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

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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.

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