FORTHEM international conference: Democratic & Responsible Bio Innovation

Wednesday, 11 March 2026 - Friday, 13 March 2026

ONLINE

Book of Abstracts

Sustainable Bioeconomy & Nature Based Solutions / 30

Sequential inoculation strategies in malolactic fermentation: impact on fermentation kinetics and volatile compounds in Catarratto wines

Author: Irene Dolce[1]

Co-authors: Vincenzo Naselli [2]; Enrico Viola [3]; Azzurra Vella [4]; Valentina Craparo [3]; Antonino Pirrone [2]; Micaela Carusi [5]; Venera Seminerio [5]; Antonio Alfonzo [2]; Luca Settanni [2]; Antonella Maggio [6]; Daniela Fracassetti [7]; Vasileios Englezos [8]; Nicola Francesca [2]

[1] *Università di Palermo*

[2] *Università degli Studi di Palermo*

[3] *University of Palermo*

[4] *Università degli studi di Palermo*

[5] *Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze Bldg. 5, Ent. C, 90128 Palermo, Italy*

[6] *Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Parco d'Orleans II, 90128 Palermo, Bldg. 17, Italy*

[7] *Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy*

[8] *Department of Agricultural, Forest and Food Sciences, University of Torino, Largo P. Braccini 2, 10095, Grugliasco, Italy*

Corresponding Authors: nicola.francesca@unipa.it, antonio.alfonzo@unipa.it, antonino.pirrone@unipa.it, azzurra.vella@unipa.it, valentina.craparo03@unipa.it, luca.settanni@unipa.it, enrico.viola01@unipa.it, vincenzo.naselli@unipa.it, irene.dolce@unipa.it

Malolactic fermentation is a key biological deacidification process in winemaking, in which L-malic acid, naturally present in grapes, is converted into L-lactic acid and carbon dioxide, resulting in a reduction of wine acidity. Lactic acid bacteria can be introduced at various stages of production, before alcoholic fermentation, during co-inoculation, or through sequential inoculation with yeasts (after 24, 48, or 72 hours). Previous studies have shown that early bacterial inoculation improves acclimatisation to stress factors such as ethanol and sulphur dioxide, bacterial resilience and accelerating fermentation. This study comparative six fermentation protocols and their respective controls to assess the impact of the non-*Saccharomyces* yeast *Starmerella lactis-condensi* MN412, sequentially inoculation with *Saccharomyces cerevisiae* QA23 and the lactic acid bacteria *Oenococcus oeni* and *Lactiplantibacillus plantarum*, on fermentation kinetics and the volatile organic compounds (VOC) profile of Catarratto wines. The fastest fermentation kinetics were observed in the sequential inoculation involving *St. lactis-condensi*, *L. plantarum*, and *S. cerevisiae.* In this protocol, malic acid showed the greatest decline between days 3 and 4, decreasing from 1.30 to 0.41 g/L and reaching a final concentration of 0.12 g/L. Concurrently, lactic acid increased to approximately 0.73 g/L. The resulting volatile profile displayed elevated alcohol levels and the highest total concentration of carboxylic acids (32.68 ppm), including 23.41 ppm of acetic acid, together with esters and fruity compounds. The protocol combining *St. lactis-condensi*, *O. oeni* and *S. cerevisiae* showed a more gradual conversion of malic acid, with a reduction of 0.11 g/L between days 3 and 4, and final lactic acid values comparable to the other treatments (approximately 0.73 g/L). VOCs in this treatment were dominated by esters (20.2 ppm), including 3-methylbutyl acetate, ethyl lactate, and multiple fatty acid esters. The treatment with *St. lactis-condensi* alone produced one of the highest total alcohol concentrations (192.49 ppm). In the protocol in which *S. cerevisiae* was inoculated prior to *O. oeni*, malic acid was degraded more slowly than in treatments involving *L. plantarum*, although medium-chain fatty acids such as hexanoic and octanoic acids, together with abundant esters, contributed positively to the aroma profile. Finally, the sequential inoculation of *S. cerevisiae* and *L. plantarum* achieved rapid malic acid degradation and generated the highest lactic acid concentration (0.96 g/L), with medium-chain fatty acids supporting overall compositional balance. Overall, sequential inoculation strategies, particularly those involving *L. plantarum*, resulted in faster fermentation kinetics and more complex aroma profiles, while treatments with *O. oeni* favoured higher ester production.

