analysis, but the difference between the two protocols were negligible.

3.2 Monitoring of yeasts in grape must

The second phase involved monitoring yeasts levels after inoculating ADY into two grape musts with varying pH levels at the start of fermentation. Additionally, two bio-activators were added to evaluate their effect during the initial fermentation stages and to compare treatments with and without bio-activators. The initial analysis (Figure 2) revealed that the treatment using standard must with one of the bio-activators (L1S1) exhibited the highest yeast levels at the first detection point (14 h). Interestingly, in stressed must fermentation, only the L1S1 treatment achieved yeast populations comparable to non-stressed must. By the 24 h detection, the differences between treatments with the bio-activators and the control group were even higher in both stressed and non-stressed fermentations. However, at 48 h detection, the differences levelled out. The control treatments had slightly lower yeast levels than the L1S1 treatment in low-pH must, while both bio-activator treatments in standard fermentation showed yeast levels slightly higher than those reported by Bordet et al. (2024) for a grape must fermented at 20 °C using a *S. cerevisiae* ADY strain.

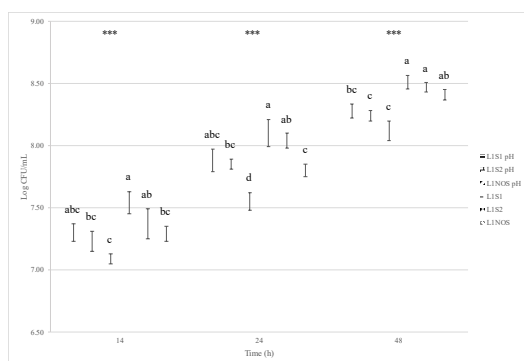


Figure 2 Microbiological monitoring of yeast levels after inoculum in standard and low pH grape must through plate counts.

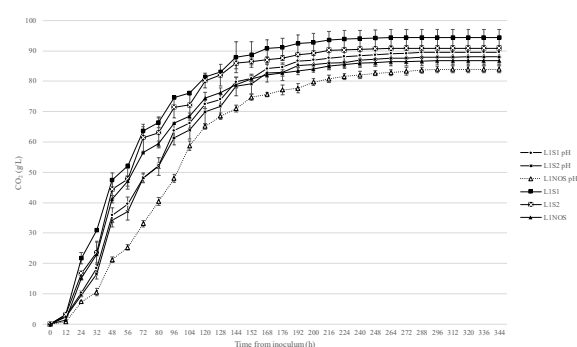


Figure 3 CO₂ emissions calculated by weight losses of the different trials representing inoculum in standard and low pH musts.

Furthermore, fermentation kinetics was evaluated by monitoring weight losses (Figure 3). The treatments with added bio-activators showed superior overall performance. In detail, the highest fermentation rate occurred in trials fermenting standard must, with no statistically significant differences between the bio-activator-added treatments and the control. However, in low-pH conditions, marked differences emerged. The experimental trails (L1S1 and L1S2) had fermentation rates of 16.05 and 16.03 g/L/day, respectively, while the control trial (L1NOS) had a rate of 11.02 g/L/d. Furthermore, the presence of the bio-activators led to higher cell viability (Figure 4). Under standard conditions, viability exceeded 90% in all treatments at each detection point, consistent with previous findings by Chaney et al. (2006). On the other hand, in low-pH conditions, the control treatment exhibited severe viability decline (< 75%) at both 14 and 24 h compared to the bio-activator-added treatments. This viability assessment suggests that the improved fermentation rate observed with the two bio-activators occurs in both standard and low-pH conditions.

4. References

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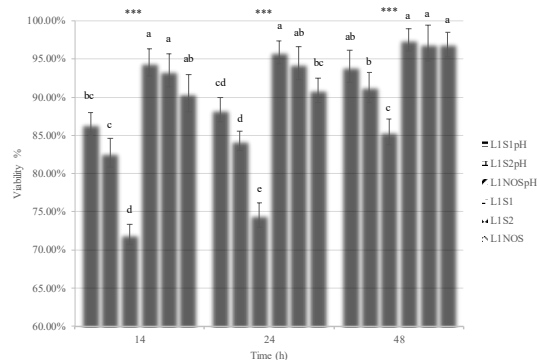


Figure 4 Viability of yeast levels after inoculum in standard and low pH grape must calculated through rate of live and dead cells determined by flow cytometry assay.

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Effects of the addition of bio-activators on wine alcoholic fermentation in challenging conditions

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INTRODUCTION

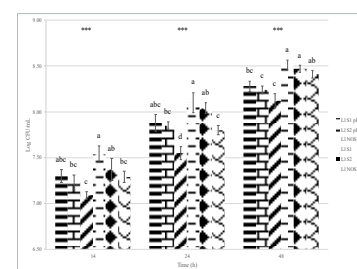
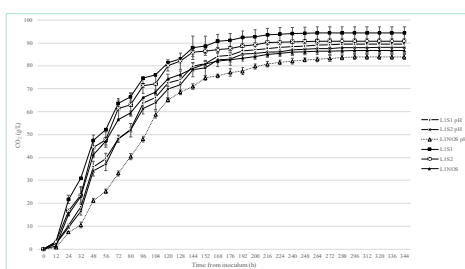
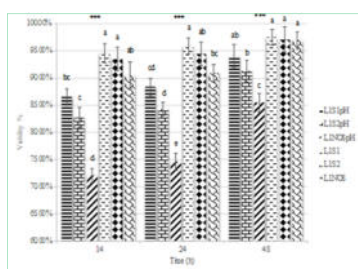
The alcoholic fermentation process is usually driven by yeast cells, which could face several issues due to numerous ecological and technological factors that could affect the final quality of the product. The metabolic activity and cell development of yeasts can be severely affected by a wide variety of stress factors. In order to protect themselves from environmental stress, cells often develop adaptive responses. These require temporary reprogramming of cellular activities to protect cell membranes from stress-induced damage, enabling cells to withstand and recover from these stresses. Among these environmental challenges, low pH can have a significant impact on yeast metabolism and viability. This factor directly affects several metabolic pathways and cellular functions in yeast. As the extracellular environment becomes more acidic, the proton gradient across the cell membrane can be severely disrupted. As a result, the rate of glucose metabolism can be reduced, reducing overall energy production and yeast cell development.

With the final aim to improve the intrinsic resistance of yeast cells, this study investigated the addition of yeast-based bio-activators and their effect on fermentation performance.

METHODOLOGY

The strain was first rehydrated and viability assessed by plate count. The yeast levels were then monitored during the first hours of alcoholic fermentation. The grape must used was cv Catarratto and the inoculum was performed in the presence of two bio-activators. Two different conditions were established, the first representing a standard must and the second simulating a challenging environment (pH 2.9). Weight loss, plate counts, and flow cytometry were used to monitor the results. The samples were collected 14, 24, and 48 hours after the inoculum to determine microbial content and yeast viability

RESULTS



- Viability > 80 % in all treatments except for L1NOS_pH within the first 24 h from the inoculum

- Standard treatments showed the highest fermentation rates
- Within low pH trials, bio-activators added ones exhibited significantly higher FR than control treatment

- Yeast levels increased along 48 h of alcoholic fermentation, showing the greatest differences within the first 24 h

CONCLUSIONS

The presence of bio-activators enhanced cell viability, which could represents a fair explanation for the improved fermentation rate observed in presence of both bio-activators. Further researches are ongoing to study the effects on the volatile organic composition and to explore how gene expressions and fatty acids profile are modulated in several challenges conditions