RESOLUTION OIV-OENO 739-2025

GUIDELINES TO EVALUATE THE FERMENTATION PROPERTIES OF *Saccharomyces cerevisiae* STRAINS

WARNING: This resolution will be added as an appendix of the following resolution:
• OIV-OENO 370-2012 GUIDELINES FOR THE CHARACTERIZATION OF WINE YEASTS OF THE GENUS SACCHAROMYCES ISOLATED FROM VITIVINICULTURAL ENVIRONMENTS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Microbiology" Expert Group,

DECIDES, at the proposal of the Commission Oenology, to add the following paragraph to preamble of the resolution OIV-OENO 370-2012:

The evaluation of fermentation properties of *S. cerevisiae* strains is requested to guarantee the quality of the alcoholic fermentation and the production of wines with expected sensory properties (See Appendix 2). Unsuitable experimental conditions can lead to inconsistent results that do not reflect the reality of these fermentation properties.

DECIDES to add the standardised protocol to evaluate the fermentation properties of *S. cerevisiae* strains as an Appendix at the end of the resolution OIV-OENO 370-2012:

Appendix 2

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INTRODUCTION

The utilization of *Saccharomyces cerevisiae* strains with selected traits is fundamental to modulating the final characteristics of the wine. The first step toward the attainment of *S. cerevisiae* wine starters is the clonal selection based on the oenological characterisation of *S. cerevisiae* wine strains performed by evaluation of

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several phenotypic traits based on fermentations carried out at a laboratory scale using synthetic media or grape musts. Natural grape musts can be considered more suitable to obtain a reliable characterisation of wine yeasts, but differences in their chemical composition influencing yeast oenological properties renders difficult the comparison of *S. cerevisiae* strains that are assayed in different laboratories or in the same laboratory at different times. Furthermore, the results of such characterisation may be extensively affected by the experimental conditions used to carry out the fermentation trials.

SCOPE

This annex reports in table 1 a validated and standardised protocol to assess the fermentative and metabolic properties of *S. cerevisiae* wine strains in a synthetic medium, which allows a direct unbiased comparison of the experimental data obtained during the characterisation of wine yeasts carried out by different laboratories.

Table 1. Protocol for standardisation of fermentation trials in synthetic must.

Step	Procedure
Yeast strain supply	When applicable, the active dry yeast (ADY) belonging to the same lot should be used after rehydration. If other sources are available prepare the liquid inoculum as follows: 100 µL from the mother culture into 5 mL of fresh medium and incubating for 12 hours repeating for three times (i.e. this operation should be repeated three times for inoculum standardisation).
Synthetic must preparation	The synthetic must composition is reported in Table 1 of the resolution OIV-OENO 370-2012.
Synthetic must distribution	500-mL Erlenmeyer flasks containing 350 mL of synthetic must and equipped with Muller valves are used. The trials are carried out in triplicates (three independent experiments).

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Yeast rehydration	According to the resolution OIV/OENO 329/2009 each ADY strain is rehydrated as follows: • Weigh 1 g of ADY under aseptic conditions, • Rehydrate in 100 mL of water at 36-40°C under sterile conditions; • Homogenize slowly using a rod or a magnetic stirrer for 5 min, • Stop stirring and allow to stand for 20 min at a temperature between 36-40°C, • Homogenize again at room temperature for 5 min, • Take 10 mL under sterile conditions and then proceed to count viable yeast cells by Thoma counting chamber and 0.1% (w/v) methylene blue solution.
Yeast strain inoculum	Inoculate the rehydrated yeast, or the precultured liquid culture in the synthetic must, to get $2X10^6$ viable cells/mL
Fermentation trial conditions	Incubate the Erlenmeyer flasks closed with Muller valves (containing sulfuric acid) at 25 °C or 17°C for red, white and rosé wines, respectively, in static conditions for 15 days
Fermentation monitoring	Check the weight loss daily after shaking each Erlenmeyer flask by hand for 1 min
Sample arrangement for analyses	At the end of the fermentation, centrifuge at 3,000 x g for 5 min at room temperature and separate the cells from the supernatant
Chemical analyses	1. Chemical analyses of the resulting wines should be performed at the end of the alcoholic fermentation (residual sugars < 2 g/L). 2. The following chemical analyses should be carried out: ethanol, glucose, fructose, glycerol and acetic acid content and the concentration of acetaldehyde, 1-propanol, 2-methyl-1-butanol, ethyl acetate, 2-methyl-1-propanol, and 3-methyl-1-butanol. 3. The yield of the fermentative products (ethanol, glycerol, acetic acid) will have to be standardised by calculating the amounts of fermentative products per unit of consumed sugar. 4. The analysis should be performed by official OIV methods at a certified laboratory.

PRINCIPLE OF THE PROTOCOL

The protocol indicates the different phases and, for each phase, the detailed procedures to be performed to define the standard conditions to be applied for the characterisation of oenological properties of *S. cerevisiae* strains. This protocol was

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validated by performing inter-laboratory-scale comparative fermentations using both synthetic medium and grape musts and through verification of obtaining repeatable, reproducible, and statistically valid results. The conditions to address replicability were the use of standardised experimental conditions (by calculating the amounts of fermentative products per unit of consumed sugar) and accurate treatment of the data, including the combination of non-parametric tests and clustering approaches.

Reference

1. Romano P. *et al.,* (2022) Validation of a standard protocol to assess the fermentative and chemical properties of *Saccharomyces cerevisiae* wine strains. *Frontiers in Microbiology,* 13: 830277. doi: 10.3389/fmicb.2022.830277

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