Sequential inoculation strategies in malolactic fermentation: impact on fermentation kinetics and volatile compounds in Catarratto wines

Irene Dolce[1], Vincenzo Naselli[1], Enrico Viola[1], Azzurra Vella[1], Valentina Craparo[1], Antonino Pirrone[1], Micaela Carusi[1], Venera Seminerio[1], Antonio Alfonzo[1], Luca Settanni[1], Antonella Maggio[2], Daniela Fracassetti[3], Vasileios Englezos[4], Nicola Francesca[1]

[1] Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze Bldg. 5, Ent. C, 90128 Palermo, Italy [2] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Parco d'Orleans II, 90128 Palermo, Bldg. 17, Italy

[3] Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy

[4] Department of Agricultural, Forest and Food Sciences, University of Torino, Largo P. Braccini 2, 10095, Grugliasco, Italy

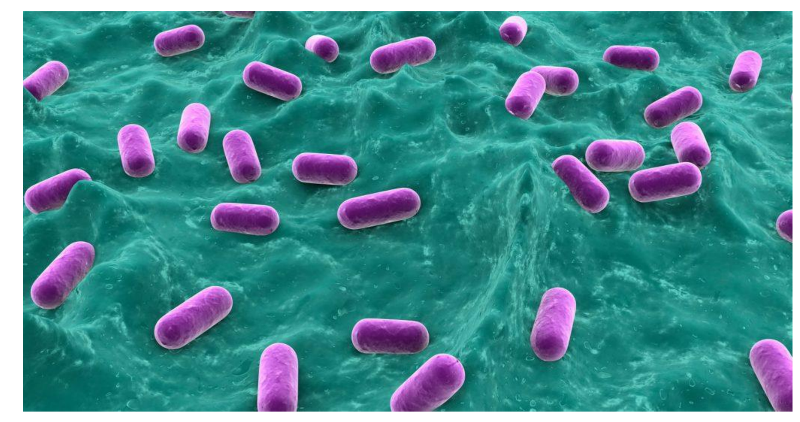
INTRODUCTION

Malolactic fermentation is a key biological deacidification process in winemaking, in which L-malic acid, naturally present in grapes, is converted into L-lactic acid and carbon dioxide, resulting in a reduction of wine acidity. Lactic acid bacteria can be introduced at various stages of production, before alcoholic fermentation, during co-inoculation, or through sequential inoculation with yeasts (after 24, 48, or 72 hours). Previous studies have shown that early bacterial inoculation improves acclimatisation to stress factors such as ethanol and sulphur dioxide, bacterial resilience and accelerating fermentation

This study comparative six fermentation protocols and their respective controls to assess the impact of:

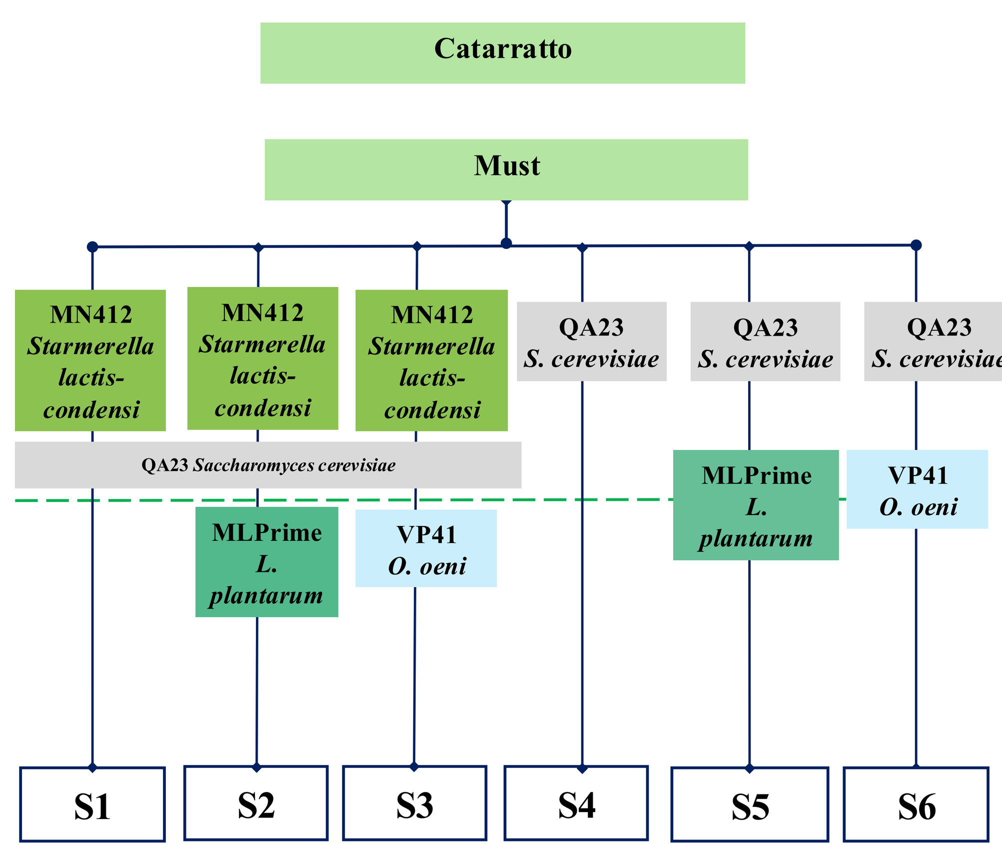
- *Starmerella lactis-condensi* MN412;
- *Saccharomyces cerevisiae* QA23;
- the lactic acid bacteria *Oenococcus oeni* and *Lactiplantibacillus plantarum*;

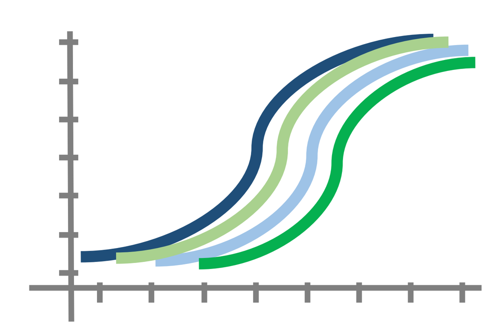
on fermentation kinetics and the volatile organic compounds (VOC) profile of Catarratto wines.



METHODOLOGY

- ❖ Analysis of **volatile compounds**: wines obtained from different combinations of yeast/bacteria starters were analysed using **SPME/GC-MS** to identify and quantify alcohols, aldehydes, ketones, carboxylic acids, esters and other volatile compounds.
- ❖ **Malolactic fermentation**: monitored by evaluating the conversion of malic acid to lactic acid (**g/L**).

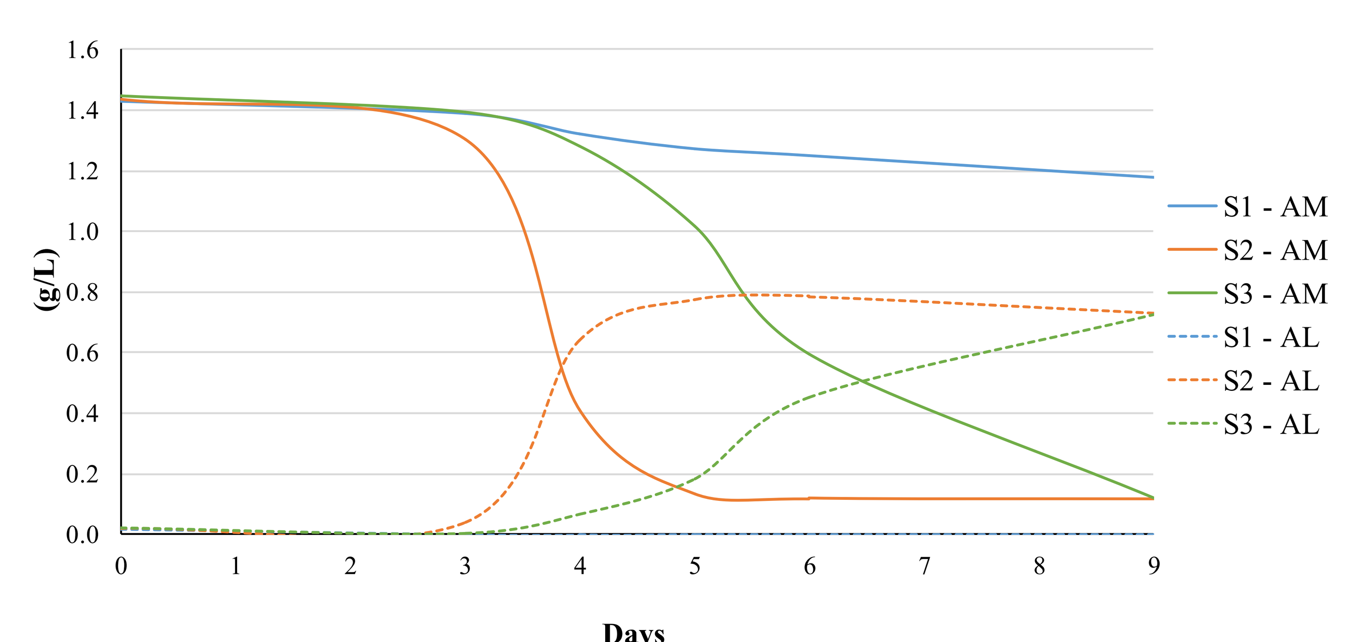


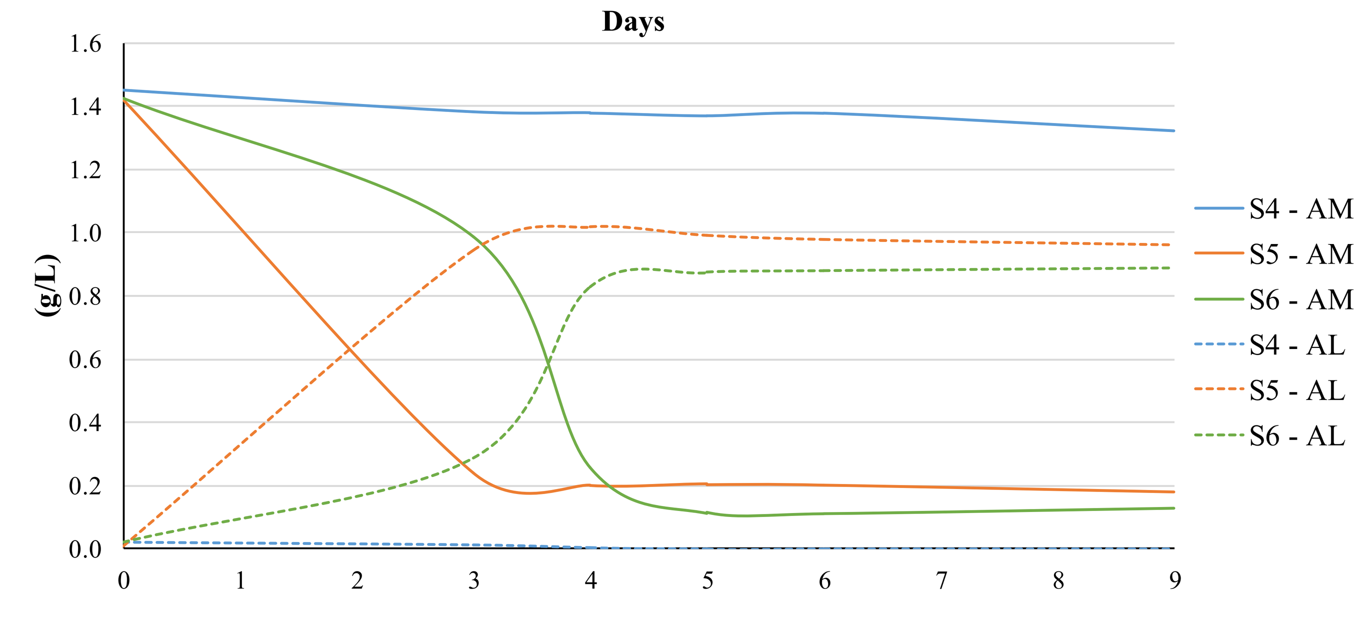


RESULTS

- ❖ Malic acid degradation was closely correlated with **cell density**, leading to final **lactic acid** concentrations of up to **0.96 g/L**.
- ❖ **Alcohols** are the most abundant compounds
- ❖ **Aromatic esters** (e.g., ethyl acetate, ethyl lactate), enhancing **fruity** notes and **aromatic complexity.**

Compounds	Trials					
	S1	S2	S3	S4	S5	S6
∑Alcohols	**192.49±9.79ab**	**215.85±14.1a**	**141.74±11.96cd**	**86.20±5.62f**	**124.34±8.4de**	**146.27±8.19cd**
∑Aldehydes	**4.9±0.36b**	**7.15±0.96a**	**2.46±0.36de**	**2.08±0.25def**	**3.12±0.44cd**	**4.29±0.54bc**
∑Ketones	**7.29±0.94a**	**2.97±0.34bc**	**3.04±0.35b**	**1.49±0.14de**	**2.47±0.28bcd**	**3.22±0.36b**
∑Carboxylic acids	**4.6±0.51de**	**32.68±3.71a**	**18.14±2.14b**	**5.17±0.67de**	**8.03±0.72cd**	**3.87±0.59de**
Acetic acid	0.00±0.00d	23.41±2.54a	11.59±1.34b	3.21±0.47c	3.23±0.24c	0.00±0.00d
∑Esters	**14.49±1.58b**	**15.74±1.80ab**	**20.2±2.44a**	**8.51±0.99c**	**7.89±0.92cd**	**19.21±2.28ab**
Ethyl acetate	10.54±1.16a	11.00±1.34a	9.74±1.12a	5.99±0.64b	3.53±0.45c	10.21±1.15a
Ethyl lactate	0.00±0.00e	0.00±0.00e	5.93±0.68a	0.27±0.03de	1.28±0.14de	3.99±0.57b
3-Methyl-butyl acetate	0.61±0.08cd	0.61±0.09cd	1.87±0.24b	0.58±0.07d	0.93±0.11cd	1.51±0.17bc
2-Phenylethyl acetate	0.52±0.04de	0.57±0.03cd	0.43±0.08de	0.36±0.04e	0.52±0.06de	0.87±0.09b





ACKNOWLEDGMENTS

Project: PRIMA 2022 - MEDIET4ALL. Title: "Transnational Movement to Support the Sustainable Transition towards a Healthy and Eco-friendly Agri-Food System through the Promotion of MEDIET and its Lifestyle in Modern Society." Code CUP: B73C23000060001; PRJ-1312